Cangrelor vs. Ticagrelor in Patients with ST Segment Elevation Myocardial Infarction Treated with Primary Percutaneous Coronary Intervention: Impact on Platelet Activity, Myocardial Micro-Vascular Function and Infarct Size

A Randomised Controlled Open Label Pilot Study



UNIVERSITY OF BIRMINGHAM

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Abstract

Background

The action of oral $P2Y_{12}$ inhibitors is delayed in patients with acute myocardial infarction. Intravenous $P2Y_{12}$ inhibition might lead to a more timely and potent antiplatelet effect in the context of primary angioplasty, improving myocardial recovery.

Objectives

To compare the efficacy of IV cangrelor vs. ticagrelor in a STEMI population treated with primary percutaneous coronary intervention (PCI).

Methods

In an open-label, prospective, randomized controlled trial, 100 subjects with STEMI were assigned to IV cangrelor or oral ticagrelor. The co-primary endpoints were platelet $P2Y_{12}$ inhibition at infarct vessel balloon inflation time, 4 and 24 hours. Secondary endpoints included indices of coronary microcirculatory function, myocardial infarct size, and angiographic, and electrocardiographic analysis. (ClinicalTrials.gov NCT02733341).

Results

$P2Y_{12}$ inhibition at balloon inflation time was significantly greater in cangrelor-treated patients (cangrelor PRU 145.2 ± 50.6 vs. ticagrelor 248.3 ± 55.1; $p<0.001$). There was no difference in mean PRU at 4 hours and 24-36 hours post dosing. IMR, final infarct size, angiographic and electrocardiographic measures of reperfusion were all similar between groups.

Conclusion

Cangrelor produced more potent $P2Y_{12}$ inhibition at the time of coronary reperfusion compared with ticagrelor. Despite this enhanced antiplatelet effect, coronary microvascular function and infarct size did not differ between groups.

Dedication

I dedicate this thesis to my parents Mohammed-Rushdi and Shiela Amin and to my wife Iman and my children, Yusuf and Leen.

Acknowledgments

Firstly, want to thank my clinical supervisor, Professor James Cotton, for giving me the opportunity to work with him and for his fantastic support and guidance throughout my project. I have found Professor Cotton to be very supportive and dedicated. The door to Professor Cotton's office was always open whenever I needed help and support. During my time working with Professor Cotton, I enjoyed a high degree of independence, and at the same time, he steered me in the right direction whenever he thought I needed it. I could not have wished for better mentorship. To Professor Cotton, I remain ever grateful.

I want to offer my special thanks and sincere appreciation to my academic supervisor, Professor Jon Townend, for his support, motivation, immense knowledge, and insightful comments. His guidance always assisted me during this research and writing of this thesis. I owe my sincere gratitude to Dr. Saib Khogali for his support throughout the study and his valuable advices. Also, I would like to show my appreciation to Dr. Mike Norell for sharing his pearls of wisdom during this study. This has been extremely useful and fruitful experience in my career development, and to all, I am most grateful.

I am thankful and fortunate enough to get support, encouragement, and guidance from the research and development department, interventional cardiology colleagues and nurses at the Heart and Lung Centre, New Cross Hospital in Wolverhampton. I was fortunate enough to be able to work with fantastic colleagues with significant expertise in the field, particularly from Dr. Vincent Amoah to whom I am very grateful. I am also very appreciative to Dr. Elisa McAlindon for her support with performing CMR studies. I would not forget Dr. McAlindon coming back to work during her maternity leave period to provide CMR scans reports. I am grateful to Dr. Mark Thomas for his excellent academic support.

I would also like to thank members of the Tripartite Charity Fund Organization for granting me sufficient funds to run this project. I genuinely want to thank all the patients who took part in my project, without whom this research would not have been possible.

I will remain endlessly grateful to my wife, Iman, for her unlimited support and putting up with the many days and nights I have spent at the hospital to accomplish this work.

Study Publications and Presentations

- Cangrelor vs. Ticagrelor in Patients Treated with Primary Percutaneous Coronary Intervention: Impact on Platelets, Microcirculation & Infarct Size
  Thrombosis and Haemostasis Journal (Published, May 2019)

- The effect of Cangrelor versus Ticagrelor on platelet activity, coronary micro-circular function and infarct size in patients suffering acute ST-segment elevation myocardial infarction treated with primary percutaneous coronary intervention: a randomized controlled trial.
  Ubaid, S. Ford, T. Berry, C. Khogali, S. Wrigley, B. Munir, S. Martins, J. Jaumdally, R. Amoah, V. Khan, N. Jon, T. McAlindon, E. [and] Cotton, J. (2018). J Am Coll Cardiol. March 2018, 71(11), Supplement, A1388.

- Cangrelor versus Ticagrelor in Primary Percutaneous Coronary Intervention: Platelets, Microcirculation and Infarct Size.
  Ubaid, S. Khogali, S. Wrigley, B. Munir, S. Martins, E. McAlindon, Ford, T. Berry, C. Townend, J. and Cotton, J. Heart. January 2018; 104: A8-A9 (Abstract)

- American College of Cardiology Conference
  Orlando, Florida, USA 10-12/03/2018 (Abstract presentation)

- BCIS Young Investigator Award (Runner up)
  RCP, London 30/11/2017 (Abstract presentation)

Extent of Personal Contribution

Dr. Elisa McAlindon and Dr. S. Hothi conducted and reported cardiac magnetic resonance scans. Interventional cardiology colleagues and I conducted IMR tests. Dr. Tom Ford and Professor Colin Berry performed an independent analysis of IMR and CFR data. Professor Cotton and Dr. Ben Wrigley performed the second blinded analysis of angiographic data. I made a significant contribution to the study protocol design. In addition to the above, I have undertaken the following:

• Application for local peer review committee approval.
• Application for ethics approval.
• Application for MHRA approval.
• Application for funding from the Tripartite Charity organisation.
• Arranging the necessary consumables needed to run the study such as VerifyNow, VASPFix materials and equipment required to perform coronary physiology studies.
• Undertaking the training necessary to learn how to run platelet reactivity test using point-of-care VerifyNow assay at New Cross Hospital and VAPFix assay provided by Platelet-solutions at Queens medical centre, Nottingham, UK.
• All the necessary communication with the Medicines Company concerning arranging cangrelor supply with the help of the pharmacy department.
• Training cardiology wards and cardiac catheterisation laboratory nurses how to set up and administer IV cangrelor infusion.
• Arranging teaching sessions for physiologist and cardiac catheterisation laboratory staff on IMR technique and setup of RadiAnalyzer.
• Traveling to Golden Jubilee Hospital in Glasgow several times and to John Ratcliff Hospital to attend “Meet the expert sessions” to learn IMR assessment technique.
• Designing case report, consent, and verbal assent forms, in addition to patients information sheets.
• Designing and distributing study posters.
• Communicating with referring hospitals to facilitate the enrolment of patients.

• Approaching 226 patients with STEMI in the space of approximately five months to eventually be able to recruit 100 patients.
• Assisting nurses in the setup and administration of study drugs.
• Conducting VerifyNow and VASFix assay and analysing them.
• Performing and supervising IMR tests.
• Performing angiographic analysis following primary PCI.
• Consenting patients and completing all the relevant research related paper works.
• Complete data collection.
• Data analysis with the help of Mrs. Heather Murray at Robertson Centre for Biostatistics, University of Glasgow.
• Reporting to local monitoring group at New Cross Hospital.
• Writing the manuscript for peer review publication.

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List of Abbreviations

ACS	Acute coronary syndrome
ADP	Adenosine diphosphate
ASA	Acetylsalicylic acid
ATP	Adenosine triphosphate
CABG	Coronary artery bypass graft
CAD	Coronary artery disease
CBFV	Coronary blood flow velocity
CFR	Coronary flow reserve
CMR	Cardiac magnetic resonance
CMVD	Coronary Microvascular dysfunction
CTFC	Corrected TIMI frame count
ESC	European society of cardiology
FDA	Food and drug administration
GP	Glycoproteins
IMR	Index of microvascular resistance
IV	Intravenous
MPG	Myocardial perfusion grade
NSTEMI	Non ST-elevation myocardial infarction

PCI	Percutaneous coronary intervention
STEMI	ST-elevation myocardial infarction
TFG	TIMI flow grade
VASP	Vasodilator-stimulated phosphoprotein
VWF	Von Willebrand factor

Introduction

In the United Kingdom, cardiovascular diseases account for more than 28% of all deaths each year, that is approximately 170,000 deaths each year, of whom 43,000 are under the age of 75.

Over 66,000 deaths each year are from coronary artery disease (CAD), of whom 22,000 deaths are under the age of 75. Acute myocardial infarction is responsible for most deaths from CAD are caused. The annual number of deaths from CAD in the UK Since the 1960s has fallen by more than 50%. Death rates have dropped more rapidly than the actual number of deaths because people are living longer. (Bhf.org.uk/statistics) (Figure 1)

Worldwide, CAD remains the leading cause of mortality. (1) More than 7 million people die every year from CAD accounting for approximately 12.8% of all deaths in western countries. (1) The west has witnessed a remarkable decline in mortality from CAD due to the significant advances in cardiovascular science and medicine. (2)

Currently, atherosclerosis is recognised to be an inflammatory condition. Inflammation plays a vital role in all stages of atherogenesis and contributes to the complications of atherosclerosis. (3) The deposition of lipids, inflammatory cells, and other materials in the coronary arteries eventually lead to the formation of atheromatous plaques that in turn cause progressive narrowing of the arteries and reduction of blood supply to the myocardium.

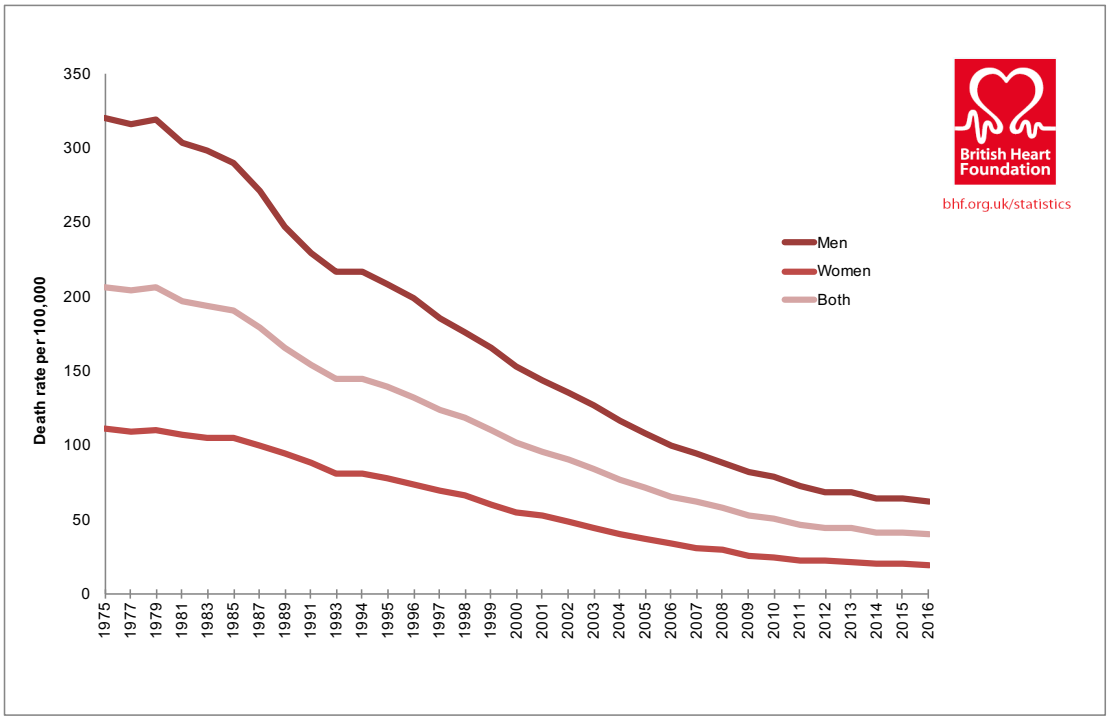


Figure 1 UK age-standardised death rate

Death rate per 100,000 from coronary heart disease (CHD), under 75, by gender, United Kingdom, 1975 to 2016 (British Heart Foundation/Statistics)

Role of Platelets in Coronary Artery Disease

The Coronary Endothelium

The vascular endothelium is a biologically active single layer of cells that lines the entire circulatory system. It acts as a dynamic barrier that separates the vascular wall from circulating blood. It controls vascular function by responding to various hormones, neurotransmitters, and vasoactive substances, which affect the vascular tone, platelet aggregation, thrombosis, and inflammation. The endothelium releases different vasoactive substances. Substances that have a vasodilatory effect include nitric oxide, prostacyclin, and endothelium-derived hyperpolarizing factor. Substances that have a vasoconstrictive effect include thromboxane (TXA2) and endothelin-1 (ET-1). (1) These substances provide an atheroprotective environment. Risk factors such as diabetes mellitus, hypertension, hyperlipidaemia and smoking can cause pathophysiological vascular changes such as overproduction of reactive oxygen species (ROS), increased oxidative stress, vasomotor tone modifications, thrombotic dysfunctions, smooth muscle cell proliferation, and migration, as well as leukocyte adhesion and migration and eventually impairment of endothelial vasodilatation, atherogenesis and later formation and progression of atherosclerotic plaque. (4, 5)

Coronary Atherosclerosis

Atherosclerosis is a disease of the vascular intima affecting medium and large-sized arteries. It involves a diffuse pathological process that includes structural changes in the intima and media of arterial vessels largely determined by cholesterol accumulation, inflammatory cell infiltration, and vascular smooth muscle cell migration. The first type of atherosclerotic lesion is a fatty streak. (6) Persistent high levels of plasma lipids, mainly, low-density lipoprotein cholesterol is considered one of the major factors responsible for the endothelial injury. Infiltration of the sub-endothelial layer with low-density lipoproteins particles containing apolipoprotein B is the essential initial process that leads to atherogenesis. (7)

Low-density lipoprotein particles stimulate the endothelium to secrete chemotactic materials and express adhesion receptors, such as integrins and selectins, which attract monocytes and lymphocytes recruitment, adhesion, and migration into the arterial wall. Cytokines or growth factor produced in the inflamed intima and macrophage colony-stimulating factor induce monocytes to differentiate into macrophages, which in turn express receptors such as scavenger receptors and toll-like receptors, that help the uptake of cholesterol molecules found in low-density lipoprotein particles resulting in the formation of foam cells, the main component of atherosclerotic plaques. (6, 8, 9) (Figure 2)

Foam cells cause the release of cytokines, growth factors, reactive oxygen species, tissue factor, and metalloproteinases that prolong and magnify the inflammatory response. (10) With time, this leads to vascular remodeling and increase the susceptibility of a plaque to become unstable and prone to subsequent rupture resulting in thrombus formation and coronary artery obstruction. Stable advanced atherosclerotic plaques usually have homogeneously dense fibrous caps. (Figure 3) The main characteristics of plaques that are unstable, vulnerable and liable to rupture include; a sizeable necrotic lipid core; a thin inflamed fibrous cap due to permanent activation of macrophages, which release proteolytic enzymes (11); reduced collagen and vascular smooth muscle cells quantity; and neovascularisation. (Figure 4)

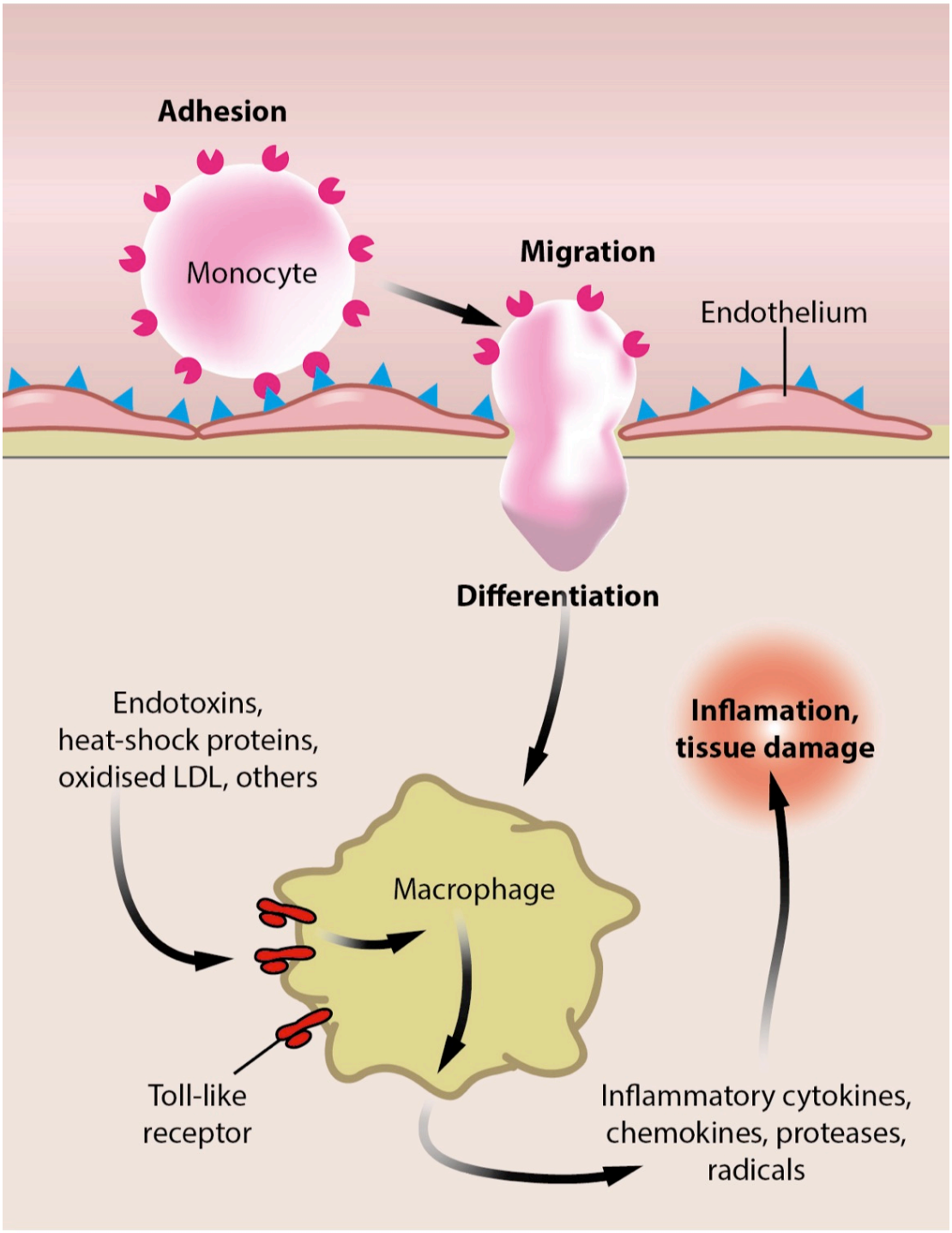


Figure 2 Pathophysiology of atherosclerosis. NEJM 2005;352:1685-95

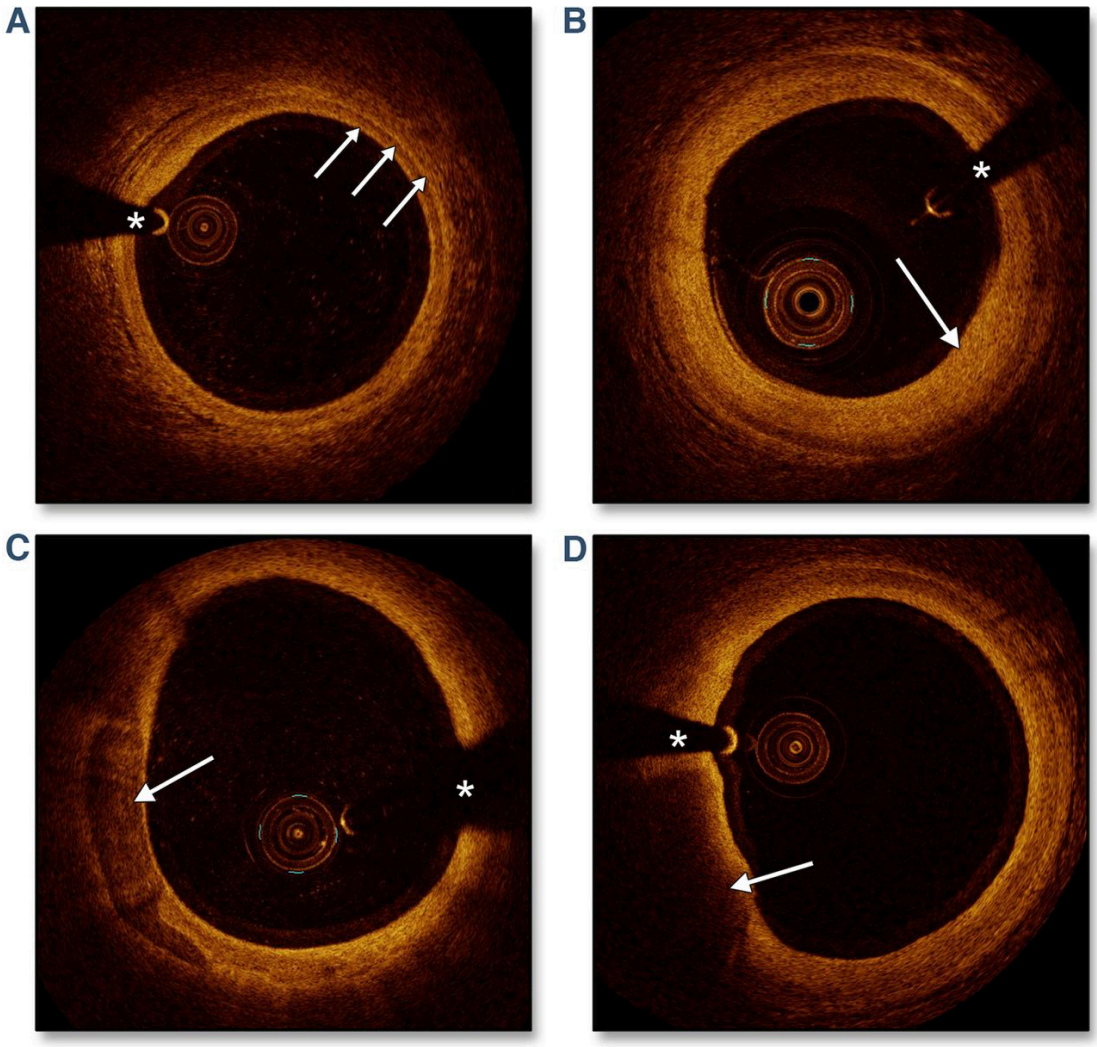


Figure 3 Plaque characterisation by Optical Coherence Tomography (OCT)

A-Composition of the normal coronary artery, with white arrows depicting the internal elastic lamina, media and external elastic lamina. B- Concentric fibrous plaque. C- Calcified plaque. D- Necrotic core. *Guidewire artifact. Source; OCT for the Identification of Vulnerable Plaque in Acute Coronary Syndrome Hannah Sinclair, Christos Bourantas, Alan Bagnall, Gary S. Mintz and Vijay Kunadian, JACC 2015

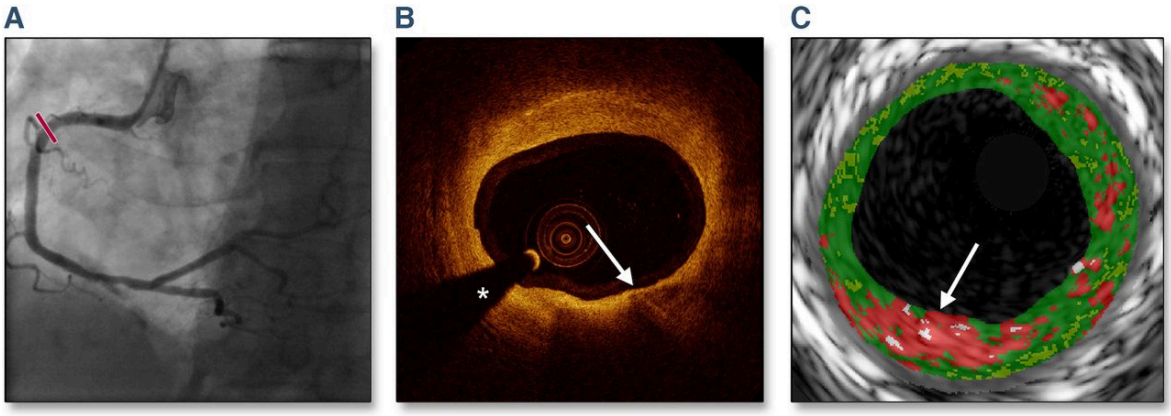


Figure 4 Thin-Cap Fibroatheroma

A-Angiogram with a nonobstructive mild atheroma in the right coronary artery (red bar). B- OCT image of the thin-cap fibroatheroma (white arrow). C- VH-IVUS image of the thin-cap fibroatheroma (white arrow). *Guidewire artifact. OCT = optical coherence tomography; VH-IVUS = virtual histology–intravascular ultrasound. Source; OCT for the Identification of Vulnerable Plaque in Acute Coronary Syndrome Hannah Sinclair, Christos Bourantas, Alan Bagnall, Gary S. Mintz and Vijay Kunadian, JACC 2015

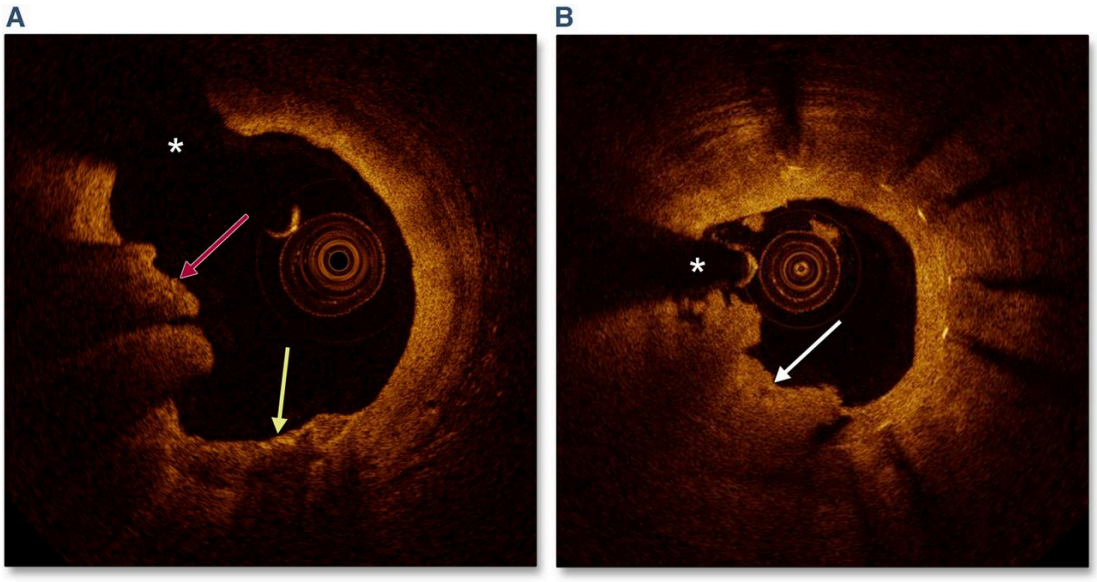


Figure 5 Ruptured atheromatous plaque

A- Red thrombus (red arrow) with high attenuation, associated with a calcified plaque (yellow arrow). B- Stent thrombosis with homogenous, low-attenuation white thrombus (white arrow). *Guidewire artifact. Source; OCT for the Identification of Vulnerable Plaque in Acute Coronary Syndrome Hannah Sinclair, Christos Bourantas, Alan Bagnall, Gary S. Mintz and Vijay Kunadian, JACC 2015

Plaque angiogenesis may contribute to plaque growth and progression through providing the necessary nutrients for cell growth; thus, creating an environment in the intima that facilitate atherosclerosis progression. (12)

In patients presenting with acute coronary syndrome (ACS) undergoing PCI, non-culprit lesions predictive of events were characterised by having a thin fibrous cap, substantial plaque burden, small luminal area and they were often angiographically mild as determined by intravascular imaging studies. (13) Therefore, the composition of the plaque, and not the severity of the atherosclerotic lesion, determines its stability and the likelihood of subsequent rupture. (14) This is supported by the presence of mild residual stenosis on coronary angiography after thrombolytic treatment for acute myocardial infarction. (9)

Studies have highlighted the limited role of coronary angiography in identifying the diffuse nature of atherosclerosis and intimal disease in patients with ACS. Intravascular imaging in ACS has demonstrated that the culprits are often proximal to the site of maximal stenosis. (15)

Plaques that have a significant lipid content and a thin fibrous cap seen on CT coronary angiography can cause outward expansion of the arterial wall (outward remodeling) without minimising the arterial lumen. (15) These types of plaques, which are usually missed by coronary angiography, are often asymptomatic and do not cause ischaemia until physical disruption occurs to the fibrous cap resulting in thrombosis and subsequently ACS. Collateral circulation, which acts as a protective mechanism is more likely to develop at arterial sites, which have severely stenotic plaques to supply the post-stenotic myocardial area with blood, which may explain the reason why some patients with severe atherosclerotic plaques develop silent vessel occlusion. (16)

Role of Platelets in Coronary Atherosclerosis

Platelets are anucleate fragments of megakaryocytes, which are produced in the bone marrow and play a key role in haemostasis and vascular integrity. (17) Under steady-state conditions, approximately 200 billion platelets are produced per day, which circulate in the blood for about ten days. (18) Their production is controlled by a glycoprotein called thrombopoietin, which is produced by the liver and kidneys. Platelets structure enables them to regulate essential and diverse biological functions both in health and disease via a complex molecular system.

Platelets play an essential role in triggering atherosclerosis through several surface glycoproteins (GP) such as GPIbα, GPIIb/IIIa, and β3 integrin. These glycoproteins enable platelet "rolling" on the endothelium, platelet-vessel wall interaction in addition to leukocyte build up and release of numerous mediators. (19) Long-lasting activation of platelets and inflammatory cells lead to vascular smooth muscle cell migration into the intima and progression of atherosclerosis from fatty streaks to intermediate and advanced lesions resulting in coronary vessel wall thickening and lumen narrowing. Physical disruption of the atherosclerotic plaque due to erosions or rupture is responsible for almost all cases of ACS. (13)

Role of Platelets in Coronary Atherothrombosis

Acute Coronary Syndrome

Based on clinical features, electrocardiogram (ECG) findings and markers of myocardial necrosis, ACS can be classified into unstable angina (UA), non-ST-elevation myocardial infarction (NSTEMI), or ST-elevation myocardial infarction (STEMI). (20) Unstable angina can be defined as myocardial ischaemia at rest or minimal exertion in the absence of myocardial cell necrosis. There is no elevation in biomarkers and ECG changes if present are usually transient. In comparison with NSTEMI, patients with UA have a significantly lower risk of death, and aggressive antiplatelet or early invasive therapy by PCI is of lesser

benefit in this group of patients. (21) Non-ST-elevation myocardial infarction can be defined as myocardial ischaemia with evidence of myocardial cell necrosis. Biomarkers are elevated, and ECG changes may include transient ST-segment elevation, ST-segment depression, and T-wave inversion. ECG can sometimes be normal. ST-elevation myocardial infarction can be defined as myocardial ischaemia with persistent (>20 minutes) ST-elevation, which occurs as a result of complete occlusion of a coronary artery. (21)

The term myocardial infarction is used when there is evidence of myocardial cell necrosis in the context of myocardial ischaemia. (20) According to the third universal definition;

Type 1 myocardial infarction: is characterized by a spontaneous rupture, ulceration, fissure, erosion or dissection of an atherosclerotic plaque with resulting intraluminal thrombus in one or more coronary arteries leading to decreased myocardial blood flow and or distal embolisation and subsequent myocardial necrosis. (20) The patient may have underlying severe coronary artery disease, and sometimes non-obstructive or no coronary artery disease.

Type 2 myocardial infarction: occurs where a condition other than coronary artery disease contributes to an imbalance between myocardial oxygen supply and or demand, e.g., coronary endothelial dysfunction, coronary artery spasm, coronary embolism, tachyarrhythmias or bradyarrhythmias, anaemia, respiratory failure, hypotension, and hypertension with or without left ventricular hypertrophy.

Type 3 myocardial infarction: is associated with sudden death before blood samples could be obtained or before cardiac biomarkers could rise.

Type 4a myocardial infarction: is related to PCI procedure

Type 4b myocardial infarction: is related to stent thrombosis.

Type 5 myocardial infarction: is related to CABG operation.

ST- elevation myocardial infarction is an acute and life-threatening manifestation of CAD. Acute STEMI occurs following an abrupt rupture of an atherosclerotic plaque, which triggers various pathophysiological processes that lead to the formation of a thrombus and the subsequent occlusion of the culprit coronary artery. (22)

Platelets play a fundamental role in atherothrombosis. Plaque rupture results in adhesion, activation, and aggregation of platelets, and eventually, the formation of a thrombus that obstructs the coronary arteries and cause myocardial ischaemic damage. (Figure 6) The P2Y12 receptor, activated by ADP, has an essential role in platelet activation and is therefore considered an attractive therapeutic target.

Role of P2Y12 in Platelet Functions

During platelet activation, platelets express three subtypes of receptors on their surface, P2X1, P2Y1, and P2Y12. (1) P2X1 is activated by ATP and is involved in collagen-mediated platelet activation and platelet morphological changes. (1) Adenosine diphosphate activates P2X1 and P2Y12 receptors. P2Y1 receptor, when activated by ADP, results in platelet shape change and initial activation and aggregation of adjacent platelets. P2Y12 receptor amplifies and sustains this activation. (23-26)

The P2Y12 receptor belongs to a G1 family of a group of G protein-coupled purinergic receptors. It has a crucial role in various platelet functions, haemostasis, and thrombosis. It enhances secretion of dense granules, activation of fibrinogen receptor, and thrombus formation. It has an essential role in platelet aggregation via ADP-mediated production of thromboxane A2 (TXA2). Additionally, activation of the P2Y12 receptor causes α-granule release and expression of P-selectin on activated platelets. (27)

Since P2Y12 receptor mediates a wide range of platelet functions, such as amplification of platelet activation, recruitment of platelets to the site of plaque rupture and endothelial injury and the augmentation of platelet activation by other agonists such as thrombin and TXA2, is therefore considered an attractive target for antithrombotic therapy.

Platelet Adhesion

Under steady-state conditions, platelets respond to any breach in the integrity of the endothelium. To be able to do that, they circulate alongside the vessel wall, pushed aside by the red blood cells and by shear forces. The endothelial surface is usually non-adhesive to the circulating platelets. However, when damage occurs to the endothelium as in the case of a ruptured atheromatous plaque, platelets, through receptors on their surface membrane, become adhesive to the exposed subendothelial matrix, which contains thrombotic materials such as collagen fibers and Willebrand factor (VWF) (circulating VWF does not usually bind to platelets). Collagen is highly thrombogenic and is, together with VWF responsible for the initiation of platelets adhesion through interaction directly or indirectly with the platelets adhesive receptors. Platelets initially bind with collagen via the integrin α2β1 (GPIa/IIa). This allows further binding with collagen via GPVI receptor leading to the initiation of transmembrane and subsequently intracellular signaling.

Another essential step in platelet adhesions involves platelets binding to VWF via the platelet GPIb-IX-V receptor complex. (23) This binding occurs in high shear stress conditions. Binding of VWF and platelet GPIb represents the first stage in the process of primary haemostasis and is followed immediately by platelets activation.

Platelet Activation

Initial adhesion stimulates downstream signaling pathways that lead to an increase in the concentration of intracellular calcium and subsequent activation of platelets, which result in aggregation. (24) During this stage, the adherent platelets undergo significant morphological and cytoskeletal changes to an irregular dendritic sphere with multiple filipodia spreading on the subendothelium to increase their surface contact area. Then the adherent platelets, via an exocytosis process, release the content of their storage granules (α and dense granules), which are essential for platelets plug formation at the site of vascular injury.

Inflammatory mediators are released from α-granules (e.g., fibrinogen and plasminogen) and dense granules such [e.g., serotonin, adenosine diphosphate (ADP) and adenosine

triphosphate (ATP)]. (25) Fibrinogen and VWF released from α granules enhance platelet adhesion and activation. Serotonin causes vasoconstriction and ADP augments platelet activation. At this stage, platelets express three subtypes of receptors on their surface, P2X1, P2Y1, and P2Y12. (26) Other receptors expressed on platelets surface include GPIIb/IIIa and P-selectin. P-selectin, an α-granule transmembrane protein, becomes exposed and acts as an adhesive receptor recruiting leucocytes to the area of vascular injury. Additionally, it up-regulates tissue factor expression on monocytes.

GPIIb/IIIa is a member of the integrin family (αIIbB3) of adhesion receptors and is present in significant concentration on the platelet surface (80,000 per platelet). (28) These receptors are essential for the regulation of platelet adhesion and aggregation. (29) Platelet activation leads to the initiation of a signalling process called "inside-out" signalling. This process leads to conformational changes within GPIIb/IIIa, which increase the affinity of the GPIIb/IIIa receptor to bind with fibrinogen. The resultant GPIIb/IIIa-fibrinogen molecule facilitates the interaction of adjacent platelets. Following fibrinogen binding, a signalling process called "outside-in" signalling occurs and cause further conformational changes within GPIIb/IIIa, eventually resulting in acceleration of the platelet aggregation. (29)

Platelets Aggregation

Activation of platelets causes the release of ADP from their storage granules and another aggregating agent, TXA2 that in turn activates neighboring platelets. (30) Thromboxane A2 and ADP production leads to rapid aggregation of platelets and the formation of platelet-rich thrombus. Different platelet receptors are involved in this process, including the P2Y12 receptor. Adenosine diphosphate activates P2Y12 receptors causing the release of potent prothrombotic and proinflammatory factors involved in platelet aggregation. (25) Parallel to activation and aggregation of platelets, the exposed and highly prothrombotic lipid-rich core of a ruptured atheromatous plaque causes activation of the coagulation system and the formation of thrombin (factor IIa) from prothrombin (factor II) on the pro-coagulant surface of activated platelets. By activating proteinase-activated receptors (PAR) on platelets, thrombin stimulates platelet activation even further, a continuous feedback loop.

Additionally, thrombin induces the formation of fibrin for the mesh that is needed to stabilise the clot (secondary haemostasis). (29, 30) In addition to stimulating platelet aggregation, thrombin triggers the release of platelets storage granules such as ADP and TXA2, both of which enhance platelet activation.

Adenosine diphosphate, TXA2, and thrombin bind to specific platelets membrane receptors and stimulate aggregation on and around the platelets adherent to the exposed subendothelium via receptor-mediated signal transduction events. Binding of these agonists to these specific membrane receptors stimulate "inside-out" signalling pathways that eventually converts GPIIb/IIIa from a resting state to an activated state, which enables them to bind to extracellular ligands such as plasma fibrinogen (a glycoprotein synthesized in the liver) and VWF.

The transmission of an intracellular signals lead to a conformational change in GPIIb/IIIa receptors resulting in the exposure of ligand binding domains, thus promoting their binding with circulating soluble fibrinogen and VWF, both of which act as bridges between GPIIb/IIIa receptors on adjacent activated platelets (fibrinogen can bind to two platelets). The occupied GPIIb/IIIa then suffers further conformational changes and signals into the platelet, "outside-in" signalling, leading to a sequence of events that further drive platelet aggregation process and thus act as a bi-directional conduit for information flow across the plasma membrane. (31) This allows the complex process of platelet aggregation to continue at the site of ruptured atheromatous plaque.

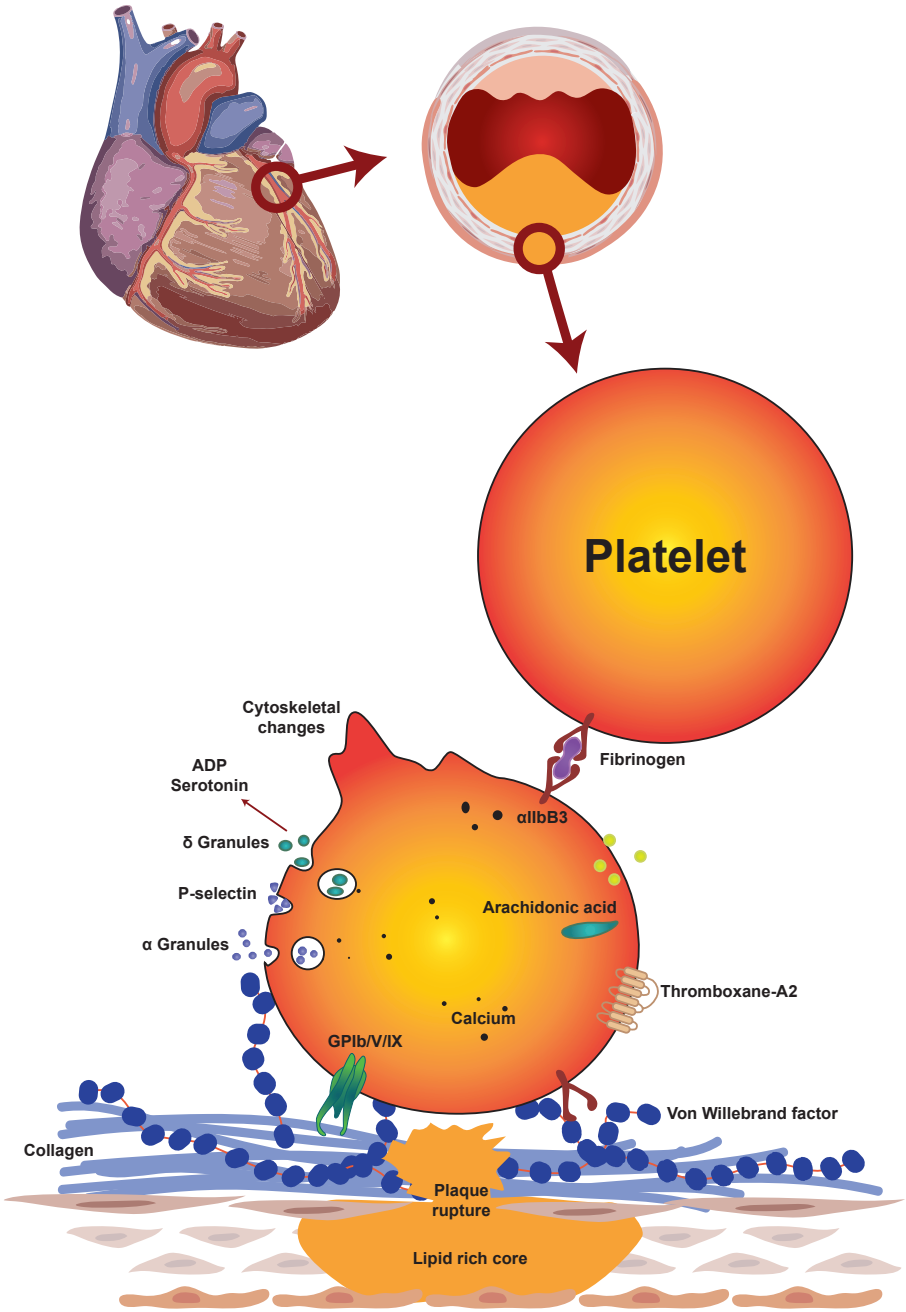


Figure 6 Pathways of platelets adhesion, activation and aggregation

Role of Platelets in Coronary Microvascular Dysfunction

Recent studies highlight the role of platelets in contributing to microvascular dysfunction in the context of acute STEMI through various mechanisms including ischaemia, reperfusion injury, and distal emobolisation. In patients with acute STEMI, platelet-derived microparticles have been linked to the pathogenesis of microvascular obstruction. (32)Also, it has been shown that platelets interact with neutrophils to form platelet-neutrophil aggregates. These aggregates obstruct the coronary microvascular lumen and cause the release of inflammatory mediators and vasoconstrictors that contribute to microvascular endothelial injury and microvascular dysfunction. (33) GPIIb/IIIa inhibition with abciximab has been shown to reduce infarct size and microvascular obstruction in patients with acute STEMI as determined by CMR scanning. (34) This further supports the role of platelets and platelet-leucocyte aggregates in coronary microvascular obstruction. Furthermore, during coronary microvascular obstruction, TXA_2 a potent vasoconstrictor and platelet agonist is generated which further aggravates microvascular obstruction. (35)

It is well established that platelets play a pivotal role in the pathophysiology of STEMI with or without microvascular obstruction. A better understanding of platelets role in this setting has led to the development of new therapies.

Antiplatelet Therapy in Acute ST- Elevation Myocardial Infarction

The success of contemporary treatment for STEMI is dependent, partly on the adequate suppression of platelet activity. Antiplatelet therapy has been shown to considerably reduce the risk of serious ischaemic events in patients with acute STEMI.

Several randomised controlled trials have demonstrated that oral platelet P2Y12 receptor antagonists (clopidogrel, prasugrel, and ticagrelor) considerably reduce the risk of adverse ischaemic events and death in patients with ACS and patients undergoing PCI. (36-38)In patients presenting with STEMI undergoing primary PCI, current guidelines recommend, in addition to aspirin, the administration of an oral P2Y12 receptor antagonist as early as possible before coronary angiography or at the time of primary PCI. (21, 22) Currently available oral P2Y12 platelet inhibitors, however, are limited by a relatively slow onset of action and so are not fully functional at the time of primary PCI. (1, 39) The bioavailability of orally administered P2Y12 inhibitors in patients with acute STEMI needing primary PCI may be limited by various other factors, such as vomiting and poor gastrointestinal absorption. (40-42) Hypotension and/or diversion of splanchnic blood flow are likely to slow drug absorption and the intrinsic increase in platelet activity seen during STEMI may also attenuate the effect of antiplatelet drugs. (41, 42) A previous pharmacokinetic study has shown reduced drug absorption during STEMI. In a randomized placebo-controlled fashion, oral disopyramide phosphate was administered within 12 hours of suspected acute myocardial infarction. Disopyramide levels 2 hours following loading dose were lower in acute myocardial infarction than in non-myocardial infarction patients (P=0.004), suggesting delayed absorption of disopyramide into the circulation. (43) Administration of morphine for pain relief appears to attenuate the antiplatelet effects of newer P2Y12 inhibitors in the hours after primary PCI. (44) Also, the best time to administer oral P2Y12 inhibitors is debatable. (45) An ideal antiplatelet would be one that is fully functional at the start of primary PCI. The ingestion of crushed oral prasugrel and ticagrelor tablets, compared with whole tablet ingestion in patients with STEMI has been demonstrated in

two studies (CRUSH and MOJITO) to lead to faster drug absorption and a potentiated antiplatelet effect being observed as early as 30 minutes following drug loading. (46, 47)

Cangrelor is a direct reversible P2Y12 antagonist. It has a rapid onset and offset of action, and consistent and reliable effects. Thus, it overcomes many of the limitations associated with oral P2Y12 inhibitors and makes its use in the setting of acute STEMI undergoing primary PCI where prompt antiplatelet inhibition is required, appealing. (48) This concept is supported by the FABOLUS PRO study which, found that in patients presenting with STEMI undergoing primary PCI who received prasugrel at a loading dose of 60mgs, the addition of a bolus only dose of GPIIb/IIIa antagonist tirofiban without a maintenance infusion led to almost complete platelet inhibition. (49)

Antiplatelet therapy can be classified according to the route of administration into either oral or IV drugs or according to their mechanism of action into cyclooxygenase enzymes inhibitors, ADP receptor inhibitors, phosphodiesterase inhibitors, Protease-activated receptor-1 (PAR-1) antagonists, GPIIb/IIIa inhibitors, adenosine reuptake inhibitors, and thromboxane inhibitors. The antiplatelet drugs commonly used in clinical practice relevant to our study are discussed below. (Figure 7)

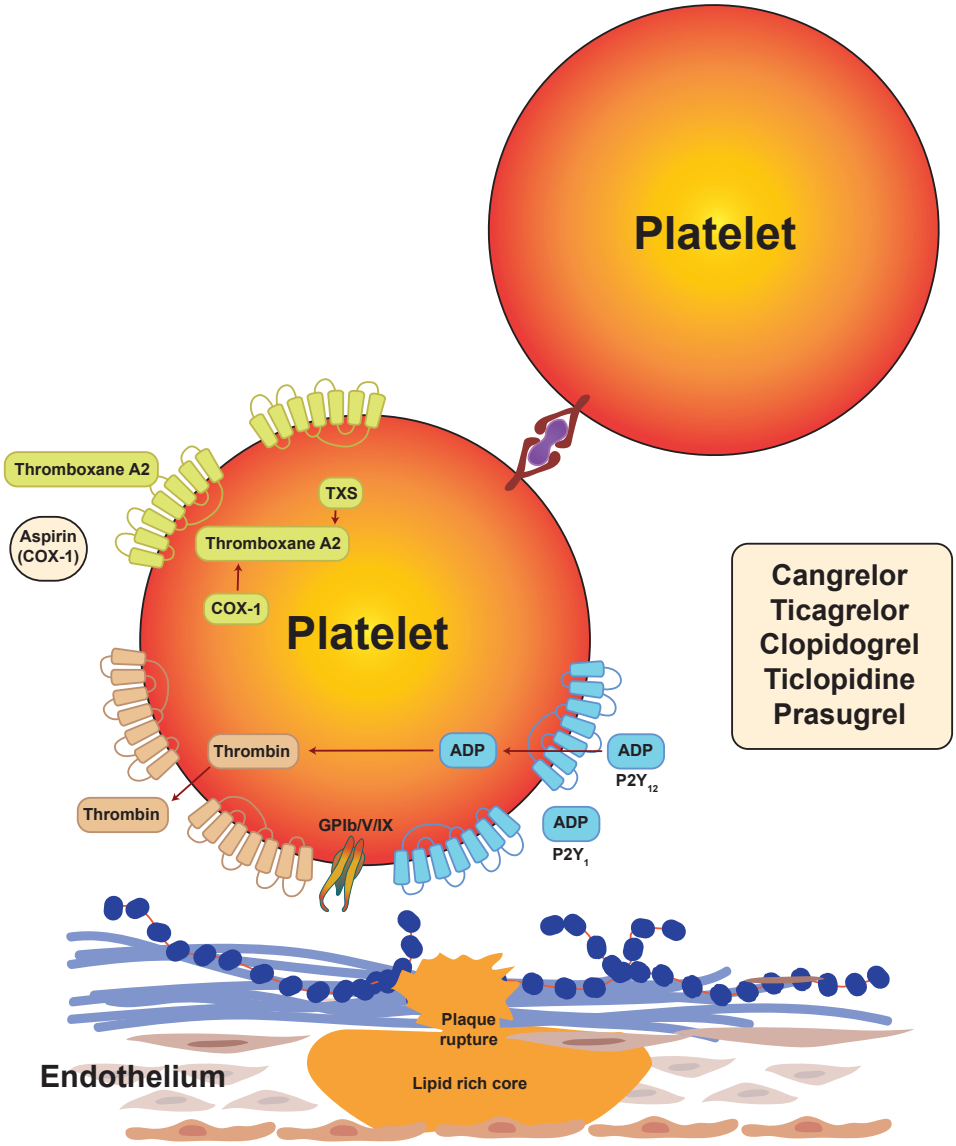


Figure 7 Mechanism of action and binding properties of P2Y12 inhibitors commonly used in clinical practice

Oral Antiplatelet Therapy

Cyclooxygenase Enzyme Inhibitors

Aspirin

Aspirin (Acetylsalicylic acid) (ASA), synthesized in the late 19th century, is the oldest antiplatelet drug. Its antiplatelet effect was first established in the mid-1960s, and since then it has become the keystone of antiplatelet therapy in the secondary prevention of ischaemic heart disease. Dr. Harvey Weiss discovered the antiplatelet benefits of aspirin in 1967. In 10 healthy men, aged 25-40 years, ingestion of 3g of aspirin per day for 54 hours resulted in significantly less platelet aggregation compared to placebo, due to the impaired release of platelet ADP. (50) The history of aspirin is summarised in table 1.

Aspirin irreversibly inhibits cyclooxygenase enzymes (COX-1 and COX-2). Low-dose aspirin preferentially inhibits COX-1, which prevents the formation of TXA2 from arachidonic acid. TXA2 is a potent vasoconstrictor and potent agonist for platelet aggregation. (51) Additionally, ASA inhibits thrombin generation and augments fibrinolysis independent of COX enzymes pathway. (50) Aspirin is quickly absorbed in the upper gastrointestinal tract and results in a measurable inhibition of platelet function within 15 minutes. (52, 53)

The benefit of aspirin as adjunctive therapy to thrombolysis was established in the ISIS-2 trial. The survival benefit of aspirin in this study was shown to be comparable to streptokinase thrombolysis alone. Aspirin has been demonstrated to reduce 30-day mortality in patients with ACS by approximately 25%, and the risks of re-infarction and stroke by about 50%. (54)

The CURRENT–OASIS 7 randomised trial failed to establish a difference in hard clinical outcomes within 30 days when a low dose Aspirin (75–100 mg/day) was compared with a higher doses Aspirin (300–325 mg/day). (55)

Aspirin (≥300 mg) was given to all patients on day 1. Patients assigned to the high dose group received aspirin 300 to 325 mg daily on days 2 through 30. Patients assigned to the lower-dose group received aspirin 75 to 100 mg daily on days 2 through 30. There was a significant increase in the rate of minor bleeding in patients who received the higher dose of aspirin (HR, 1.13; 95% CI, 1.00 to 1.27; P=0.04). There were fewer gastrointestinal bleeds with lower doses compared to a higher dose (29 patients [0.2%] vs. 47 patients [0.4%], P=0.04). Six patients in each aspirin dose group developed intracranial bleeding.

In the treatment of patients with acute STEMI, the European Society of Cardiology (ESC) guidelines recommends administration of oral aspirin (including chewing) at a loading dose of 150-300mgs followed by a daily maintenance dose of 75–100mg (class I recommendation) and then continued indefinitely regardless of the treatment strategy. (1)

In certain centres, the IV preparation is available and can be administered in patients who are unable to swallow oral aspirin. Aspirin inhibits platelet aggregation via one pathway (COX1). However, platelet activation is mediated by other pathways; therefore, aspirin is not effective in inhibiting platelet activation mediated by separate pathways involved in the formation of arterial thrombus. This increases the risk of thrombotic events and underscores the need for additional therapy.

Table 1 Aspirin timeline, D. Connelly, the pharmaceutical journal, 26 Sep 2014

Year	Event
c3000 – 1500 BC	Sumerians and Egyptians used Willow as a medicine. The Ebers papyrus, an ancient Egyptian medical text, refers to willow as an anti-inflammatory or pain reliever for non-specific aches and pains.
c400 BC	In Greece, Hippocrates administers willow leaf tea, which contains the natural compound from which aspirin is derived, to women to ease the pain of childbirth.
1763	The Royal Society publishes a report detailing five years of experiments on the use of dried, powdered willow bark in curing fevers, submitted by Edward Stone, a vicar in Chipping Norton, Oxfordshire.
1828	Joseph Buchner, professor of pharmacy at Munich University, Germany, succeeds in extracting the active ingredient from willow, producing bitter tasting yellow crystals that he names Salicin.
1830	Salicin is also found in the meadowsweet flower by Swiss pharmacist Johann Pagenstecher and later by German researcher Karl Jacob Löwig.
1853	French chemist Charles Frédéric Gerhardt determines the chemical structure of salicyclic acid and chemically synthesises acetylsalicylic acid.
1876	The first rigorous clinical trial of salicin finds that it induces remission of fever and joint inflammation in patients with rheumatism (Lancet 1876;1:383).
1897	While working for pharmaceutical company Bayer, German chemist Felix Hoffmann, possibly under the direction of colleague Arthur Eichengrün, finds that adding an acetyl group to salicylic acid reduces its irritant properties and Bayer patents the process.
1899	Acetylsalicyclic acid is named Aspirin by Bayer. The letter 'A' stands for acetyl, "spir" is derived from the plant known as Spiraea ulmaria (meadowsweet), which yields salicin, and "in" was a common suffix used for drugs at the time of the first stable synthesis of acetylsalicylic acid.
1967	Dr. Harvey Weiss discovered the antiplatelet effect of aspirin.
1971	John Vane, professor of pharmacology at the University of London, publishes research describing aspirin's mechanism of action (dose-dependent inhibition of prostaglandin synthesis) (Nature New Biology 1971;231:232). He later wins a Nobel prize (1982) for this work, along with Bengt Samuelsson and
1974	Data from the first randomised controlled trial of aspirin in the secondary prevention of death from myocardial infarction showed a reduction in total mortality of 12% at six months and 25% at twelve months, but the results were statistically inconclusive (BMJ 1974;1:436).

Thienopyridines

The use of newer antiplatelet drugs such as P2Y12 antagonist as adjunctive therapy in addition to aspirin has led to a reduced risk of adverse cardiovascular events in patients with ACS undergoing PCI, albeit at an increase in the rate of bleeding complications. (56) These agents have a synergistic effect on platelet function with the addition of clopidogrel to aspirin leading to approximately 20% relative risk reduction in adverse cardiovascular events. (57)

Thienopyridines are a class of antiplatelet drugs that irreversibly block P2Y12 receptors expressed on the platelet surface, causing an irreversible inhibition of platelet function for the lifetime of the platelet, which is around 7-10 days. They include ticlopidine (first generation), clopidogrel (second generation) and prasugrel (third generation). All thienopyridines are pro-drugs and require biotransformation via the hepatic cytochrome P450 (CYP) enzymes into metabolically active drugs to allow binding to the P2Y12 receptor. (58) The pharmacokinetic and pharmacodynamic properties of each drug are different due to the difference in the activations steps and the hepatic enzymes involved in the activation process.

Ticlopidine

Ticlopidine is a first-generation thienopyridine. It acts by irreversibly inhibiting P2Y12 receptors, thus preventing ADP from activating platelets. It was the first P2Y12 receptor inhibitor, which was shown to improve outcomes in patients undergoing PCI. (59) Ticlopidine significantly reduced the risk of stent thrombosis in patients receiving bare-metal stents at 30 days when administered in addition to aspirin. However, its use was limited by severe haematologic side effects such as agranulocytosis and aplastic anaemia. The adverse effect profile of ticlopidine (severe bone marrow toxicity and skin rashes) led to the introduction of the safer second-generation thienopyridine, clopidogrel.

Clopidogrel

Clopidogrel is a prodrug and requires two-step oxidation by the hepatic cytochrome P450 (CYP) isoenzymes to transform it into an active metabolite. Once clopidogrel is absorbed from the intestine, approximately 85% of it is transformed into an inactive metabolite. The residual 15% is rapidly metabolized by hepatic cytochrome P450 (CYP) isoenzymes in a two-step oxidation process leading to the formation of an active metabolite which binds irreversibly and competitively to the P2Y12 receptor, thus exerting its antiplatelet effect. (60) The active metabolite of clopidogrel is not stable, has a very short half-life (≈ 30 minutes), and is rapidly eliminated from the circulation if binding to P2Y12 receptors does not occur. The half-life of the parent drug is approximately 6 hours. The onset of action of clopidogrel is 2-8 hours, and the recovery time after discontinuation is around 5-7 days. This corresponds with the life span of the platelets due to the irreversible nature of binding. (58, 61). The CURE (Clopidogrel in Unstable Angina to Prevent Recurrent Ischemic Events) trial investigated the efficacy and safety of clopidogrel when given in combination with aspirin to patients with ACS without ST-elevation. (57) In this study, 12,562 patients were randomly assigned to clopidogrel 300mg immediately, followed by 75mg once daily (n=6259) or placebo (n=6303) in addition to aspirin for 3 to 12 months. The composite of death from cardiovascular death and nonfatal MI occurred in 9.3% of patients in the clopidogrel arm vs. 11.4% in the placebo group (RR 0.80; 95% CI 0.72-0.90; P<0.001; NNT=4). The composite of death from a cardiovascular cause, nonfatal myocardial infarction or refractory ischaemia occurred in 16.5% of the patients in the clopidogrel group vs. 18.8% in the placebo group (relative risk, 0.86, P<0.001). The incidence of major bleeding was significantly more in the clopidogrel group (3.7% vs. 2.7%; relative risk, 1.38; P=0.001). It was concluded that the addition of clopidogrel to aspirin was beneficial but at the expense of increased bleeding risk.

In the CURE trial, 2658 patients had PCI (CURE- PCI). The addition of clopidogrel to aspirin in this group of patients significantly reduced the composite of death from cardiovascular cause, nonfatal myocardial infarction and target vessel revascularization within 30 days (4.5% in the clopidogrel group vs. 6.4% in the placebo group; an absolute

risk reduction of 1.9% and a relative risk reduction of 30%), without a significant difference in major bleeding between the two groups. (56) The effectiveness of early and long-term treatment with clopidogrel had been established in patients with ischaemic heart disease undergoing PCI. The CREDO (Clopidogrel for the Reduction of Events During Observation) trial showed the benefit of clopidogrel pre-treatment and long-term dual antiplatelet therapy in stable patients undergoing elective PCI. (37, 62)

In patients with STEMI, the beneficial effect of clopidogrel given in addition to aspirin was demonstrated in the CLARITY–TIMI 28 (Clopidogrel as Adjunctive Reperfusion Therapy-Thrombolysis In Myocardial Infarction) and COMMIT-CCS (Clopidogrel and Metoprolol in Myocardial Infarction Trial/Second Chinese Cardiac Study) trials. (63, 64)

The CLARITY–TIMI 28 was conducted to find out whether the addition of clopidogrel to aspirin in patients who have STEMI receiving fibrinolytic therapy is beneficial.

The CLARITY–TIMI 28 was an international, multi-center, double-blinded, randomised, placebo-controlled trial that enrolled 3491 patients presenting with STEMI within 12 hours of symptom onset. Eligible patients were treated with standard fibrinolytic therapy, including aspirin and heparin. Patients were randomized to either clopidogrel with a loading dose of 300mg followed by 75mg/day for 30 days (n=1752) or to placebo (n=1739). Coronary angiography was performed 2-8 days after randomisation. The study drug was stopped after angiography, and for those patients who underwent PCI, a loading dose of clopidogrel was recommended. Clinical follow-up was for 30 days duration. Primary endpoints were composite of occluded infarct-related artery (TIMI flow 0/1) on angiography, death, or myocardial infarction by the time of angiography. Clopidogrel caused a significant reduction in the rate of ischaemic events without a significant increase in major bleeding. The primary endpoint of the study was significantly lower in patients randomized to clopidogrel compared with placebo (15% vs. 21.7%, respectively) (95% CI, P= .00000036). (63) This benefit was seen across all pre-specified groups. The trial investigators concluded that the addition of clopidogrel to aspirin and standard fibrinolytic

therapy in patients with acute STEMI improves the patency rate of the infarct-related artery and reduces ischaemic complications.

In the COMMIT trial, 45,852 patients within 24 hours of acute myocardial infarction, mainly STEMI were randomised to receive either 75mg clopidogrel once daily or placebo for up to 4 weeks, in addition to aspirin. (64)Clopidogrel treatment significantly decreased thrombotic events (9.2% vs. 10.1%; OR 0.91, 95% CI 0.86–0.97, P=0.002), and all-cause mortality (7.5 vs. 8.1; OR 0.93 95% CI 0.87-o.99; p=0.03).

ESC guidelines suggest administration of clopidogrel at a loading dose of 300 or 600mg followed by a maintenance dose of 75mg daily (or 150mg until day 8) in patients with STEMI and NSTEMI when it is not possible to administer the newer antiplatelet medications (prasugrel, ticagrelor). (21)

Despite the established benefits of dual antiplatelet therapy with aspirin and clopidogrel, a significant number of patients still encounter thrombotic events. (60) This may be partly explained by the considerable variability in the patient's response to clopidogrel. (65) It has been demonstrated that up to 40% of patients treated with clopidogrel show high on-treatment platelet reactivity, which could be responsible for late thrombotic events, including stent thrombosis. (65) This broad inter-individual efficacy of clopidogrel can be attributed to several other factors such as the pharmacokinetic and the pharmacodynamic properties of the drug, clinical (obesity, diabetes, ACS), genetic (i.e., CYP polymorphisms) and cellular mechanisms. (66) Additionally, clopidogrel has a delayed onset of action (2-6 hours). (67) The maximum antiplatelet effect of clopidogrel is achieved approximately two hours after administration of a loading dose of 600mg. (68) Limitations encountered with the use of clopidogrel emphasize the need for the use of more potent antithrombotic treatment, especially in high-risk patients. Currently, due to the availability of a new generation of thienopyridines, clopidogrel is less frequently used.

Prasugrel

Prasugrel is a prodrug and requires bio-activation by the hepatic CYP isoenzymes to produce an active drug that blocks P2Y12 receptor irreversibly. Unlike clopidogrel, this process involves single-step oxidation to produce its active metabolite, resulting in a more rapid onset of action and a more potent platelet inhibitory effect with less inter-individual variability. (60) The plasma level of the active metabolite of prasugrel drops rapidly due to distribution into the extravascular compartments. The distribution half-life of prasugrel is 30-60 minutes. Compared to clopidogrel, the active metabolites of prasugrel stay longer in the circulation due to the longer elimination half-life, which is approximately 2-15 hours. The half-life of the parent drug is < 5 minutes. The onset of action of prasugrel is 30 minutes-4 hours, and the recovery time after discontinuation is approximately 7-10 days. This corresponds with the life span of the platelets due to the irreversible nature of binding. (58) Despite the established clinical benefits of dual antiplatelet therapy with aspirin and clopidogrel in patients who have ACS and undergo PCI, recurrent thrombotic events continue to occur.

The TRITON-TIMI 38 trial compared the efficacy of prasugrel against clopidogrel in 13608 patients with moderate-high risk ACS with scheduled PCI. (69) Patients were randomised to receive either prasugrel, 60mg loading dose, followed by 10mg maintenance dose or clopidogrel 300mg loading dose, followed by a 75mg maintenance dose. This study demonstrated that prasugrel was superior to clopidogrel in patients with unstable angina, NSTEMI, and STEMI, but at the expense of significantly higher risk of major bleeding, including fatal bleeding. Overall mortality was not significantly different between treatment groups. Death from cardiovascular causes, non-fatal myocardial infarction and non-fatal stroke (primary endpoints) occurred in 9.9% of prasugrel group vs. 12.1% of the clopidogrel group (HR 0.81 [95% CI: 0.73–0.90]; $p<0.001$). Additionally, prasugrel therapy was associated with significantly reduced rates of myocardial infarction, target-vessel revascularisation, and stent thrombosis ($p<0.001$). (69) However, the rate of major, life-threatening, non-fatal and fatal bleeding was higher in the prasugrel group vs. clopidogrel (major bleeding 2.4% vs. 1.8%; $p=0.03$, life-threatening bleeding 1.4% vs.

0.9%; p=0.01, non-fatal bleeding 1.1% vs. 0.9%; p=0.23 and fatal bleeding 0.4% and 0.1%; p=0.002) respectively. Patients aged ≥75 years, weighing <60kg and patients who had a prior stroke or transient ischaemic attack were mainly at high risk of bleeding. No significant difference was observed in major bleeding when these high-risk patients were excluded (HR 1.24 [0.91–1.69]; p=0.17). (69)

In a subgroup analysis of STEMI patients undergoing primary PCI (n=3534), prasugrel compared to clopidogrel, showed a significant reduction in the primary endpoint (6.5% versus 9.5%; HR 0.68, 95% CI 0.54–0.87; P=0.002) and the secondary endpoints of myocardial infarction, urgent target-vessel revascularization, stent thrombosis, and death from cardiovascular cause at 30 days. (70) There was no difference in non-coronary artery bypass operation (CABG) related major bleeding in the prasugrel and clopidogrel arms. However, in this sub-group of patients, the benefit of prasugrel over clopidogrel was demonstrated in patients undergoing secondary PCI (12 hours-14 days after symptom onset). The risk reduction achieved by prasugrel in patients presenting with STEMI undergoing primary PCI was similar to that demonstrated in the whole trial cohort (HR 0.80, 95% CI 0.60–1.08, P= 0.144). Additionally, the rate of stent thrombosis was significantly reduced. (70)

Specific concerns have been highlighted about this trial. In the TRITON-TIMI trial, prasugrel was administered after performing coronary angiography, knowing the coronary anatomy and immediately before PCI and not at the time of clinical diagnosis, which is not in line with clinical practice. Also, clopidogrel was administered at a loading of 300mg rather than 600mg. Most of the benefit seen with prasugrel appeared to occur early on, but the bleeding accumulated over time.

These limitations, therefore, mean that the TRITON study did not address whether treatment with prasugrel prior to coronary angiography is of benefit compared to pre-treatment with a contemporary 600mg dose of clopidogrel. The effect of pre-treatment with prasugrel at the time of clinical diagnosis was examined in the ACCOAST (Comparison of

Prasugrel at the Time of Percutaneous Coronary Intervention or as Pre-treatment at the Time of Diagnosis in Patients with Non-ST Elevation Myocardial Infarction) trial. (71)

It demonstrated no significant difference in the composite primary endpoint of cardiovascular death, MI, stroke, urgent revascularisation, and bailout use of GPIIb/IIIa inhibitors therapy at seven days. Additionally, there was an increase in the rate of major bleeding complications. Current guidelines, therefore, restrict the use of prasugrel to patients presenting with acute STEMI undergoing primary PCI and patients with ACS after the coronary anatomy is identified. (1, 21)

ISAR REACT 5 was an investigator-initiated, open-label, multicenter, randomized clinical trial that compared the efficacy and safety of ticagrelor and prasugrel in patients with acute coronary syndromes. (72) This trial tested the hypothesis that ticagrelor is superior to prasugrel in reducing the primary composite endpoint of death, myocardial infarction, or stroke within 12 months in acute coronary syndrome patients intended for an invasive strategy. A total of 4,018 patients were enrolled from 23 centres in Germany and Italy and randomly allocated to prasugrel or ticagrelor.

Patients assigned to ticagrelor received a loading dose as soon as possible after randomisation. In the prasugrel group, the timing of study drug initiation depended on clinical presentation. Patients who presented with STEMI received prasugrel as soon as possible after randomisation. Patients who presented with NSTEMI received prasugrel loading dose following identification of the coronary anatomy.

The primary composite endpoint of death, myocardial infarction, or stroke at 12 months occurred in 9.3% of patients in the ticagrelor group and 6.9% in the prasugrel group (hazard ratio [HR] 1.36; 95% confidence interval [CI] 1.09–1.70; p = 0.006).

The incidence of the individual components of the primary endpoint in the ticagrelor and prasugrel groups was 4.5% versus 3.7% for death, 4.8% versus 3.0% for myocardial infarction, and 1.1% versus 1.0% for stroke. Definite or probable stent thrombosis occurred

in 1.3% of patients assigned to ticagrelor and 1.0% assigned to prasugrel and definite stent thrombosis in 1.1% versus 0.6% of patients, respectively.

The increase in anti-ischaemic efficacy with prasugrel was not accompanied by a raised bleeding risk. Bleeding (BARC class 3 to 5) was observed in 5.4% of patients in the ticagrelor group and 4.8% of patients in the prasugrel group (HR 1.1; 95% CI 0.8–1.5; p = 0.46). (72)

Nucleotide/Nucleoside Analogues

These are a group of drugs that are active, do not require biotransformation and therefore produce a faster platelet inhibitory effect.

Ticagrelor

Ticagrelor is direct acting, reversible P2Y12 receptor inhibitor that belongs to the cyclopentyl-triazolopyrimidine class. Ticagrelor differs from the thienopyridines class of antiplatelet agents, as it does not exist as a prodrug. Ticagrelor has a half-life of 7-12 hours and around 36% bioavailability. The short half of ticagrelor together with the reversible binding to P2Y12 receptors requires twice-daily dosing. (73) Ticagrelor is biotransformed by CYP3A4/A5 to the main active (AR-C124910XX) and inactive (AR-C133913XX) metabolites. AR-C124910XX is equally pharmacologically active as ticagrelor and contributes to around 30% of the antiplatelet effect. (73) Ticagrelor is not affected by variations in CYP450 and binds reversibly to P2Y12 receptors leading to a faster onset and offset than clopidogrel with the antiplatelet effect of ticagrelor being observed within 2 hours of administration. (74) More significant platelet inhibition and a faster onset and offset of action are achieved with a 180mg loading dose of ticagrelor compared to 600mg loading of clopidogrel. (74)

Reticulated platelets (new circulating platelets) are more reactive than older platelets and act as nuclei for the development of platelet aggregates even in the presence of antiplatelet

therapy. (75) This subgroup of platelets remains un-inhibited even in the presence of aspirin and thienopyridines. The short half-lives of these antiplatelet agents could explain this phenomenon, which is absent with ticagrelor because of its longer half-life and the reversible inhibition of platelets P2Y12 receptors. This gives ticagrelor the opportunity in between doses to target and inhibit new circulating platelets (reticulated platelets). (75)

In addition to P2Y12 inhibition, ticagrelor also inhibits adenosine uptake by blood cells, thus increasing extracellular adenosine levels. (76) Adenosine offers additional antiplatelet effect to that of ticagrelor by increasing cyclic AMP levels acting via A2A receptors. (77) Furthermore, the increase in local adenosine levels enhances coronary microvascular blood flow. (78-80) Adenosine has also been found to provide myocardial protection by ischaemic preconditioning and also to protect against myocardial reperfusion injury when coronary blood flow is re-established after a period of myocardial ischaemia. (81, 82) Ticagrelor is also shown to be effective in clopidogrel non-responders, thus overcoming some of the limitations encountered with clopidogrel. (83) The IMPRESSION trial has demonstrated that morphine, which is commonly administered to ease chest pain in patients with STEMI, delays ticagrelor exposure, and action in patients with acute myocardial infarction. (84) IMPRESSION was a randomized, placebo-controlled trial that assessed 70 patients with STEMI and NSTEMI. Patients (35 in each arm) received aspirin 300mg and were then randomized to receive morphine 5mg IV or placebo followed by a 180mg loading dose of ticagrelor. Compared to the placebo group, patients who received morphine had a 36% and 37% reduction in total drug exposure for active ticagrelor, and it's metabolite, respectively. Peak plasma ticagrelor concentrations were considerably delayed in the morphine group compared to placebo (4 hours vs. 2 hours, P=0.004). (84)

Ticagrelor is associated with adverse effects such as increased rates of bradyarrhythmias, shortness of breath, and decreased clearance of creatinine and uric acid.

Current guidelines recommend the addition of aspirin or clopidogrel to aspirin in patients who have ACS. Clopidogrel has many limitations, and prasugrel is associated with an increased risk of major bleeding.

The PLATO (Platelet Inhibition and Patient Outcomes) trial was conducted to evaluate whether ticagrelor is more efficient than clopidogrel for the prevention of vascular events and death in patients presenting with ACS. (39) In this trial, 18,624 patients admitted with STEMI and high-risk NSTEMI were randomised within the first 24 hours of symptoms regardless of the planned treatment strategy (invasive or non-invasive) to receive aspirin with either ticagrelor 180mg loading followed by 90mg twice-daily maintenance dose (9333 patients) or clopidogrel 300-600mg followed by 75mg per day maintenance dose (9291 patients). Both drugs were administered following randomisation and before any potential PCI procedure. At 12 months follow up, the primary composite endpoint of death from cardiovascular causes, myocardial infarction and stroke occurred significantly less often in the ticagrelor group than in the clopidogrel group (9.8% vs. 11.7% respectively; HR, 0.84; 95% confidence interval [CI], 0.77 to 0.92; P<0.001) (16% relative risk reduction and 1.9% absolute risk reduction). The positive treatment effect of ticagrelor was evident within the first 30 days of therapy and persisted throughout the study duration.

Similarly, a reduction in the secondary endpoints of all-cause death, death from vascular causes, and myocardial infarction was observed in patients receiving ticagrelor (10.2% vs. 12.3%, P<0.001). The composite endpoint of death from vascular causes, myocardial infarction, stroke, recurrent ischaemia, transient ischemic attack, or other arterial thrombotic events (14.6% vs. 16.7%, P<0.001); myocardial infarction alone (5.8% vs. 6.9%, P=0.005); and death due to vascular causes (4.0% vs. 5.1%, P=0.001).

Additionally, a 50% reduction in mortality was observed in patients receiving ticagrelor who underwent CABG operation. There was no increase in major nonfatal bleeding as defined by PLATO criteria in patients receiving ticagrelor (11.6 vs. 11.2%; p= 0.43) but there was however an increase in the rate of major bleeding not related to cardiac surgery using PLATO criteria, (4.5% vs. 3.8%; P = 0.03) and the rate of fatal intracranial bleeding. (39) Based on the results of the PLATO trial, current guidelines recommend ticagrelor as a first-line treatment option for patients with moderate to high-risk ACS. (1, 21)

In PLATO STEMI subgroup, 8430 patients with STEMI planned to have primary PCI were randomized to receive either ticagrelor at a loading dose of 180mg during PCI, followed by a maintenance dose of 90mg twice daily thereafter for 6-12 months (n=4201) or clopidogrel at a loading dose of 300-600mg during PCI, followed by 75mg daily thereafter for 6-12 months (n=4229). Aspirin was given to all patients. Out of 8430, PCI was performed in 75% of patients, mainly using bare metal stents. After 12 months, ticagrelor therapy was associated with a significant reduction in the rate of the primary composite endpoint of death from vascular causes, myocardial infarction and stroke (9.4% in ticagrelor group vs. 10.8% in clopidogrel group; HR 0.87 [0.75–1.01]; p=0.07).

Furthermore, compared with clopidogrel, ticagrelor reduced the rate of myocardial infarction alone (HR 0.80 [0.65 to 0.98]; p=0.03), death from cardiovascular causes (HR, 0.83 [0.67 to 1.02] p=0.07), overall mortality (HR 0.82; p=0.05) and definite ST (HR 0.66; p=0.03) without an increase in the rate of major bleeding (HR 0.98; p=0.76) regardless of which bleeding definition was used. (85, 86)

Lack of increased rate of bleeding events seen with ticagrelor (consistent with the STEMI subgroup of the TRITON-TIMI 38 trial) could be explained by the younger age and low bleeding risk of this cohort of patients. Patients in PLATO primary PCI subgroup were younger; had less comorbidity and were less likely to have a previous medical history than the overall STEMI subgroup and the larger study patient cohort. They also were more likely to be assigned a treatment earlier after symptom onset and receive more intensive antithrombotic therapy. (86)

PLATO investigators faced much criticism by few investigators after looking at the Food and Drug Administration (FDA) documents that revealed concerns about the reliability of the trial published results. James DiNicolantonio and Ales Tomek examined the information from FDA complete response review to assess the reliability of published PLATO trial and highlighted several issues. (87) During the trial, there were 864 primary endpoint events in the ticagrelor arm and 1,014 in the clopidogrel arm, a difference of 150. Of the events in favor of ticagrelor, 46% came from two countries (Poland and Hungary),

when only 21.1% of the enrolled patients came from those areas. This finding questions the ability to generalize the result of PLATO trial to other geographical regions. AstraZeneca monitored some of the trial recruiting centres, and some other centres were monitored by an independent clinical research organization (Worldwide Clinical Trials). The FDA documents indicated that there was a substantial gain for ticagrelor over clopidogrel for the primary endpoint at centres monitored by the drug maker (odds ratio 0.74, 95% CI 0.64-0.85), but not among the centres monitored by the independent organization (OR 1.21, 95% CI 0.91-1.59), which included the U.S., Russia, and Georgia.

Additionally, among patients taking ticagrelor, those treated at centres monitored by AstraZeneca were less likely to have a primary endpoint event than those treated at centres monitored by the independent organization (OR 0.67, 95% CI 0.54-0.83). Also, the FDA indicated that cardiovascular events or deaths on ticagrelor were either not submitted for adjudication, inactivated, deleted or were downgraded to "softer" endpoints. Subsequently, 4 out of 10 FDA reviewers recommended ticagrelor non-approval. Based on these multiple serious deficiencies, the FDA stated in their report that individual clinicians might, therefore, wish to reconsider their practice of ticagrelor prescription for this indication carefully. Guideline bodies should also evaluate the information in its totality.

The ATLANTIC (Administration of Ticagrelor in the Cath Lab or in the Ambulance for New ST-Elevation Myocardial Infarction to Open the Coronary Artery) was a study conducted to assess whether early, in-ambulance administration of ticagrelor could safely improve coronary reperfusion in patients with STEMI transferred for primary PCI. (88)

It was a phase 4, international, randomized, double-blind study. Patients were randomly assigned to receive either pre-hospital (in the ambulance) or in-hospital (in the catheterization laboratory) treatment with ticagrelor, in addition to aspirin and standard care. In the pre-hospital group, patients received a 180-mg loading dose of ticagrelor before transfer and then a matching placebo in the catheterization laboratory. Patients in the in-hospital group received a placebo before transfer and then a 180-mg loading dose of ticagrelor in the catheterization laboratory. All patients subsequently received ticagrelor at a

dose of 90 mg twice daily for 30 days, with a recommendation that treatment is continued for a total of 12 months.

The co-primary endpoints were the number of patients who did not have ≥70% ST-segment elevation resolution on ECG before PCI and the number of patients who did not meet the criteria for TFG 3 in the infarct-related artery at angiography before PCI. Secondary endpoints involved the composite of death, myocardial infarction, stent thrombosis, stroke, or urgent revascularization at 30 days; definite stent thrombosis at 30 days; thrombotic bailout with GP IIb/IIIa inhibitors; TFG 3 at the end of the PCI procedure; and complete (≥70%) ST-segment elevation resolution at 60 minutes after PCI.

There was no significant difference between the pre-hospital group and the in-hospital group in terms of the number of patients who did not have a ≥70% ST-segment elevation resolution before PCI (odds ratio with pre-hospital vs. in-hospital administration of the loading dose of ticagrelor, 0.93; 95% confidence interval [CI], 0.69 to 1.25; P=0.63) and the number of patients who did not have a TFG 3 in the infarct-related artery at initial angiography (odds ratio, 0.97; 95% CI, 0.75 to 1.25; P=0.82). Also, there were no significant differences between the two treatment groups concerning the composite endpoint of death, myocardial infarction, stroke, urgent coronary revascularization, or stent thrombosis. However, definite stent thrombosis was reduced in the pre-hospital group both at 24 hours (0 of 906 patients [0%] in the pre-hospital group vs. 8 of 952 [0.8%] in the in-hospital group, P=0.008 by Fisher's exact test) and at 30 days (2 of 906 [0.2%] vs. 11 of 952 [1.2%], P=0.02). (88)

The ATLANTIC investigators concluded that pre-hospital administration of ticagrelor a short time before PCI in patients presenting with ongoing STEMI seemed to be safe but did not improve pre-PCI coronary reperfusion. (88)

Intravenous Antiplatelet Therapy

Adenosine Triphosphate Analogues

Cangrelor

Cangrelor is an ATP analogue that directly, competitively and reversibly binds to and inhibits the P2Y12 receptor (sparing P2Y1 or P2X1 receptors) with a rapid and a predictable onset and offset of action. Cangrelor does not require hepatic biotransformation and has a very short half-life of 3 to 6 minutes. (48) Administration of an IV bolus followed by a continuous infusion rapidly achieves extensive platelet inhibition within 2 minutes. This effect is maintained throughout the infusion period with a near-full recovery of platelet function within sixty to ninety minutes of terminating the infusion. (89) (Figure 8)

Cangrelor being a direct reversible P2Y12 inhibitor with rapid onset and offset of action and consistent and reliable effects overcomes many of the limitations associated with oral P2Y12 inhibitors and make its use in the setting of acute STEMI undergoing primary PCI where prompt antiplatelet inhibition is required, appealing. (48)Additionally, cangrelor is not affected by renal function, which is usually not known at the time of STEMI presentation, and therefore does not need dose adjustment in patients with impaired renal function presenting with STEMI. This may be considered as an added value for cangrelor in this setting. (86, 90)

One of the limitations of cangrelor use is its interaction with thienopyridine active metabolites. These active metabolites compete for the same ADP-binding site on the P2Y12 receptor, and they cannot bind to P2Y12 receptors in the presence of bound cangrelor; therefore, it is important to administer thienopyridine after cangrelor infusion discontinuation to avoid this interaction. (91, 92) The transition from cangrelor to ticagrelor is of less concern, given the prolonged presence of ticagrelor in plasma. No cangrelor-ticagrelor interaction has been described during the transition; therefore, it can be administered at any time. (93)

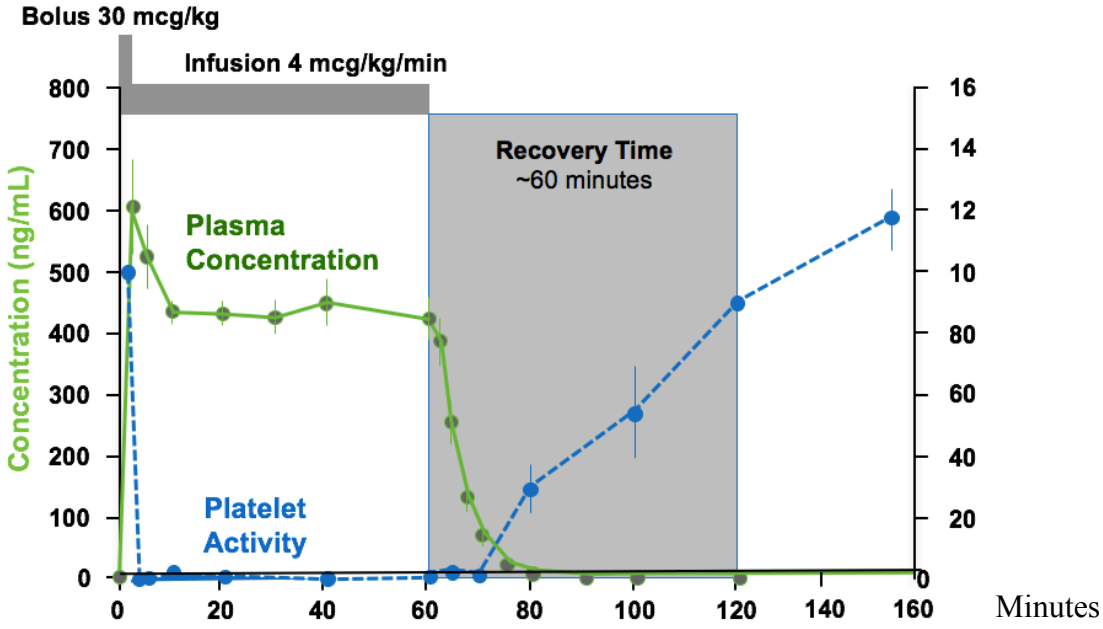


Figure 8 Pharmacokinetics and pharmacodynamics of cangrelor

Source: Akers et al. Clin Pharmacol 2010;50:27-35

The clinical benefit of cangrelor as adjunctive antiplatelet therapy in patients needing PCI has been studied in three phase III randomized clinical trials, namely the cangrelor versus Standard Therapy to Achieve Optimal Management of Platelet Inhibition (CHAMPION) PCI, CHAMPION PLATFORM, and CHAMPION PHOENIX studies. (48, 94, 95) CHAMPION PCI was a randomised, double-blind, double-dummy, active-control trial. In this study, 8877 patients undergoing PCI were enrolled (15 % of patients had stable angina, 25 % had unstable angina, 49 % had NSTEMI, and 11 % had STEMI). Patients were randomized to cangrelor infusion (30 μg/kg bolus then 4 μg/kg infusion) started within 30 minutes prior to PCI and continued for two hours or for the duration of the index procedure, whichever was longer, or clopidogrel 600mg orally, again given within 30 minutes before PCI. After the infusion, patients in the cangrelor group received clopidogrel 600mg, and patients who had already received clopidogrel were given placebo tablets.

Cangrelor was not superior to 600mg of clopidogrel with regard to the primary composite endpoint of death from any cause, myocardial infarction, or ischaemia-driven revascularization at 48 hours (7.5% of patients receiving cangrelor and 7.1% of patients receiving clopidogrel, odds ratio, 1.05; 95% CI, 0.88 to 1.24; P=0.59). The secondary endpoints of stent thrombosis and death were lower in the cangrelor group, with no significant increase in the rate of transfusion. (48)

In the CHAMPION PLATFORM study, 5362 patients (5 % stable angina, 35 % unstable angina and 60 % NSTEMI; STEMI was an exclusion criterion) with angiographically established coronary artery disease amenable for PCI were randomly assigned to receive either cangrelor infusion (30 μg/kg bolus then 4 μg/kg infusion) followed by clopidogrel 600mg after a maintenance infusion of approximately 2-4 hours or to receive placebo throughout the PCI procedure. Patients in the placebo arm received clopidogrel 600mg at the end of PCI procedure. The primary endpoint was a composite of death, myocardial infarction, or ischaemia-driven revascularization at 48 hours. Previous clopidogrel therapy was not allowed in CHAMPION PLATFORM but was permitted in CHAMPION PCI. Clopidogrel 600mg was given at the end of PCI in CHAMPION PLATFORM and the beginning of PCI in CHAMPION PCI.

The CHAMPION PLATFORM study, too, failed to show a significant reduction in periprocedural complications in the 48 hours after the PCI. The primary endpoint occurred in 185 of 2654 patients receiving cangrelor (7.0%) and in 210 of 2641 patients receiving placebo (8.0%) (OR in the cangrelor group, 0.87; 95% CI, 0.71 to 1.07; P=0.17) There was no significant between-group difference in the overall rate of myocardial infarction or ischaemia-driven revascularization. The study, however, demonstrated a substantial reduction in the secondary endpoint of stent thrombosis and all-cause death associated with cangrelor when compared with placebo at 48 hours. In both studies, there was a higher rate of groin haematoma as expected for a potent IV P2Y12 inhibitor. (94)

In summary, both the CHAMPION PCI and CHAMPION PLATFORM trials on their own did not show any ischaemic benefit of cangrelor compared to clopidogrel; therefore, both studies were discontinued early due to lack of evidence of clinical benefit.

The investigators partly attributed the negative result of both the CHAMPION PCI and CHAMPION PLATFORM to the definition of MI, which was used in both studies. Myocardial infarction was defined as the appearance of new Q waves (duration >0.03 seconds) in 2 contiguous ECG leads or cardiac biomarkers ≥three times the local upper limit of normal, together with a rise >50% above the baseline when biomarkers were initially elevated prior to PCI.

In the CHAMPION PHOENIX trial, the third universal definition of myocardial infarction was used. This required patients with normal baseline cardiac biomarkers to have an elevation of > three times upper limit of normal and those with abnormal, but stable or falling, cardiac biomarkers at baseline to have a re-elevation of ≥ 20 % between peak and nadir with a maximum of > three times upper limit of normal.

A pooled analysis of both CHAMPION PCI and CHAMPION PLATFORM trials, using the universal definition of myocardial infarction, demonstrated that treatment with cangrelor did show a significant decrease in the primary endpoint. (96) With the use of the universal myocardial infarction definition, the primary endpoint was decreased with cangrelor (odds ratio 0.82, 95% CI 0.68-0.99, P= .037). Stent thrombosis was reduced from

0.4% to 0.2% (odds ratio 0.44, 95% CI 0.22-0.87, P = .018). TIMI major bleeding and transfusions were not increased with cangrelor. This led the CHAMPION investigators to the design of the CHAMPION PHOENIX trial, where they used the third universal definition of MI.

The CHAMPION PHOENIX trial was designed to investigate whether cangrelor could lead to a reduction in the rate of periprocedural complications of PCI, including stent thrombosis. In this trial, 11,145 patients (56% stable angina, 26% NSTEMI and 18% STEMI) were enrolled. After coronary angiography, patients were assigned to cangrelor received an IV bolus of 30μg/kg, followed immediately by an IV infusion of 4 μg/kg. At the end of cangrelor infusion, these patients received clopidogrel 600mg. Patients randomised to clopidogrel received a loading dose of 300mg or 600mg after identifying their coronary anatomy. A quarter of patients in the clopidogrel group received a loading dose of 300mg, while the rest received 600mg. (95)

Clopidogrel loading dose was administered before PCI was started (63.4% of patients), before PCI was completed (6.4% of patients), within 1 hour of PCI completion (30.1% of patients) or >1 hour after PCI completion (0.1 % of patients). After that, aspirin 75–325mg, and clopidogrel 75mg were administered to all patients during the first 48 hours following PCI. The newer P2Y12 inhibitors ticagrelor and prasugrel were not used. In this study, 2059 patients (18.8%) received bivalirudin. GP IIb/IIIa inhibitors were allowed only as a bailout therapy during PCI in cases of high thrombus burden, slow or no reflow, side branch compromise, dissection or distal embolisation.

The primary composite efficacy endpoint was myocardial infarction, death from any cause, ischaemia-driven revascularization or stent thrombosis in the 48 hours following randomization. The main secondary endpoint was stent thrombosis. The rate of the primary efficacy endpoint was 4.7% in the cangrelor group and 5.9% in the clopidogrel group (21% risk reduction) (adjusted odds ratio with cangrelor, 0.78; 95% CI, 0.66 to 0.93; P=0.005). (Figure 9) Stent thrombosis developed in 0.8% of the patients in the cangrelor group and in 1.4% in the clopidogrel group (odds ratio, 0.62; 95% CI, 0.43 to 0.90; P=0.01). The

reduction in the rate of stent thrombosis (0.6%) and myocardial infarction (0.9%) mainly contributed to this positive result (38% risk reduction). (95) (Figure 10)

At 30 days, cangrelor therapy was also associated with significantly lower rate of the composite efficacy endpoint compared with clopidogrel (6.0% vs. 7.0%; OR, 0.85; 95% CI, 0.73 to 0.99; P=0.03). Cangrelor was also associated with lower rates of stent thrombosis (1.3% vs. 1.9%; OR, 0.68; 95% CI, 0.50 to 0.92; P= 0.01). Additionally, the need for bailout GP IIb/IIIa inhibitors was similarly lower with cangrelor compared with clopidogrel (1.4% vs. 3.1%, p= 0.010). There was no remarkable increase in the rate of bleeding with cangrelor compared to clopidogrel using GUSTO and TIMI major bleeding criteria. In contrast, there was an increase in ACUITY-defined major bleeding ($p<0.001$). This was mainly driven by the higher frequency of haematoma at the site of vascular access. Site investigators determined the choice of access site, with 26% of patients receiving radial PCI and 74% of patients receiving femoral PCI. In the CHAMPION PHOENIX trial, STEMI subgroup represented 18% of the total patients' cohort. In this subgroup of patients, cangrelor demonstrated a 25% reduction in primary endpoints (2.8% versus 3.7%; OR 0.75, 95% CI 0.46–1.25, P=0.98 for interaction). (95)

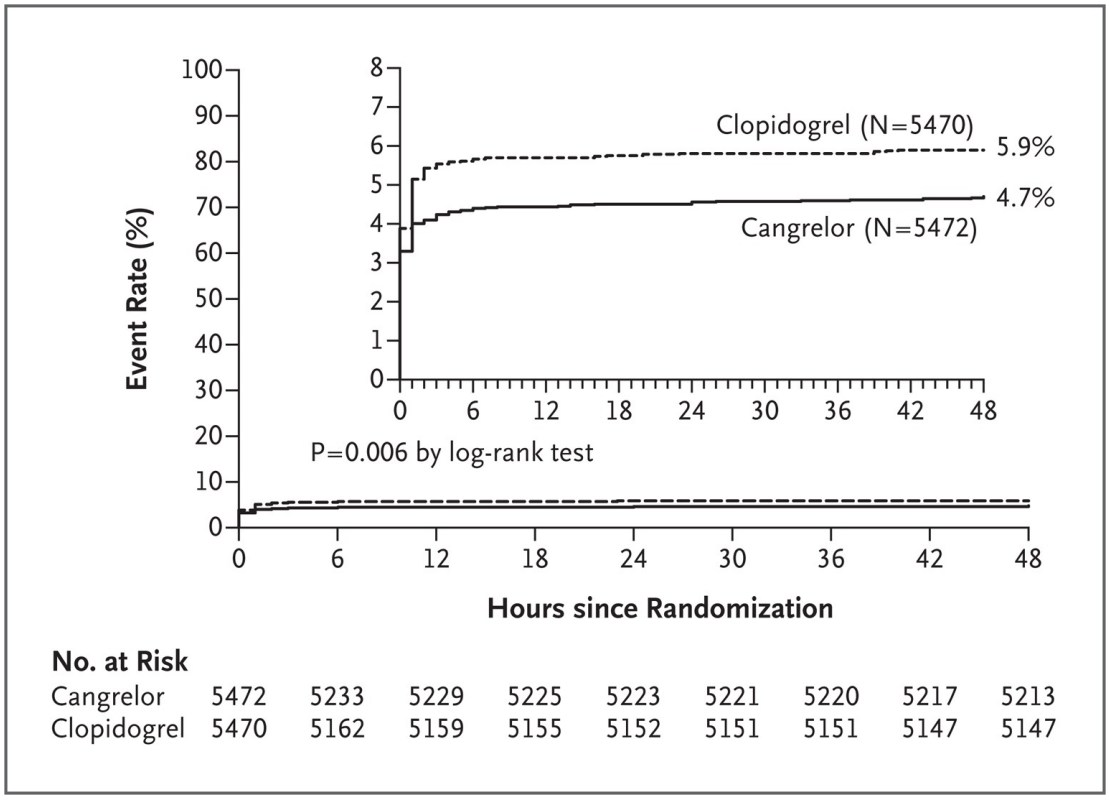


Figure 9 CHAMPION PHOENIX primary efficacy endpoints

Source : Bhatt DL, et al. N Engl J Med 2013; 368:1303-13.

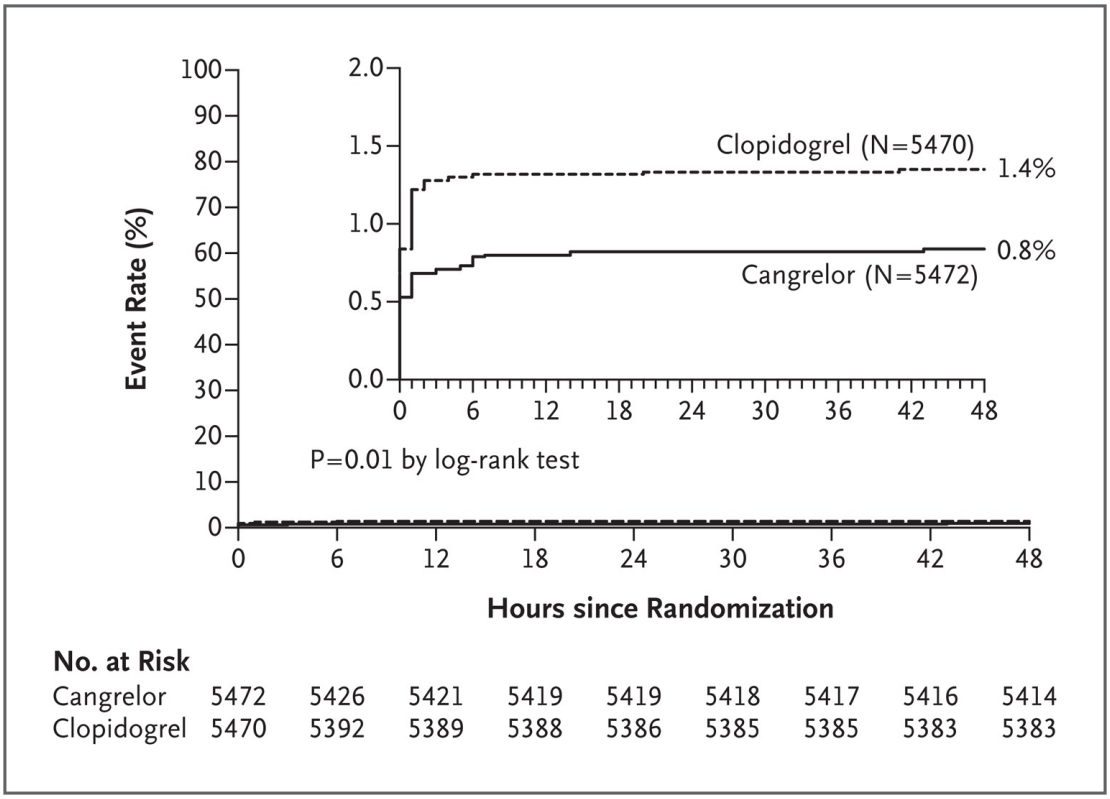


Figure 10 CHAMPION PHOENIX secondary endpoints

Source : Bhatt DL, et al. N Engl J Med 2013; 368:1303-13.

Based on a pooled analysis from 12140 patients in the 3 phase 3 CHAMPION trials, cangrelor alone compared with GPIIb/IIIa inhibitors in addition to clopidogrel was associated with similar ischaemic risk and lower risk-adjusted bleeding risk. (97) Out of 12140 patients included in this pooled analysis, 72.3% were men with a mean age of 63.2 years. Patients in the clopidogrel-GPIIb/IIIa group were more likely to be younger male patients with lower co-morbidities who presented with ACS and were enrolled from the USA. Also, they were less likely to receive bivalirudin.

The clinical benefit of cangrelor over clopidogrel was significant at 30 days irrespective of a clopidogrel loading dose (300mg or 600mg) or the time of its administration (before or after PCI). No mortality benefit was demonstrated at 48 hours. These positive findings were further supported by a pooled analysis conducted on approximately 25,000 patients recruited in all the three CHAMPION trials. It showed the consistency of the results using the PHOENIX definition of MI. (98) The results of CHAMPION PHOENIX trial led to the approval of cangrelor for clinical use as an adjunctive therapy in addition to dual antiplatelet therapy in patients with stable angina, NSTEMI and STEMI undergoing PCI to reduce the occurrence of acute ischaemic events.

The US Food and Drug Administration approved cangrelor (Kengrexal, the Medicines Company) on 22nd June 2015 for reducing thrombotic events in patients undergoing PCI. The European Medicines Agency approval for the drug was granted in March 2015. As per this approval, co-administration of cangrelor with aspirin is indicated for reducing the risk of adverse cardiovascular events in adult patients with coronary artery disease undergoing PCI who have not received an oral P2Y12 inhibitor before the PCI procedure and in whom oral therapy with P2Y12 inhibitors is not feasible or desirable.

Although ESC guidelines recommend cangrelor for patients with NSTEMI (class IIb, level of evidence A), there are no clear recommendations concerning the use of cangrelor in patients with STEMI. (21)

The following are examples of other circumstances where cangrelor can be considered a reasonable alternative to oral P2Y12 inhibitors due to its pharmacological properties: haemodynamically unstable, intubated or sedated patients, patients with STEMI complicated by cardiogenic shock where intestinal absorption is poor or with nausea or vomiting which might occur as a result of morphine administration. Cangrelor could also be beneficial during PCI-related complications and in circumstances where the diagnosis of ACS is mistaken, such as for aortic dissection where bleeding needs to be controlled rapidly. Additionally, it could be used as a bridging therapy if an emergency CABG operation is required. (99)

GPIIb/IIIa Inhibitors

Depending on the size of molecules, IV GPIIb/IIIa inhibitors can be classified into large and small molecules. Abciximab is a large molecule (monoclonal antibody) GPIIb/IIIa inhibitor. Tirofiban and Eptifibatide are small molecule GPIIb/IIIa inhibitors. (100) Eptifibatide is modelled on a compound insulated from the venom of the pygmy rattlesnake. (101) Tirofiban is modelled on a mixture insulated from saw-scaled viper venom. (102)The structure of the GPIIb/IIIa inhibitors determines their pharmacological properties.

Abciximab is a part-murine, part-human chimeric Fab fragment of the monoclonal 7E3 IgG3 antibody against the GP IIb/IIIa receptor. Its structure is based on a murine monoclonal antibody. (103) It binds with high affinity to the GP IIb/IIIa receptors in a non-competitive way.

GPIIb/IIIa inhibitors in ST Segment Elevation Myocardial Infarction

The ReoPro and Primary PTCA Organization and Randomized Trial (RAPPORT) trial was the first to assess the benefits of abciximab in the setting of primary PCI. (104) In this study, abciximab in patients undergoing primary PCI without routine stent implantation compared with placebo significantly reduced the incidence of reinfarction, urgent target vessel revascularisation and death (9.9% versus 3.3%, P=0.003, at 7 days; 11.2% versus 5.8%, P=0.03, at 30 days; and 17.8% versus 11.6%, P=0.05, at 6 months) at the expense of higher rates of bleeding (3.7 vs. 12.0 %, p=0.001). Most of the difference in bleeding was related to the access site. In the current era of the radial approach, one would expect this difference in bleeding to be negligible.

Parallel results were seen in the Abciximab Before Direct Angioplasty and Stenting in Myocardial Infarction Regarding Acute and Long-term follow-up (ADMIRAL) study. (105) In this study, 300 patients with acute myocardial infarction were randomised to receive abciximab plus stenting (149 patients) or placebo plus stenting (151 patients) before undergoing coronary angiography. Abciximab substantially reduced the incidence of death, reinfarction and target vessel revascularisation (6% in the abciximab group vs. 14.6% in the placebo group; P=0.01, at 30 days, and 7.4% in the abciximab group vs. 15.9% in the placebo group; P=0.02, at six months). The improved clinical outcomes in the abciximab group were attributed to the higher occurrence of TIMI III flow in the infarct-related artery before coronary stenting. In addition to better clinical outcomes, abciximab improved coronary patency at six months and left ventricular function.

On the other hand, the Controlled Abciximab and Device Investigation to Lower Late Angioplasty Complications (CADILLAC) was a 2-by-2 factorial design trial investigating the effect of abciximab in the setting of primary PCI. (106) Patients with acute myocardial infarction (total n=2082) were randomised to undergo balloon angioplasty alone (n=518), balloon angioplasty plus abciximab therapy (n=528), stenting alone (n=512), or stenting plus abciximab therapy (n=524). The primary endpoints of death, reinfarction, target vessel revascularisation, and stroke did not differ between the patients who received abciximab or

placebo and as such is considered a negative study. However, an essential limitation of this study was the relatively low-risk population. In other studies, abciximab demonstrated a reduction in the incidence of death and reinfarction when less strict patient selection criteria were applied. (107)

A meta-analysis of 11 randomized trials (n=27115) demonstrated that adjunctive abciximab therapy in patients undergoing primary PCI significantly reduced the 30 days and long-term mortality and the rate of reinfarction, with the degree of clinical advantage being mainly related to patients selection based on their risk profile. (108) Furthermore, a systematic review of randomized clinical trials performed by Kandzari et al. showed that treatment with abciximab as adjunctive therapy in the setting of primary PCI significantly reduced early adverse ischaemic events, a benefit that was maintained at six months follow up. Additionally, there was a positive trend toward a reduction in mortality and reinfarction. (109)

In the ON-TIME 2 trial (Ongoing Tirofiban in Myocardial Infarction Evaluation Trial), the benefit of routine pre-hospital administration of high-bolus dose tirofiban in patients with acute STEMI undergoing primary PCI was investigated. (110) In this multicenter double-blinded randomised controlled trial, 984 patients with STEMI undergoing primary PCI were randomised to either pre-hospital high bolus dose of tirofiban in the ambulance (n=491) or placebo (n=493). All patients were given a loading dose of aspirin (500mg), 5000 IU of unfractionated heparin, and a loading dose of clopidogrel (600mg).

The primary endpoint of this study was the degree of ST-segment resolution. Pre-hospital administration of tirofiban led to improved ST-segment elevation resolution 60 minutes following PCI (10.9 ± 9.2 vs. 12.1 ± 9.4 mm; p=0.028) and a significant reduction in the combined incidence of myocardial infarction, urgent target vessel revascularisation, death and thrombotic bailout (tirofiban 26.0 % vs. placebo 32.9 %; p=0.020) at 30 days without a significant increase in the incidence of major bleeding (p=0.36). Thus, differences among trials can undoubtedly lead to different results, and despite the negative results of the

FINESSE trial, a multitude of data still support the benefits from facilitation with GP IIb/IIIa inhibitors.

In conclusion, the currently available data suggest that upstream administration of GPIIb/IIIa inhibitors versus in the catheterization laboratory might be considered in high-risk patients undergoing primary PCI such as those with anterior STEMI or advanced Killip, or patients presenting within the first 3 hours on the basis of the advantage indicated in a post-hoc analysis of the FINESSE trial. (111)

In a small randomised controlled trial, abciximab as a bolus only has been found to be non-inferior to abciximab bolus followed by 12 hours infusion in patients with NSTEMI. (112)

The ESC guidelines give a class IIa recommendation for GPIIb/IIIa inhibitors for bailout use only (for example, in thrombotic complications or no reflow). (1) The ACC/AHA guidelines give a class IIa recommendation for GPIIb/IIIa inhibitors use at the time of primary PCI (abciximab, double-bolus eptifibatide, or high-bolus-dose tirofiban) in selected patients with STEMI receiving unfractionated heparin and a class IIb indication for intracoronary abciximab. (40)

Timing of Pre-treatment with P2Y12 Inhibitors in Acute Coronary Syndrome

The mainstay of treatment in patients with ACS involves aspirin in addition to a P2Y12 receptor inhibitor. Pretreatment is defined as the administration of the loading dose before assessment of coronary anatomy. Timing of pretreatment with P2Y12 inhibitors in the setting of ACS remains the subject of an on-going debate. Currently, there are no strong data in favour of a pretreatment approach. At the same time, there are no data suggesting harm and the decision to choose one approach over another seems to be guided by clinician's evaluation of a patient's risk of thrombosis and bleeding. (113) All the randomised controlled trials in this field have proven negative when it comes to the primary endpoints. They did not provide sufficient evidence that helps clinicians in the process of decision-making about the timing of treatment with P2Y12 inhibitors whether they are initiated in the pre-hospital setting, emergency department, or anywhere else. (113)

The CREDO trial was a randomized, double-blind; placebo-controlled trials conducted among 2116 patients who were to undergo elective PCI or were considered at high likelihood of undergoing PCI. (37) Patients were randomised to receive a 300mg clopidogrel loading dose (n=1053) or placebo (n=1063) 3-24 hours before PCI. After that, all patients received clopidogrel 75mg/day, through day 28. From day 29 through 12 months, patients in the loading-dose group were given clopidogrel 75mg/day, and those in the control group were given a placebo. Both groups received aspirin throughout the study. The authors have concluded that following PCI, long-term (1-year) clopidogrel therapy significantly reduced the risk of adverse ischaemic events. However, a loading dose of clopidogrel given at least 3 hours before the procedure did not reduce events at 28 days, but subgroup analyses suggested that longer intervals between the loading dose and PCI may reduce events. (37) The clinical benefits of pretreatment with prasugrel in patients with NSTEMI and ticagrelor in patients with STEMI were evaluated in the ACCOAST and ATLANTIC trials, respectively. (88, 114) Both trials did not meet their primary endpoints and did not demonstrate strong evidence in favor of a pretreatment approach.

It is intuitive to think that pretreatment with oral P2Y12 inhibitors in the setting of STEMI might leave patients at an increased risk of bleeding in circumstances when primary PCI is not undertaken such as in patients ultimately diagnosed with aortic dissection or patients needing CABG operation. (115) Less benefit is expected from pretreatment if the time between pretreatment and PCI is short; the P2Y12 inhibitor is less potent and has a slow onset of action. Lack of apparent benefit of pretreatment with the currently available oral P2Y12 inhibitors highlights the need for additional trials designed to evaluate pretreatment with cangrelor, an IV, rapid and directly acting P2Y12 inhibitor, in comparison with either ticagrelor or prasugrel.

The ESC 2017 guidelines state "A reasonable approach is to start treatment with a P2Y12 inhibitor based on the timing with which the drug was investigated in approval studies (i.e., start as soon as possible and deemed safe for clopidogrel and ticagrelor or after the indication for PCI is established based on coronary anatomy for prasugrel). The decision to withhold early administration of P2Y12 inhibitors may also depend on the planned use of cangrelor in the catheterization laboratory, which ensures immediate inhibition of the target receptor in oral P2Y12 inhibitor-naive patients. Timing of administration of P2Y12 inhibitors in patients receiving cangrelor infusion at the time of PCI should be drug-specific. While ticagrelor can be given any time before, during or at the end of cangrelor infusion, it is recommended that clopidogrel or prasugrel be given at the time of cangrelor infusion discontinuation (or within 30 minutes before the end of infusion in the case of prasugrel administration). If coronary anatomy is known or the probability of PCI is high (such as for STEMI patients), there is evidence and a consensus that early administration of oral P2Y12 inhibitors outweighs any potential risks. On the other hand, there are no convincing data that the benefits of early administration of a P2Y12 inhibitor outweigh the possible risks in stable CAD patients undergoing diagnostic angiography".

Guidelines for P2Y12 Pretreatment in non-ST elevation Myocardial Infarction

In patients with NSTEMI undergoing invasive management, ticagrelor administration (180mg loading dose, 90mg twice daily), or clopidogrel (600mg loading dose, 75mg daily dose) if ticagrelor is not an option, should be considered as soon as the diagnosis is established (Class IIa, C).

In patients with ACS, ticagrelor (180mg loading dose, 90mg twice daily) on top of aspirin is recommended, regardless of initial treatment strategy, including patients pretreated with clopidogrel (which should be discontinued when ticagrelor is commenced) unless there are contraindications (Class I, B). In patients with ACS undergoing PCI, prasugrel (60mg loading dose, 10mg daily dose) on top of aspirin is recommended for P2Y12 inhibitor-naive patients with NSTEMI. (Class I, B) In patients in whom coronary anatomy is not identified, it is not recommended to administer prasugrel. Clopidogrel (600mg loading dose, 75mg daily dose) on top of aspirin is recommended in ACS patients who cannot receive ticagrelor or prasugrel, including those with prior intracranial bleeding or an indication for oral anticoagulants (Class I, A).

Guidelines for P2Y12 Pretreatment in ST- Elevation Myocardial Infarction

A potent P2Y12 inhibitor (prasugrel or ticagrelor), or clopidogrel if these are not available or are contraindicated, is recommended before (or at latest at the time of) PCI and maintained over 12 months, unless there are contraindications such as the excessive risk of bleeding (Class I, A). (1) Prasugrel (60mg loading dose, 10mg daily dose) on top of aspirin is recommended for initially conservatively managed STEMI if the indication for PCI is established, or in STEMI patients undergoing immediate coronary catheterization unless there is a high risk of life-threatening bleeding or other contraindications (Class I, B). Clopidogrel (300mg loading dose in patients aged ≤75, 75mg daily dose) is recommended

on top of aspirin in STEMI patients receiving thrombolysis. (116) Cangrelor may be considered in patients who have not received P2Y12 receptor inhibitors (Class IIb, A). (1)

Bleeding Criteria in Clinical Trials

The recent advances in antithrombotic therapy in patients with ACS, including those who undergo PCI have improved outcomes. This has been reached at the expense of increased bleeding risk, which is associated with subsequent adverse outcomes. (117) On the other hand, reducing bleeding complication is associated with improved outcomes. (21) While there is a consensus agreement on defining ischaemic endpoints in clinical trials such as death, stroke, myocardial infarction or stent thrombosis (20, 118), there is significant variation in defining bleeding parameters amongst different clinical trials.

Lack of standardised bleeding endpoints and the use of various terms such as serious, severe, catastrophic, major or life-threatening, makes it difficult to accurately interpret safety versus efficacy results of various antithrombotic treatments in clinical trials. (119)

Adopting different bleeding scales in clinical trials can produce different results. Meta-analyses of bleeding rates associated with cangrelor administration in all the CHAMPION trials (CHAMPION-PLATFORM, CHAMPION-PCI and CHAMPION-PHOENIX), at 48 hours, demonstrated statistically no significant difference in the rate of severe bleeding as defined by the Global Use of Strategies to Open Occluded Coronary Arteries (GUSTO) bleeding scale (RR: 1.21, 95% CI: 0.70-2.11, p=0.49; TIMI major: RR: 1.00, 95% CI: 0.59-1.68, p=0.99). However, when the more sensitive, Acute Catheterization and Urgent Intervention Triage (ACUITY) major bleeding scale was used, bleeding risk was increased with cangrelor versus clopidogrel irrespective of the vascular access used, (RR: 1.51, 95% CI: 1.32-1.72, p<0.00001), (femoral group; OR 1.69; 95% CI 1.35-2.12 vs. radial group OR 2.17; 95% CI 1.02-4.62). (120) Therefore, it is essential to consider these observations when interpreting results of bleeding events in clinical trials.

In an attempt to standardise bleeding endpoints definition in cardiovascular clinical trials, the Bleeding Academic Research Consortium (BARC) scale was developed in 2010.

Coronary Microvascular Dysfunction

The Anatomy and Function of the Coronary Circulation

The coronary arterial system can be divided into the macrocirculation, represented by the large epicardial coronary arteries, and the microcirculation represented by prearterioles and arterioles. Each of these compartments has a distinct structure and function.

Macrocirculation (Large Epicardial Coronary Arteries)

The large coronary arteries have a diameter between 500μm to 2-5mm, and they function as conductance tubes offering minimal resistance to coronary blood flow (10%). These conductive arteries exhibit endothelial-dependent dilatation in response to changes in flow. During systole, they dilate and accumulate elastic energy, leading to an increase in their blood content by approximately a quarter. During diastole, this elastic energy is then transformed into kinetic energy in the blood leading to a swift reopening of the intramyocardial vessels, which were squeezed closed, during systole. (121)

Microcirculation (Prearterioles and Arterioles)

The pre-arterioles have a diameter between 100-500μm and account for 30% of the total coronary resistance. Prearterioles are not affected by myocardial metabolite concentrations due to their wall thickness and their extra myocardial location. Similar to large arteries, pre-arterioles are reactive to flow-dependent dilatation. Blood pressure drops significantly when it passes through prearterioles. They keep pressure within a narrow range at the origin of the arterioles, whenever there is a change in coronary perfusion flow and pressure. (121) Proximal pre-arterioles are mainly responsive to changes in flow, whereas distal pre-arterioles are mostly reactive to intravascular pressure changes and are responsible for blood flow autoregulation. Arterioles have a thinner wall (<100 μm in diameter) and are intramyocardial vessels. Arterioles account for approximately 50% of the total coronary resistance. The primary function of arterioles is to maintain adequate oxygen supply to the myocardium through metabolic control of blood flow.

The vascular tone of the arterioles is regulated by metabolic and hormonal factors, changes in the autonomic nervous system, and endothelial modulation. This function is dependent on the concentration of diffusible metabolites released by the myocardium such as adenosine, and hydrogen peroxide, through their effect on the arteriolar vascular tone (vasoconstriction and vasodilatation). Endogenous substances such as angiotensin, vasopressin, and thromboxane induce vasoconstriction. Adenosine, nitric oxide, and prostacyclin cause vasodilatation. (122)

At rest, the vascular tone is very high, whereas, during conditions where there is increased oxygen demand, metabolites are released from the myocardium, which cause dilatation of the arterioles leading to a subsequent reduction in pressure and resistance, thus regulating blood flow and maintaining adequate oxygen supply to the myocardium. The maximal increase in coronary blood flow (maximal hyperaemia) in response to various stimuli is called coronary flow reserve (CFR). Additionally, the resultant dilatation of distal prearterioles and arterioles leads to an increase in shear stress, which in turn activates flow-dependent dilatation in larger prearterioles and conductance epicardial arteries. (123)

This adaptation of the vascular resistance regulates blood flow to different parts of the heart according to need. This explains the essential role that the microcirculation plays in the pathophysiology of myocardial ischaemia. (Figures 11 and 12)

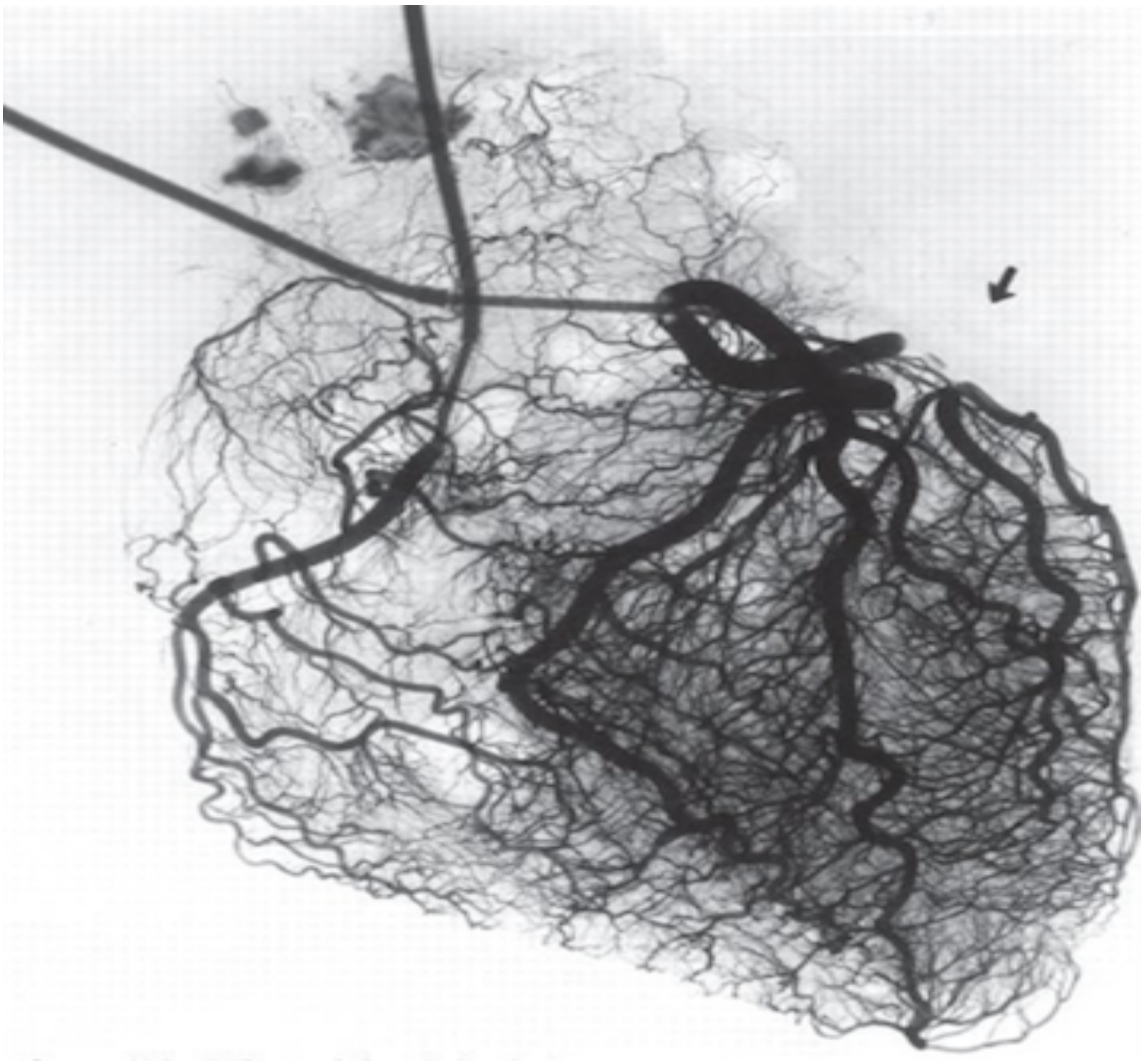


Figure 11 Coronary microcirculation

Source: Stereo-arteriogram by the late Dr. William Fulton, 1963, University of Glasgow

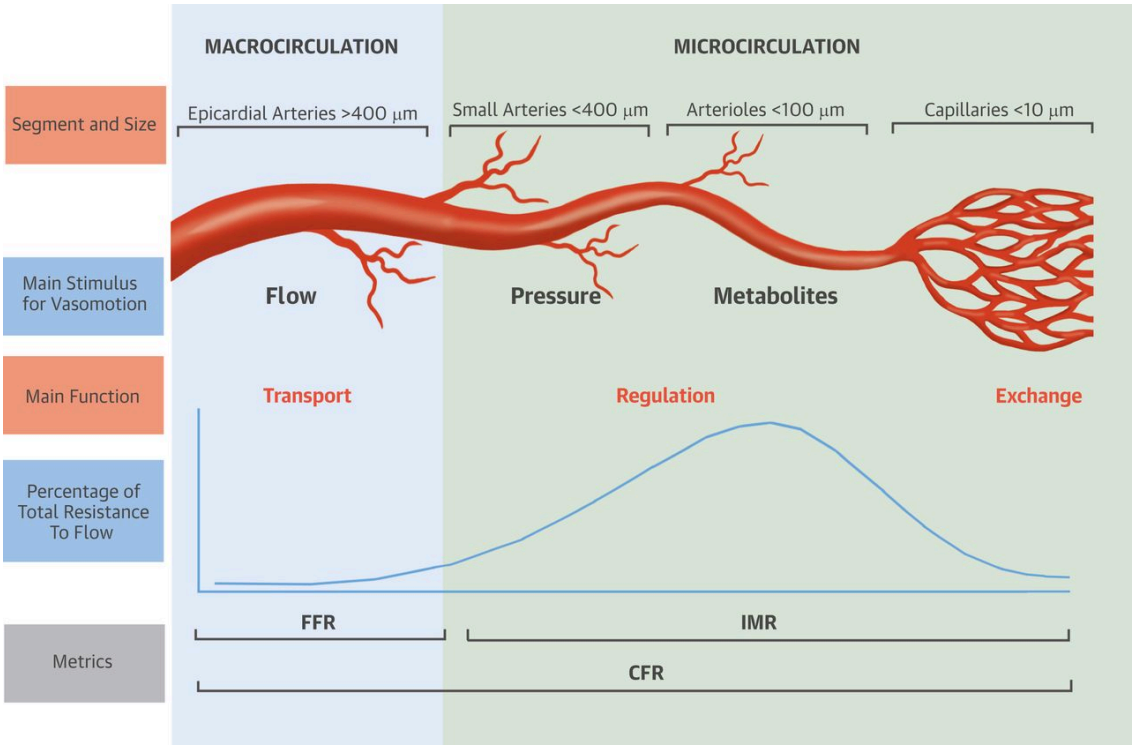


Figure 12 Macrocirculation and microcirculation across segments and sizes of the coronary tree

Source: Bernard De Bruyne et al. JACC 2016;67:1170-1172

Definition and Prognostic Significance of Coronary Microvascular Dysfunction

Coronary microvascular dysfunction can be defined as a mismatch of myocardial blood supply and oxygen consumption as a result of abnormal coronary microvascular resistance (arteriolar or pre-arteriolar) that is clinically evident as inappropriate coronary blood flow response, impaired myocardial perfusion and/or myocardial ischaemia that cannot be accounted for by abnormalities in the epicardial coronary arteries. (124)

Coronary microvascular dysfunction is associated with an increase in the risk of cardiovascular events regardless of the epicardial disease status and can occur in the presence or absence of coronary or myocardial diseases. (125, 126) In a single-centre observational study conducted by Britten et al, coronary flow reserve was assessed by intracoronary Doppler and quantitative coronary angiography in 120 patients with angiographically normal or minimally diseased coronary vessel.

Cardiovascular events during follow-up (6.5+/-3 years) were defined as sudden death, myocardial infarction, unstable angina, ischaemic stroke or the need for revascularization by PCI or coronary as well as peripheral bypass surgery.

Reduced coronary flow reserve was significantly associated with a poor long-term outcome: cardiovascular events occurred in 7 (18%) patients in the lowest tertile of coronary flow reserve compared with 4 patients in the middle tertile (10%) and 2 patients in the upper tertile (5%) (P=0.019).

It was concluded that CFR, as a measure of microvascular dysfunction in angiographically normal or mildly diseased coronary arteries was an independent predictor of long-term cardiovascular outcomes. (126) It is essential to highlight that coronary artery disease and ischaemic heart disease do not necessarily correlate. Some patients with severe coronary artery disease that is diagnosed angiographically are asymptomatic and do not have evidence of myocardial ischaemia, whereas, many patients with evidence of myocardial ischaemia do not have apparent coronary atherosclerosis. (127, 128) One possible explanation for this mismatch is microvascular dysfunction.

Myocardial ischaemia can be due to many causes, including, but not restricted to, narrowing of the coronary arteries, microvascular disease or changes in cardiac morphology due to a poorly responsive microcirculation, such as in left ventricular hypertrophy and heart failure. Certain other conditions such as diabetes mellitus, hypertension, hyperlipidaemia, and old age can increase individual's susceptibility to CMVD. (129) Microvascular dysfunction has been well documented in many studies after acute myocardial infarction and is indicative of poor prognosis. In a study by Morishima et al, the long-term prognostic importance of angiographic no-reflow phenomenon after PCI for acute myocardial infarction was assessed. One hundred and twenty patients with their first acute myocardial infarction treated by PCI without flow-restricting lesions were studied. The patients were classified as either no-reflow (n=30) or reflow (TIMI-3) (n=90) to follow up (5.8 +/- 1.2 years) for cardiac death and nonfatal events. Patients with no-reflow had congestive heart failure ($p < 0.0001$), malignant arrhythmia ($p = 0.038$), and cardiac death ($p = 0.002$) more often than did those with reflow. (130)

Classification of MVD and Responsible Mechanisms

Camici and Crea proposed a clinical and pathogenic classification of CMD into four types based on clinical data. (131)

Type 1: Microvascular dysfunction in the absence of myocardial diseases and obstructive coronary artery disease (Functional)

- **Mechanism:** microvascular re-modeling, endothelial dysfunction, and smooth muscle dysfunction.
- **Conditions:** smoking, hypertension, hyperlipidaemia, diabetes mellitus, insulin-resistant states, and microvascular angina.
- **Assessment:** noninvasive assessment of CFR.

Type 2: Microvascular dysfunction in myocardial diseases without obstructive coronary artery disease

- **Mechanism:** microvascular remodeling, smooth muscle dysfunction, extramural compression, reduced diastolic perfusion time (increased intramyocardial pressure or tissue oedema), vascular wall infiltration, vascular rarefaction, and perivascular fibrosis.
- **Conditions:** hypertrophic cardiomyopathy, dilated cardiomyopathy, Anderson-Fabry's disease, amyloidosis, myocarditis, and aortic stenosis.
- **Assessment:** invasive or noninvasive assessment of CFR.

Type 3: Microvascular dysfunction in obstructive coronary artery disease

- **Mechanism:** endothelial dysfunction, smooth muscle dysfunction, and luminal obstruction (microembolization).
- **Conditions:** stable CAD or ACS (STEMI and NSTEMI).
- **Assessment:** invasive and noninvasive techniques.

Type 4: Iatrogenic microvascular dysfunction

- **Mechanism:** luminal obstruction (microembolization by plaque and thrombus debris), autonomic dysfunction (coronary recanalisation).
- **Conditions:** PCI and CABG operation caused mainly by vasoconstriction or distal embolisation.
- **Assessment:** invasive or noninvasive assessment of CFR, which is reduced, but returns spontaneously in the weeks after revascularization.

Overall, these pathophysiological mechanisms cause either impaired vasodilatation or increased vasoconstriction of the microvascular vessels.

Microvascular Dysfunction in Acute ST- Elevation Myocardial Infarction (The Slow Flow or No-Reflow Phenomenon)

Mechanisms

In acute STEMI, the main objective of therapy is the early reopening of the blocked infarct-related coronary artery to restore myocardial perfusion. This is usually partly achieved with primary PCI and adjunctive pharmacotherapy. However, even after reopening the vessel and re-establishing coronary blood flow, normal myocardial reperfusion remains inadequate in up to half of patients with acute STEMI as assessed by corrected TIMI frame count (CTFC), myocardial perfusion grade (MPG), ST-segment resolution (STR) and myocardial contrast echocardiography, which is associated with a high in-hospital mortality. (132-136)

Thrombolysis In Myocardial Infarction Flow Grade classifies antegrade blood flow in the infarct-related coronary artery into three grades (grades 0–3) (table 2). TIMI myocardial perfusion grade measures the time for contrast medium to penetrate and or exit the microcirculation. (137)

It assesses microvascular clearance of the contrast medium and is scored on a scale of 0–3, with higher scores indicating better perfusion (table 3). Grade 3 represents normal entry and

exit of contrast from the microvasculature in a way, which is similar to that of an uninvolved artery. At the end of a successful PCI, the culprit artery should exhibit TIMI 3 flow rate and normal myocardial tissue perfusion (grade 3). The most severe manifestation of microvascular dysfunction is referred to as the "slow or no-reflow phenomenon" which is a predictor of long-term complications such as death, life-threatening arrhythmia and heart failure leading to worse long-term outcomes. No-reflow can be defined as the insufficient myocardial reperfusion (abnormal TFG and MPG) of a given coronary segment of the coronary circulation in the absence of angiographic evidence of epicardial coronary vessel obstruction, flow-limiting dissection, vessel spasm, or apparent in situ thrombosis despite the successful recanalisation of the infarct-related artery. (138, 139)

The pathophysiology of microvascular obstruction and no-reflow in patients with acute STEMI undergoing primary PCI is multifactorial and complex. Four possible mechanisms have been suggested: ischaemia-related injury, reperfusion-related injury, distal coronary embolization, and individual susceptibility of the microcirculation to injury. (34)

Table 2 TIMI Flow Grades

TIMI 0 flow	Absence of any antegrade flow beyond a coronary occlusion
TIMI 1 flow	Faint antegrade coronary flow beyond the occlusion, with incomplete filling of the distal coronary bed (penetration without perfusion).
TIMI 2 flow	Delayed or sluggish antegrade flow with complete filling of the distal territory (partial reperfusion).
TIMI 3 flow	Normal flow, which fills the distal coronary bed completely (complete perfusion).

Table 3 Myocardial Perfusion Grades

Grade 0	Either minimal or no ground glass appearance ("blush") of the myocardium in the distribution of the culprit artery
Grade 1	Contrast slowly enters but fails to exit the microvasculature. Contrast staining is present on the next injection.
Grade 2	Delayed entry and exit of dye from the microvasculature
Grade 3	Normal entry and exit of dye from the microvasculature similar to that in an uninvolved artery

Ischaemia Related Injury

The pathophysiological changes that happen to the myocardial cells during ischaemia are well recognized and described in both animal and human studies with the first studies being made by Kloner et al. in animal models. (138, 140, 141) After 20 minutes of coronary occlusion in dogs, ischaemic myocardial cell death starts at the subendocardium and progress towards the subepicardium in a time-dependent manner. Myocardial ischaemic injury is reversible until 90 minutes from coronary obstruction. After this time, the ability to re-perfuse the previously necrotic myocardium is impaired, as a result of progressive microvascular obstruction. After approximately 6 hours of coronary occlusion and myocardial ischaemia, the process of myocardial cell necrosis becomes completed. (140)

Under aerobic conditions (mitochondrial metabolism) majority of the cardiac energy is derived from fatty acids, and up to 90% of this energy is used for ATP synthesis. The rest of the cardiac energy comes from pyruvate oxidation. Pyruvate is the product of glycolysis and lactate oxidation. Within seconds of sudden coronary artery occlusion (early phase of ischaemia where myocardial injury is reversible), aerobic metabolism changes into anaerobic metabolism. Metabolism of pyruvate triggers the production of lactic acid, subsequent acidosis, reduction in myocardial contractile function, and a more significant need for ATP synthesis to maintain metabolism. (142) Adenosine triphosphate formation is reduced, which leads to an increase in the intracellular levels of sodium chloride causing cell swelling. This imbalance in the cellular transport system eventually leads to intracellular calcium overload.

As a result of this osmotic overload condition, the myocardial cells become oedematous, larger in size, and deficient in glycogen. This resultant interstitial myocardial oedema compresses capillaries and small arterioles leading to further reduction of blood flow to these vessels and poor myocardial perfusion. Parallel to the changes that the myocardial cells sustain during ischaemia, the endothelial cells also undergo alterations, which are not usually seen until approximately an hour from ischaemia. (141) Myocardial ischaemia leads to severe endothelial cells injury, capillary damage, adhesion of neutrophils to the injured

endothelium and activation and accumulation of platelets that eventually lead to blockage of the capillary lumen and reduction of blood flow to these already compressed vessels by interstitial oedema. Coronary microvascular obstruction and myocardial tissue hypoperfusion could happen even in the presence of patent epicardial coronary arteries. The longer the duration of myocardial ischaemia, the more severe and irreversible the damage to the myocytes becomes. (141)

Reperfusion Related Injury

In patients with STEMI, it is of prime importance to re-establish coronary blood flow to the ischaemic myocardium promptly to reduce infarct size and improve outcomes. The intended benefits with reperfusion have to be balanced with the damages that might occur as a result of reperfusion injury. The pathophysiological changes seen during prolonged myocardial ischaemia (longer than 3 hours) are further potentiated by reperfusion injury; thus, it has now become recognised that reperfusion acts as a double-edged sword. Reperfusion injury can be defined as the reperfusion-related deterioration of ischaemic cardiac injury leading to decreased myocardial contractility, an increased arrhythmogenic threshold, change of reversible to irreversible cardiac myocyte damage, and microvascular dysfunction. At the time of myocardial reperfusion, there is a sudden increase in the intracellular calcium, causing disruption in the usual mechanisms that control calcium in the myocardial cells, referred to as the calcium paradox. This abrupt increase in intracellular calcium prompts cell death by causing overactive contracture of cardiac myocytes and opening of mitochondrial permeability transition pores. (34, 140, 143, 144)

Other mechanisms involved in reperfusion injury include the excessive enlargement, protrusion, and destruction of the vascular endothelial cells. Platelets interact with neutrophils to form platelet-neutrophil aggregates. These aggregates obstruct the coronary microvascular lumen and cause the release of inflammatory mediators and vasoconstrictors that contribute to microvascular endothelial injury and microvascular dysfunction. Red blood cells and neutrophil-platelet aggregates occlude capillary lumens leading to rupture of these microvessels, which in turn induces fibrin and platelet deposition and the

subsequent embolisation of the small arteries and arterioles. Activation of neutrophils happens early during myocardial ischaemia before myocardial tissue injury becomes histologically apparent. (144, 145) Infiltration of the ischaemic myocardium by neutrophils is significantly enhanced by reperfusion. (146) Neutrophils adhere to the endothelial cells, become activated, and pass through walls of the capillaries in the first few hours and then migrate to the surrounding infarcted myocardium during the following 24-48 hours. Neutrophils also amplify the initial inflammatory response through the production of chemo-attractants. Additionally, neutrophil activation causes a significant increase in oxygen uptake by the myocardial cells, leading to the creation of large amounts of reactive oxygen species in numbers much higher than the anti-oxidant defense mechanisms can counteract thus, causing disruption of endothelial cells. (147) This oxidative cellular damage and stress caused by high levels of reactive oxygen species are considered a significant factor in the development of lethal myocardial reperfusion injury. Furthermore, oxidative stress and ischaemia decrease the level of nitric oxide, leading to dysfunction of the myocardial microcirculation. (148) Intramural haemorrhage, which can occur as a result of reperfusion injury, can be exacerbated by consumption of the coagulation factors. (149)

In summary, myocardial ischaemia due to acute coronary artery occlusion is responsible for vascular endothelial destruction and formation of platelets/fibrin and neutrophils emboli. Reperfusion injury causes endothelial damage, interstitial and myocardial oedema, contracture of cardiac myocytes, the formation of platelets/fibrin and neutrophils emboli, formation of free radicals and embolisation of the small arteries and arterioles. All these mechanisms contribute to CMVD and the no-reflow phenomenon.

Distal Coronary Emobolisation

The pathophysiological changes and events that occur as a result of ischaemia and reperfusion can be aggravated by microembolisation of emboli downstream into the microcirculation of the infarct-related artery. (Figure 13) These microemboli are of different sizes and originate from ruptured atherosclerotic plaques or thrombotic material. They are formed either spontaneously or as a result of balloon inflation and stent

deployment in the culprit coronary artery during primary PCI and can significantly contribute to microvascular obstruction observed in the setting of acute myocardial infarction. (150, 151)

The phenomenon of distal embolisation in patients with STEMI undergoing primary PCI may not be detected by coronary angiography; therefore other methods of imaging are required such as intravascular ultrasound (IVUS), angioscopy, optical coherence tomography (OCT). (152-154) A Doppler wire technique was used by Okamura et al. to count the number of embolic particles via tracing high-intensity transient signals. The average number of emboli throughout primary PCI was 25. (155) Katayam et al. conducted a study using intravascular ultrasound to investigate the no-reflow phenomenon in 70 patients with acute myocardial infarction undergoing coronary stenting. (156) In 12 out of 70 patients who developed no-reflow as measured by TFG, intravascular ultrasound demonstrated high atherosclerotic burden and reduced plaque volume as a result of stenting suggesting distal embolisation of part of the atheromatous plaque. Additionally, no-reflow was less likely to occur with the use of manual aspiration and distal protection devices. (157, 158)

In an analysis by Yip et al., distal embolisation was usually seen after stenting large coronary arteries, possibly because stenting small coronary arteries might push the thrombus to the vessel wall, particularly if it is an old thrombus. (152) Residual thrombus might persist after stent deployment and may still contribute to distal embolisation, as suggested by a recent OCT study. (159) Thus, the amount of thrombus predicts the risk of distal embolisation as well as the presence of a lipid-rich plaque. (160)

In addition to the mechanical obstruction they cause to the distal capillaries, studies have found that these microemboli are biologically active. These emboli stimulate the release of inflammatory and vasoactive substances such as endothelin-1. This further potentiates the undesirable effects of reperfusion on the microcirculation. (161) Myocardial blood flow is not absent in areas of microvascular obstruction; instead, it is reduced to less than 50% of the baseline level and continues to drop with time.

An irreversible reduction in myocardial perfusion starts to happen when more than 50% of the capillary lumen is obstructed. (162, 163) This progressive reduction of myocardial blood flow, which is likely to occur as a result of reperfusion injury, leads to a two-fold rise in microvascular obstruction. (164)

Individual Liability of the Microcirculation to Injury

Certain conditions such as aging, obesity, diabetes mellitus, hypertension, hyperlipidaemia, genetic variability, and chronic inflammatory diseases such as chronic rheumatoid arthritis predispose to impairment of the CFR and microvascular dysfunction. (165) Such pre-existing microvascular dysfunction in patients presenting with acute STEMI has recently been more recognised as a predictor of future ischaemic events and a marker of inadequate response to reperfusion therapy. (34)

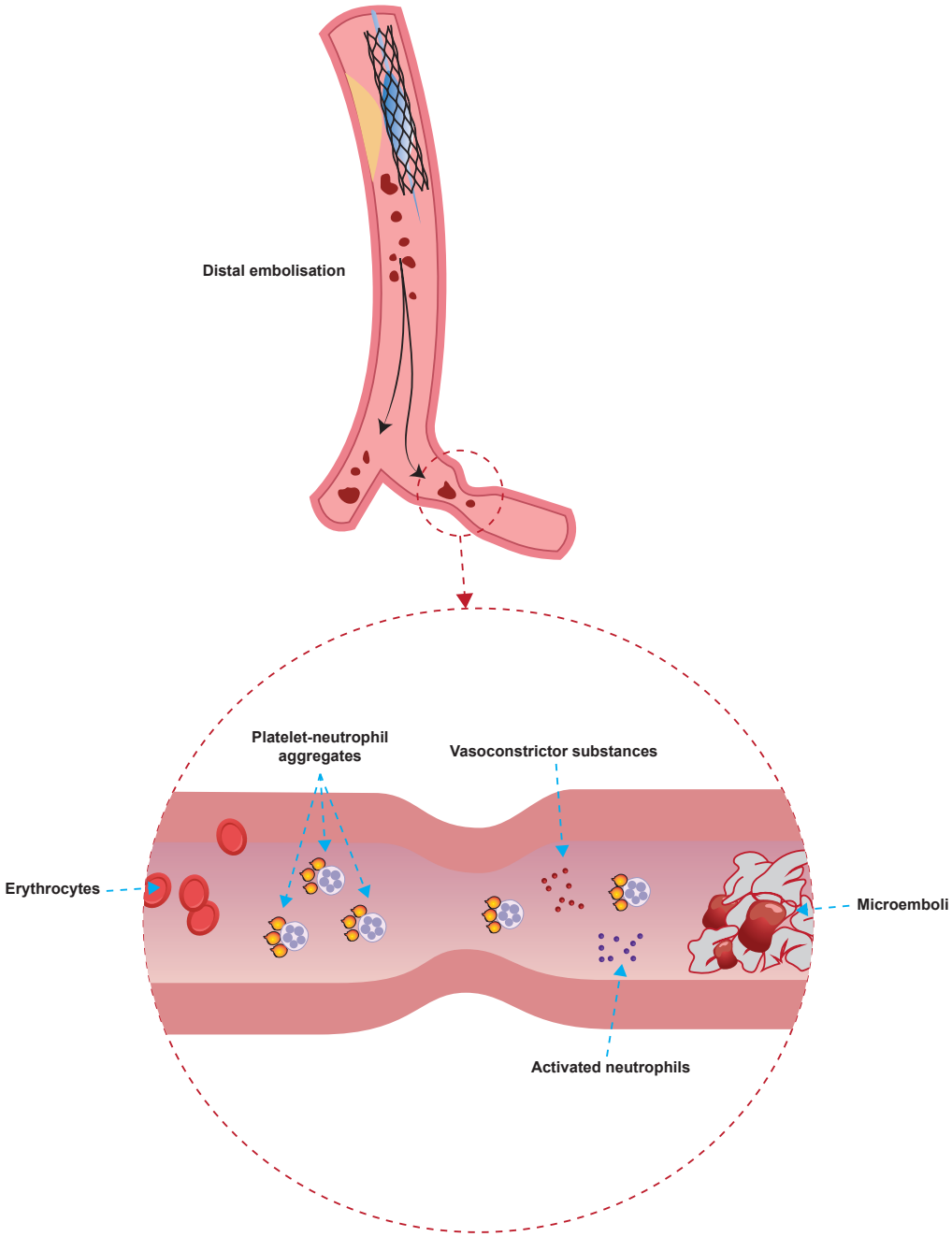


Figure 13 Coronary distal embolisation

Prevention and Treatment of Coronary Microvascular Obstruction and Dysfunction

Several factors contribute to microvascular obstruction and dysfunction such as myocardial ischaemia, distal embolisation from epicardial coronary arteries, reperfusion injury, thrombosis, vasospasm, and inflammation. Therefore, therapeutic interventions need to target the epicardial coronary artery, the microcirculation, and the myocardial tissue. Currently, no therapy has been proven to be effective in the prevention or treatment of coronary microvascular obstruction. Nonetheless, mechanical and pharmacological approaches have been studied for this purpose. In acute STEMI, the benefit of thrombectomy devices to manually aspirate coronary thrombus to protect the distal coronary bed against micro embolisation has been tested in several trials. Routine use of manual aspiration thrombectomy did not lead to improved 30-days infarct size (INFUSE-AMI) (166), 30-days mortality (TASTE) (167) and was associated with an increased risk of stroke within 30-days. (168)

Coronary microvascular obstruction may be prevented by the administration of antiplatelet and antithrombotic therapy. Abciximab has been shown to reduce infarct size and microvascular obstruction in patients with acute STEMI as determined by CMR imaging. (169, 170) This further supports the role of platelets and platelet-leucocyte aggregates in coronary microvascular obstruction.

Coronary Flow Reserve

Coronary blood flow measures the amount of blood flow through a given coronary artery per unit of time and is generally expressed in milliliters per minute. (171) When there is an increased demand for myocardial oxygen supply, such as during physical exercise, the coronary vasculature increases the resting blood flow to a maximal level to keep up with the metabolic demand. This maximal increase is referred to as coronary flow reserve, which represents the ability of the vasculature to respond with a rise in coronary flow after maximal coronary vasodilatation as compared to basal coronary flow. Under stress conditions in healthy individuals without coronary artery disease, coronary blood flow

increases more than 3-fold. Because flow resistance is mainly determined by the microvasculature (microcirculation accounts for approximately 70% of the total coronary resistance), CFR represents the ability of the microvasculature to respond to various stimuli and as such, can be regarded as a surrogate for the function of the microcirculation. Coronary flow reserve is calculated as the ratio of maximal blood flow during maximal coronary hyperaemia divided by the resting flow. A coronary flow reserve of less than 2.0 is considered abnormal.

In healthy individuals, however, CFR varies according to age and sex. (171) Certain conditions might reduce CFR without microcirculatory dysfunction being present such as tachycardia, hypoxaemia, anaemia, or ventricular hypertrophy. These conditions lead to an increase in myocardial oxygen demand and subsequently, basal blood flow. After excluding such clinical situations, impaired CFR is an essential marker of microvascular dysfunction. (131) Obstructive coronary artery diseases decrease maximal blood flow. In patients with CAD, the degree of the reduction in CFR is directly related to the severity of stenosis, whereas in individuals with angiographically normal arteries it is an indicator of microvascular dysfunction. (171) Following PCI procedure in patients with acute myocardial infarction, CFR can be assessed invasively using thermodilution or Doppler-flow measurements.

The thermodilution technique was first applied in clinical practice in determining cardiac output using a Swan-Ganz catheter before being implemented in the evaluation of blood flow in the coronary arteries. The thermodilution method is based on the principle that volume and flow could be measured using an indicator dilution (Fick principle), which is injected into the blood, and then the concentration of the indicator is measured over time distal to the site of injection. (172, 173) This indicator could be saline of a given temperature. The flow will be equal to volume over mean transit time. For this purpose, a coronary wire was developed. The distal part of the wire acts as pressure and temperature sensor, and the shaft of the wire acts as a proximal temperature sensor. (174-176)

Thermodilution derived CFR can be measured by relying on the ratio of flow at baseline and maximal hyperaemia and is thus defined as a ratio of hyperaemic and resting mean transit times. (174) Assessing CFR using the principle of coronary thermodilution has been

validated in vitro, in animal models, and human clinical studies and can be used to assess coronary microvascular obstruction. (174)

In an in vitro model, the absolute flow was compared with the inverse mean transit time (1/T(mn)) of a thermodilution curve obtained after a bolus injection of 3 mL of saline at room temperature. A very close correlation (r>0.95) was found between absolute flow and 1/T(mn) when the sensor was placed >/=6 cm from the injection site. In 6 chronically instrumented dogs (60 stenoses; FFR from 0.19 to 0.98), a significant linear relation was found between flow velocity and 1/T(mn). A significant correlation was found between CFR (Doppler), which was calculated from the ratio of hyperaemic to resting flow velocities, and CFR (thermo), which was derived from the ratio of resting to hyperaemic T(mn) (r=0.76; SEE=0.24; P<0.001). The present findings prove the validity of the thermodilution principle to assess CFR. (174)

Assessing coronary microvascular obstruction by calculating CFR using thermodilution technique has two main limitations. Firstly, as CFR interrogates the entire coronary circulation, it is therefore difficult to differentiate between epicardial coronary arteries and microcirculation's contribution to the total coronary vascular resistance. (177) Secondly, CFR which is calculated as the ratio of hyperaemic to baseline flow, is affected by haemodynamic factors such as heart rate and blood pressure because of the patients' haemodynamic status influences baseline flow. (178) These limitations have led Fearon et al. to the development of a new tool called IMR. (179) Index of microcirculatory resistance enables examination of the coronary microcirculation function directly with high reproducibility and reliability.

Exogenous adenosine induces microvascular vasodilatation through a direct effect on the vascular smooth muscle cell A2 receptors. (180) Also, adenosine induces shear stress on the coronary arteries, which causes further dilatation of the arteries. Wilson et al. investigated the effect of adenosine on coronary arterial circulation in humans. (181) Thirty-nine patients undergoing coronary angiography for chest pain were studied. Adenosine was administered by intracoronary bolus (2-16 micrograms), intracoronary infusion (10-240 micrograms/min), or IV infusion (35-140 micrograms/kg/min). The effect

on coronary and systemic haemodynamic status and the ECG was evaluated. Coronary blood flow velocity (CBFV) was measured with a 3F coronary Doppler catheter. The maximal coronary blood flow velocity was determined with intracoronary papaverine (4.5 +/- 0.2 resting CBFV). In normal left coronary arteries (n=20), 16-micrograms boluses of adenosine caused coronary hyperaemia similar to that caused by papaverine (4.6 +/- 0.7.resting CBFV). In the right coronary artery (n=5), 12-micrograms boluses caused maximal hyperaemia (4.4 +/- 1.0 resting CBFV). Intracoronary boluses caused a small, brief decrease in arterial pressure (similar to that caused by papaverine) and no changes in heart rate or the ECG. The duration of hyperaemia was much shorter after adenosine than after papaverine administration. Intracoronary infusions of 80 micrograms/min or more into the left coronary artery (n=6) also caused maximal hyperaemia (4.4 +/- 0.1 resting CBFV), and doses up to 240 micrograms/min caused a minimal decrease in arterial pressure (-6 +/- 2mmHg) and no significant change in heart rate or in ECG variables. Intravenous infusions in normal patients (n=25) at 140 micrograms/kg/min caused coronary vasodilation similar to that caused by papaverine in 84% of patients (4.4 +/- 0.9 resting CBFV). In eight patients with microvascular vasodilator dysfunction (delta CBFV, less than 3.5 peak/resting velocity after a maximally vasodilating dose of intracoronary papaverine), the dose-response characteristics to intracoronary boluses and IV infusions of adenosine were similar to those found in normal patients. The data of this study demonstrated that adenosine, given by intracoronary or IV routes, could cause near-maximal coronary vasodilation without causing clinically important changes in systemic haemodynamics or on the ECG. (181)

Adenosine could be administered via IV or intracoronary routes. Intracoronary adenosine is very safe to induce maximal hyperaemia. (182) The peak effect is observed less than 10 seconds after administration, and its effect lasts for the duration of action of less than 20 seconds. Intracoronary adenosine is currently administered at a dose of 40mcg into the right coronary artery (to avoid transient atrioventricular block) and a dose of 60mcg into the left coronary artery. (182) Hyperaemia is achieved in about one minute and lasts for up to one minute after the infusion is stopped. Transient atrioventricular block is less commonly

encountered with IV than intracoronary adenosine. Adenosine is contraindicated in patients with asthma and chronic obstructive pulmonary disease. The effective delivery of adenosine and adequate vasodilator effect of adenosine could be marked by a 10-20% increase in the heart rate and a 10-20% drop in blood pressure.

Aims and Hypothesis

This study was designed to address the hypothesis that compared to oral ticagrelor; IV cangrelor causes earlier and more potent $P2Y_{12}$ inhibition (primary endpoint). The surrogate endpoint was to assess whether IV cangrelor would lead to improved microvascular function and reduced myocardial infarct size in patients with acute STEMI undergoing primary PCI.

Study Outcomes

The primary outcome measures for this trial were the between group difference in $P2Y_{12}$ inhibition at the time of vessel reperfusion, 4 hours and 24 hours following initial dosing. Surrogate outcome measures were the assessment of microcirculatory and epicardial reperfusion in addition to myocardial infarct size.

Methods

Ethical Conduct of the Study

The National Research Ethics Service (Leicester) approved this study (reference 16/EM/0094) on the 17/03/2016. Clinicaltrials.gov identifier is NCT02733341. All patients provided written consent and continued in the study for three months. All identifiable patient information was anonymised prior to analysis. All data were recorded in case report forms. All data collected on patients were stored in an anonymised fashion on fully encrypted password-protected computers at New Cross Hospital.

Study Population

The Heart and Lung Centre is a tertiary specialist cardiac centre for the Black Country and surrounding areas serving a population of approximately 1.2 million and carrying out nearly 500 primary PCI cases per year. Data were collected for 100 patients who were treated at the Heart and Lung Centre, The Royal Wolverhampton Hospitals NHS Trust, following activation of the primary PCI pathway.

Study Design and Management

This study was an open-label, prospective, randomized controlled trial enrolling patients with acute STEMI undergoing primary PCI. Acute STEMI was defined as chest pain lasting for >30 minutes associated with ST-segment elevation >2 mm in 2 contiguous chest leads or 1mm in 2 contiguous limb leads. Soon after arrival at the Heart and Lung centre, a member of the research team approached patients, and verbal assent was sought if they deemed eligible for enrolment in the study. Patients who agreed to participate were then randomised 1:1 to routine care (aspirin and ticagrelor) or aspirin and IV cangrelor immediately prior to primary PCI, using an online randomisation software (sealedenvelop.com).

Patients were randomised to one of two treatment groups ('A' for cangrelor and 'B' for ticagrelor) and the result recorded. Randomisation was blocked (using random permuted

blocks) to ensure that the groups were balanced periodically. There was a single password for a trial, and anyone with the password could carry out new randomisations for that trial. The randomisation results were shown on-screen and emailed to both the administrator of the trial and the randomiser. All primary PCI procedures were performed in keeping with international guidelines.

Patients were eligible to be enrolled in this study if they had an indication for primary PCI, were 18 years or older and able to give informed consent, were P2Y12 receptor antagonist naïve, and had no contra-indication to ticagrelor or cangrelor. Exclusion criteria included significant active bleeding, current oral anticoagulation therapy, established cardiogenic shock defined as a systolic blood pressure of < 90mmHg, previous myocardial infarction, and contraindications to CMR imaging (e.g., presence of a pacemaker, severe renal dysfunction requiring renal replacement therapy). Patients treated with GP IIb/IIIa receptor antagonist therapy during the primary PCI were withdrawn from the analysis.

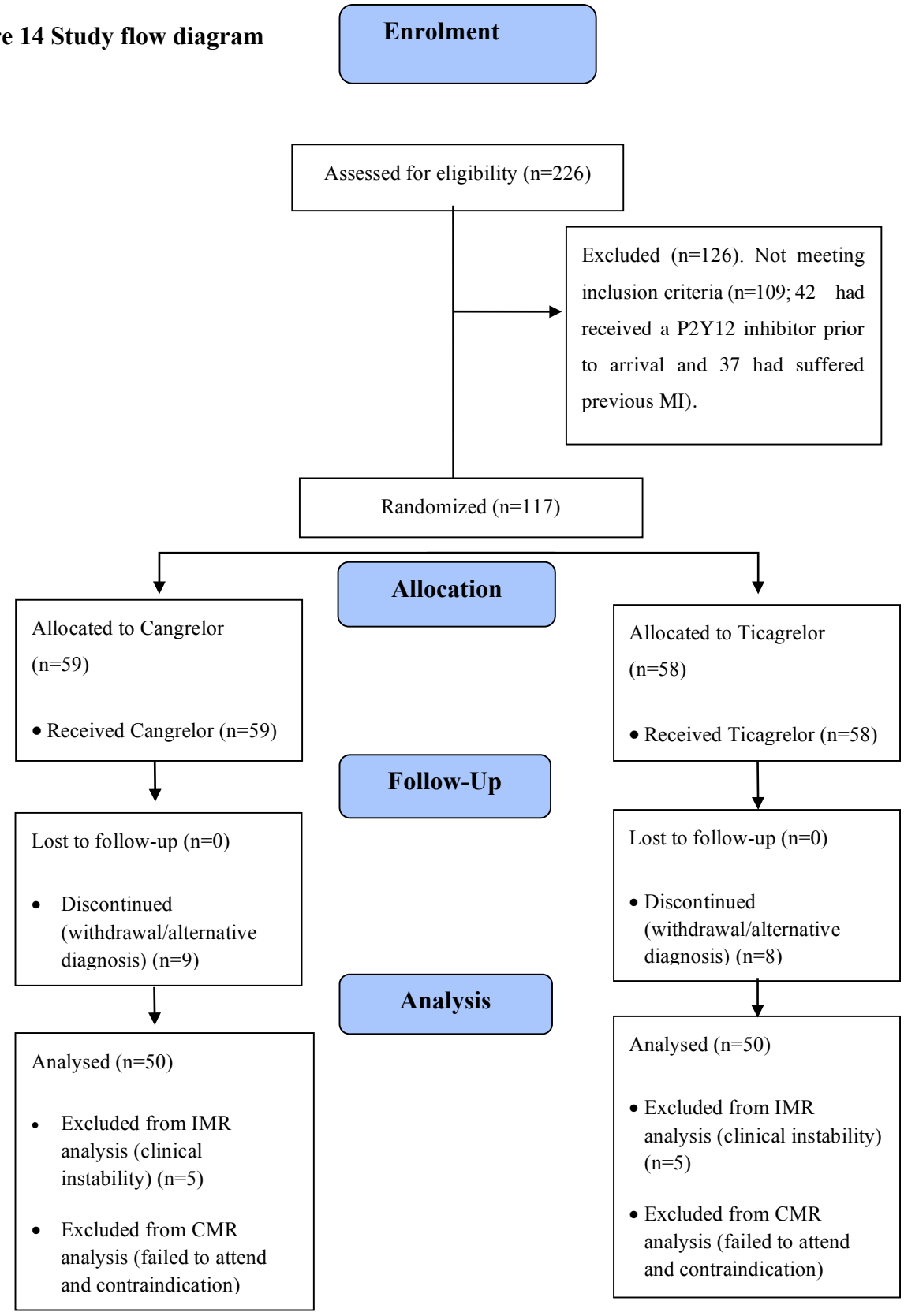
Consent

All patients provided written consent. Consent was a two-stage process for patients undergoing primary PCI for STEMI. Verbal assent was brief and taken prior to primary PCI and first blood sample. Following primary PCI and IMR measurement, when the patient became pain-free and was able to rest, a full patient information sheet was proffered and written consent sought. If the patients did not want to be part of the trial, they were withdrawn from the study, and future tests were not performed, and treatment was continued in accordance with clinical guidelines. Patients' participation in the trial lasted for three months. At the end of three months, CMR imaging was performed unless there were specific contraindications.

Drug Therapy

A total of 100 patients were enrolled, 50 patients in the cangrelor arm and 50 patients in the ticagrelor arm. All patients received a loading dose of aspirin 300mg at the time of first medical contact, prior to randomisation. Patients allocated to ticagrelor received a loading dose of 180mg of the drug orally immediately following randomization and prior to admission to the catheter suite. They then continued a maintenance dose of 90mg twice daily for 12 months as per standard clinical practice. Patients in the cangrelor arm were treated with a bolus of 30mcg/kg then 4mcg/kg/min IV infusion immediately following randomization and then transferred to the cardiac catheter suite to undergo primary PCI. Cangrelor infusion was continued for 2 hours or duration of the procedure; whichever was longer. Ticagrelor 180mg was given 30 minutes prior to stopping the infusion, as per manufacturers instructions, followed by a dose of 90mg twice daily for 12 months. Use of morphine was recorded prospectively. (Figure 14)

Figure 14 Study flow diagram



Statistics and Data Analysis

Sample Size

Sample size calculation was performed by Heather Murray, consultant statistician, at the Robertson Centre for Biostatistics, Institute of Health and Wellbeing, University of Glasgow. From preliminary data on 15 patients, the mean (SD) for P2Y12 Reaction Units (PRU) was 257 (61.1). A sample size of 50 in each group would have 80% power to detect an effect size of 0.566 of a standard deviation using a two-group t-test with a 5% two-sided significance level. This would be equivalent to a difference of 34.6 units of PRU between the cangrelor and ticagrelor. To show a reduction of 30% in PRU in the cangrelor group compared to ticagrelor group at first coronary balloon inflation time, 50 patients per group would provide > 95% power.

Descriptive Statistics

Categorical variables are reported as number and percentage (n (%)). Continuous variables are summarised by mean and standard deviation (SD) if normally distributed and median and interquartile range (IQR) if non-normally distributed. Continuous outcome measures were compared between the cangrelor and ticagrelor groups with two unpaired sample t-tests or Wilcoxon rank sum tests as appropriate. Categorical outcome measures were compared using Chi-squared tests or Fisher Exact tests. All p-values are two-sided and statistical significance was considered as $p \leq 0.05$. The Robertson Centre for Biostatistics, University of Glasgow and myself analysed data using SAS for Windows v9.3 (SAS Institute Inc., Cary, North Carolina). Graphs were produced using Prism software (GraphPad Prism version 5.00 for Windows, GraphPad Software, La Jolla California USA).

Primary Endpoint Measures

Platelet Function Testing

P2Y12 inhibition was measured using VerifyNow ™ (ACCRIVA diagnostics, San Diego, California, USA) rapid platelet function analyzer at infarct vessel opening following reperfusion (first coronary treatment) to assess the rapidity of onset and function at culprit coronary artery reperfusion, 4 hours to determine the impact of transitioning from cangrelor to ticagrelor compared to ticagrelor alone and 24-36 hours post antiplatelet dosing to assess the steady state in the two groups.

VerifyNow Assay

VerifyNow ™ is a near-patient test comprising a turbidimetric based optical detection system that measures platelet aggregation as an increase in light transmittance. (183) (Figure 14) The VerifyNow point-of-care assay has been used in the assessment of platelet reactivity following the administration of P2Y12 inhibitors. (183, 184) The VerifyNow system comprises an analyser instrument and a single-use assay device.

The instrument controls all assay sequencing, the temperature, and reagent sample mixing and performs self-diagnostics. The assay device contains human fibrinogen-coated beads, a buffer, and platelet activators. The VerifyNow P2Y12 assay contains 20micromol/L of ADP, which acts as the principal agonist of platelet aggregation via both the P2Y1 and P2Y12 receptors. In addition, the assay comprises 22nmol prostaglandin E1 to decrease platelet activation via the P2Y1 receptor. (185)

During primary PCI, blood was drawn from the coronary guide catheter, 5mls were discarded, and then 2mls was drawn into Vacuette tubes containing 3.2% sodium citrate (Greiner bio-one, Kremsmünster, Austria). P2Y12 inhibition was measured immediately after first coronary balloon inflation. Following primary PCI, samples were taken and analysed at 4 hours and then 24-36 hours post P2Y12 receptor inhibitors dosing using a 21-gauge butterfly needle in the antecubital fossa. Samples taken were utilised for both VerifyNow assay (in all patients) and VASPFix analysis in a group of patients (n=23). For

VerifyNow assay, the Vacuette tube that now contains 2ml of whole blood was carefully inverted and then left at room temperature for a minimum of 10-15 minutes (improper blood sampling may cause platelets activation) up to a maximum of 4 hours prior to P2Y12 activity assay. The tube was then loaded into a specific assay device and analysed. The system was calibrated electronically, and full quality control was performed with each batch of assay devices to ensure the integrity of the products being used. (Figure 15) After the test device was inserted into the instrument, the whole blood sample collected from a patient dosed with an antiplatelet therapy was inserted into the round opening of the test device, which contains a needle that aids in drawing a blood sample from the tube into the test device. The samples were then aspirated into the staging well, which heats the sample to 37 degrees Celsius. After heating, the staging well is pressurised pushing the blood out into the four mixing chambers simultaneously. Contained in each of these chambers, there are a chrome-plated mixing ball and a palette. The mixing ball mixes the sample while the test is running.

The palette contains fibrinogen-coated beads and an agonist (ADP) that serves to activate the platelets and facilitate aggregation. As the heated sample comes into contact with the palette in the individual mixing chambers, the palette dissolves immediately. Simultaneously the mixing ball starts to mix the solution.

As the sample is mixing, platelets begin to activate and bind to the fibrinogen creating aggregates. Inside the instrument, light shines through the individual mixing chambers. The instrument then measures the amount of light transmitted through the blood sample. As blood platelet continue to aggregate, more light passes the blood sample. Over a few minutes, the instrument measures the transmission profile of light passing through the blood sample as a function of time. In patients who respond well to the antiplatelet therapy, because of the drug-induced inhibition of platelet activity, the sample forms fewer aggregates allowing less light to pass through. In patients who do not respond adequately to antiplatelet therapy, the drug does not sufficiently inhibit platelets from activating, and as

aggregation continues to build, the blood sample becomes clearer allowing more light to pass through. Using a proprietary algorithm, the instrument analyses the different light transmission waveforms and determines the patient's platelet activity and response to their antiplatelet therapy.

Platelet reactivity is assessed as a function of an increase in light transmission through whole blood as ADP activates platelets. Results are expressed as P2Y12 reaction units (PRU), indicating the degree of ADP-mediated aggregation specific to the P2Y12 receptor. Higher PRU value reflects greater P2Y12-mediated reactivity. PRU values of ≥230 have been associated with poor clinical outcomes such as death, myocardial infarction, or stent thrombosis. PRU values of ≥208 are indicative of a suboptimal response and are associated with poor clinical outcomes. (186)

Data from 2796 (99.98%) patients enrolled in the Gauging Responsiveness With A VerifyNow P2Y12 Assay: Impact on Thrombosis and Safety (GRAVITAS) trial was analysed to examine the relationship between outcomes and on treatment platelet reactivity throughout the trial using VerifyNow assay. (187)

On treatment platelet reactivity was measured at 12-24 hours and 30±7 days post PCI. On treatment platelet reactivity <208 P2Y12 reaction units was significantly associated with a lower risk of the primary endpoint of cardiovascular death, myocardial infarction, and stent thrombosis at 60 days (HR, 0.18; 95% CI, 0.04 to 0.79; P=0.02) and at 6 months (HR, 0.43; 95% CI, 0.23 to 0.82; P=0.01). The investigators of the GRAVITAS trial concluded that achievement of on-clopidogrel reactivity <208 P2Y12 reaction units at 12-24 hours following PCI or during follow-up was associated with a lower risk for cardiovascular events. (187)

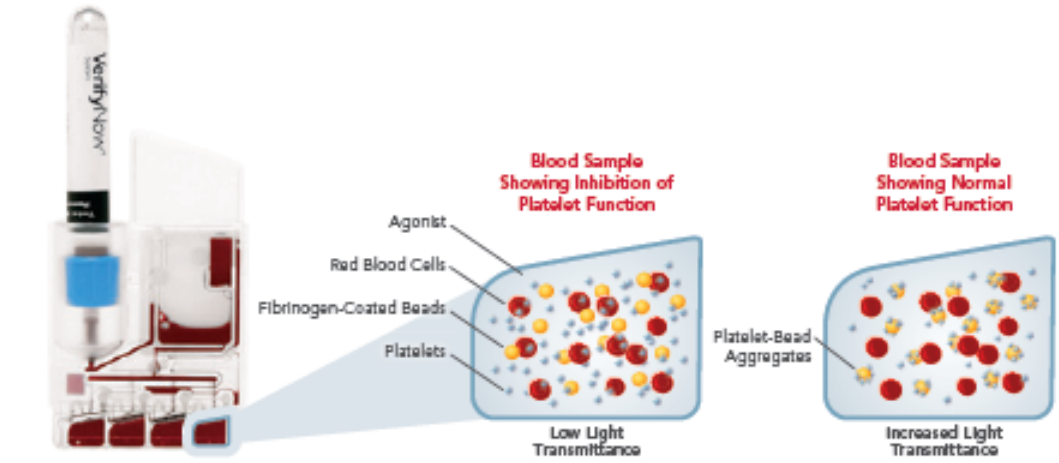
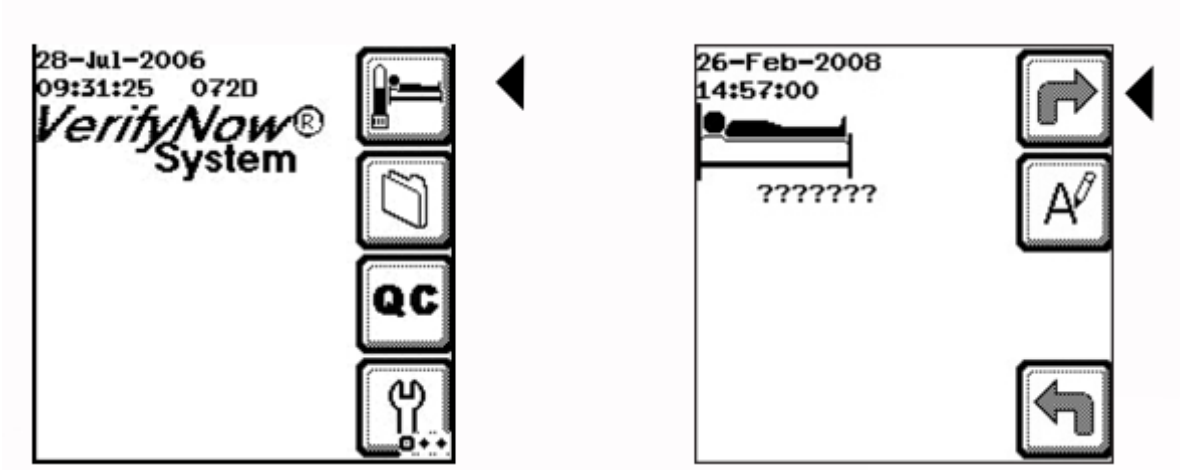


Figure 15 Measuring platelet reactivity using VerifyNow assay

Figure 16 Steps of performing VerifyNow assay

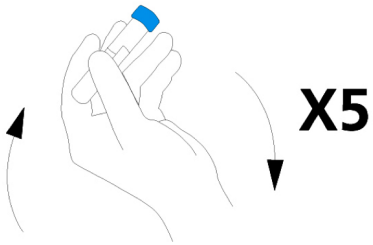


Step 1 Blood sample is collected and transferred into Greiner Bio-One Vacuette partial-fill blood collection tube.

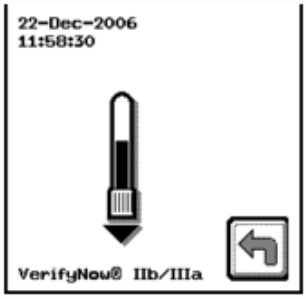


Step 2 A patient is selected and trial ID is entered.

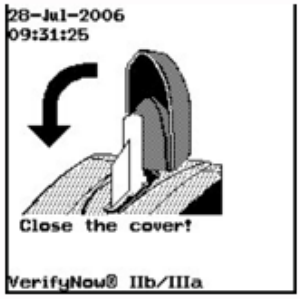




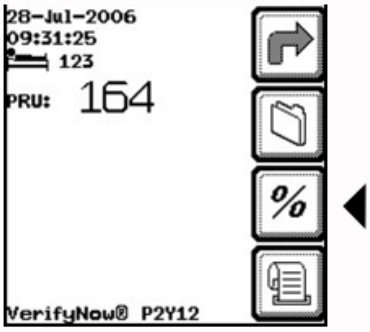
Step 3 The assay device is inserted and the sample is inverted 5 times before insertion in the device







Step 4 The tube is inserted into the VerifyNow machine and the cover is closed.





Step 5 Assay result is provided and reported in the study case report form.

VASP Flow Cytometry

Vasodilator-stimulated phosphoprotein (VASP), an intracellular actin regulatory protein, is a substrate of both cyclic adenosine monophosphate and cyclic guanosine monophosphate-dependent protein kinases. (188) Vasodilator-stimulated phosphoprotein flow cytometric analysis measures activity at the P2Y12 receptor very effectively and thereby indirectly the activity of P2Y12 inhibitor and has been shown to be of value in several clinical trials including one carried out by our research group. (189, 190) Dephosphorylation of VASP follows P2Y12 stimulation. Conversely, inhibition of the P2Y12 receptor by cangrelor or ticagrelor and stimulation of prostaglandin E1-activated adenylyl cyclase induces phosphorylation of VASP by cyclic adenosine monophosphate-dependent protein kinases. Thus levels of VASP phosphorylation/dephosphorylation reflect P2Y12 inhibition/activation. In our study, we adopted VASPFix assay for VASP phosphorylation as described by Glenn et al. (191) VASPFix can be used both as a research tool and for clinical investigations and can provide better specificity for P2Y12 receptor inhibition, and frozen VASPFix-treated samples are stable for at least six months prior to analysis. In our study, whole blood was transferred into tubes containing sodium citrate (3.2%). Citrated blood was incubated with iloprost (1 nM), iloprost (1 nM) plus ADP (5 μM), or saline for 5 minutes. 990μl of whole blood was dispensed into polystyrene tubes containing each of the agents under investigation (5μl). Samples were incubated unstirred at room temperature. After 5 minutes, 10μl blood was mixed with 25μl VASPFix in a FACS tube. The samples were then briefly vortexed and stored in -20 degrees freezers in our clinical chemistry laboratory. (Figure 16) Frozen samples were at a later stage taken out of the freezer and incubated in the dark at room temperature for two hours before flow cytometric analysis, which was performed at Nottingham University. Immediately prior to analysis, samples were re-suspended in 40μl wash buffer (Becton Dickinson, Oxford, UK). Flow cytometry was performed using an LSRII flow cytometer. A total of 300 bead events, identified by their APC fluorescence, were collected for each sample. The level of VASP-P was detected as FITC fluorescence and is expressed either as median fluorescence (mf) or a percentage of a maximally stimulated sample containing a maximal level of VASP-P (obtained, for example, by treatment of platelets with a high concentration of the IP receptor agonist

iloprost). Data obtained from our patients receiving P2Y12 antagonists was expressed as platelet reactivity index (PRI). PRI was calculated using the median fluorescence (mf) values obtained as follows

$$PRI = \frac{\text{iloprost (mf)-iloprost+ADP (mf)}}{\text{iloprost (mf)-saline (mf)}} \times 100\%$$

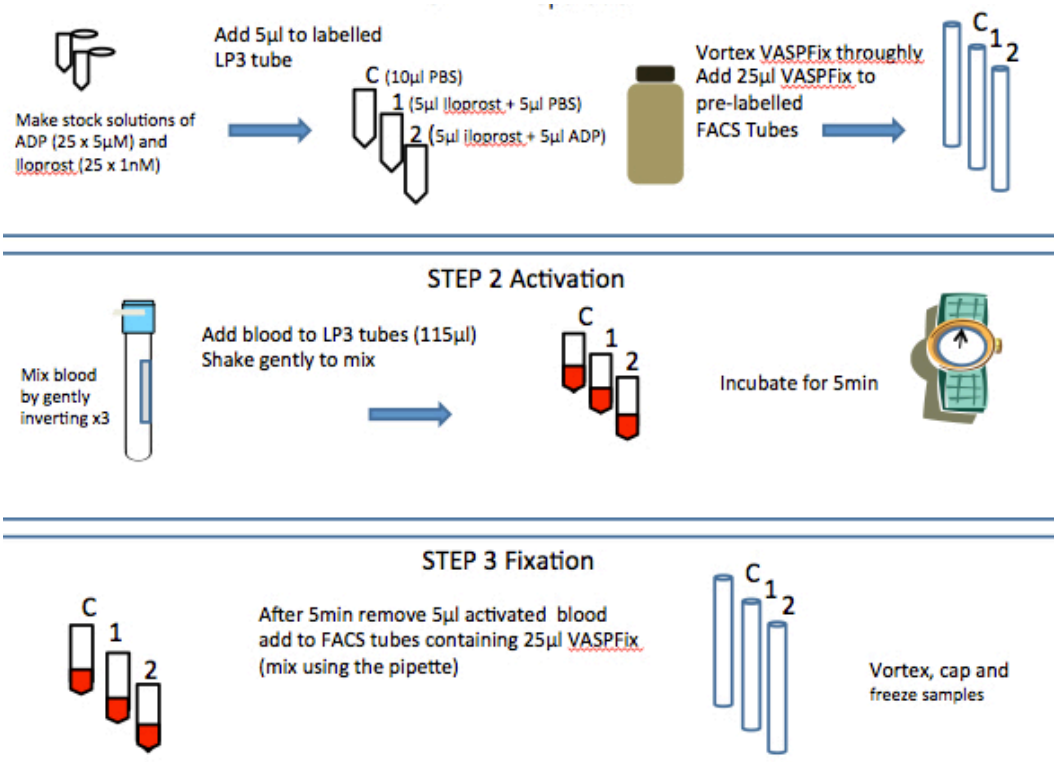


Figure 17 steps of performing VASPFix assay

Surrogate Endpoint Measures

Index of Micro-vascular Resistance and Coronary Flow Reserve

In healthy individuals and in the absence of coronary obstruction, the coronary and myocardial blood flow will be assumed to be normal, and the resistance in the epicardial coronary arteries will be minimal. The coronary vascular resistance, which is dynamic and distributed throughout the myocardium, arises mainly from the small arteries and arterioles. These small vessels contribute significantly to the regulation of coronary blood flow because of their diameter and subsequently, resistance change in a dynamic manner. During changes in the vasomotor tone, the resistance in the capillaries and venules remain relatively constant. (192) Thus, changes in vasomotor tone enable significant variations in coronary blood flow in the healthy heart. When there is > 50% stenosis in the epicardial coronary arteries, arterial resistance will start to contribute to the total coronary vascular resistance. When there is >90% stenosis in the epicardial coronary arteries, resting blood flow may reduce. (193)

Index of microvascular resistance represents the pressure drop across the microcirculation divided by coronary flow during maximal hyperaemia. (179) Blood flow is inversely proportional to the mean transit time, and therefore an index of microvascular resistance is defined as the distal coronary pressure multiplied by the mean transit time of a 3-mL bolus of saline at room temperature measured simultaneously during maximal coronary hyperaemia. (179) As such, IMR can measure coronary artery pressure and flow. Index of microvascular resistance can be measured invasively using a combined pressure-/temperature-tipped guidewire (Certus, ST Jude Medical, St Paul Minnesota). This technique has been validated in animals and human studies. (178, 179)

Index of microvascular resistance can predict myocardial infarct size as shown by peak troponin and also the severity of myocardial damage as shown by LV volume and LV function. Index of microvascular resistance measured at the end of primary PCI is an independent predictor of left ventricular ejection fraction as assessed by echocardiography or CMR imaging three months after PCI. (194) Also, it is linked to myocardial damage and predicts microvascular damage after STEMI, both acutely and in short term follow-up. (195, 196) Bulluck et al. conducted a meta-analysis of 6 studies comprising a total of 288 patients to investigate the role of IMR in detecting the presence of microvascular obstruction at the time of primary PCI in reperfused STEMI patients. (197) Microvascular obstruction data by CMR was available for 246 patients. Microvascular obstruction was present in 130 of 246 patients (53%). This study suggested that patients with a weighted mean IMR of <32 U (upper limit of the 99% CI in the group without microvascular obstruction) were far less likely to have microvascular obstruction, whereas patients with a weighted mean IMR of >41 U (lower limit of the 99% CI in the group with microvascular obstruction) were much more likely to have microvascular obstruction. The investigators concluded that IMR at the time of primary PCI could identify those patients with microvascular obstruction, allowing the implementation of treatment to minimize this complication. Additionally, IMR>40 has been found to be a multivariable associate of left ventricular and clinical outcomes following STEMI independently of the infarct size and a predictor of death. (198) Therefore, IMR can be considered a useful tool for assessing microvascular function in patients with acute STEMI. An IMR <25 is considered to be normal in healthy individuals. (199) Index of microvascular resistance is higher in patients who have subsequent evidence of procedure-related MI following PCI. (200) In our study, we set out to assess the absolute IMR values in each group and the proportion of subjects in each group with an IMR > 40.

Coronary flow reserve is calculated as the ratio of maximal blood flow during maximal coronary hyperaemia to resting flow. Both epicardial arterial and microvascular function influences it. A CFR < 2.0 is considered abnormal and is associated with cardiovascular disease states. In our study, we reported the mean CFR in each group. (201)

Index of microvascular resistance is a direct invasive assessment of microvascular resistance, whereas CFR, on the other hand, is a measure of epicardial and microcirculatory function. Thermodilution-derived IMR overcomes some of the limitations encountered with thermodilution-derived CFR. Firstly, IMR is not affected by the severity of epicardial coronary artery stenosis, and therefore, it is regarded as a measure of microvascular resistance. Secondly, IMR, unlike CFR, is independent of the haemodynamic status. This is likely because IMR is measured at maximal hyperaemia during which the haemodynamic status and the vascular tone cannot influence coronary blood flow. Index of microvascular resistance can, therefore, provide a more reproducible evaluation of the coronary microcirculation. (178) In our study, we assessed IMR and CFR in the culprit coronary artery at the end of the primary PCI procedure.

IMR Technique

At the end of primary PCI procedure and after coronary stent implantation in the culprit coronary artery, a pressure-/ temperature-sensitive guide wire (Certus, St. Jude Medical, St. Paul, Minnesota) was used to measure IMR and CFR. (Figure 17) The distal part of the wire acts as pressure and temperature sensor, and the shaft of the wire acts as a proximal temperature sensor. The guidewire was calibrated outside the body then equalized within the coronary guide catheter; a 6 French guide catheter was usually used because the accuracy of IMR using a smaller guide catheter has not been adequately assessed.

The proximal pressure sensor of the guidewire was positioned at the ostium of the guide catheter. The distal microsensor of the guidewire (pressure and temperature sensor) was placed in the distal third of the culprit artery and maintained in the same position during repeated measurement because transient mean time (Tmn) can be affected by the distance of the thermistor from the ostium of the coronary artery. Next, 3 ml of room-temperature saline was rapidly injected through the guide catheter. The transit time of the saline, which has been injected into the coronary artery, was automatically determined with (Radi Analyzer, St. Jude Medical) software.

Injection of saline was repeated three times in total. The resting mean transit time (TmnRest) was calculated from the average transit time of the three room-temperature saline injections. Following this, maximal coronary hyperaemia was induced with IV adenosine at a dose of 140 micrograms/kg/min via a large peripheral line. The dose was increased at the operator's discretion if there was a sub-optimal symptomatic or haemodynamic response at the standard dose. Maximal hyperaemia was induced before measuring IMR to obtain accurate IMR results. In order to ensure maximal delivery of the vasodilator agent into the coronary artery, a guide catheter without side holes was used. The guide catheter was kept co-axial. Using a guide catheter that is too large can cause aortic pressure damping and lead to inaccuracies and thus was avoided. It is vital to administer nitroglycerine to help dilate the epicardial coronary arteries fully to avoid flow-mediated vasodilatation; therefore, before measurements were performed; patients received 200 mcg of intracoronary isosorbide dinitrate.

After inducing maximal hyperaemia, 3 ml of room-temperature saline injections into the coronary guide catheter was repeated three times in total in a similar fashion to the resting phase. The hyperaemic mean transit time (TmnHyp) was calculated from the average transit time of the three room-temperature saline injections.

The mean aortic/proximal coronary (Pa) and distal coronary pressures (Pd) were recorded simultaneously using the same pressure wire during rest and maximal hyperaemia phases. Fractional flow reserve (FFR) is defined as Pd divided by Pa during maximal hyperaemia. Coronary flow reserve is defined as TmnHyp divided by TmnRest. Index of microvascular resistance is defined as Pd multiplied by TmnHyp (automated on the St Jude Medical RadiAnalyzer TM Xpress). (Figures 18-20) All physiological metrics were independently assessed by three observers (SU, CB, and TF). Observers were blinded to treatment group assignment and the clinical and angiographic data.

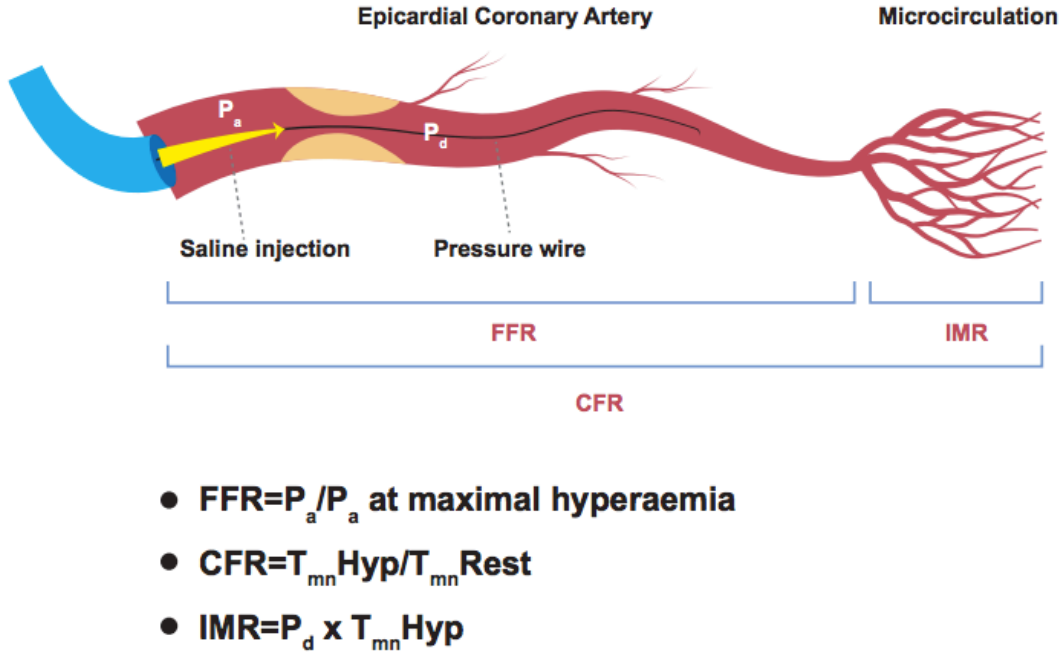


Figure 18 invasive assessments of coronary physiology parameters

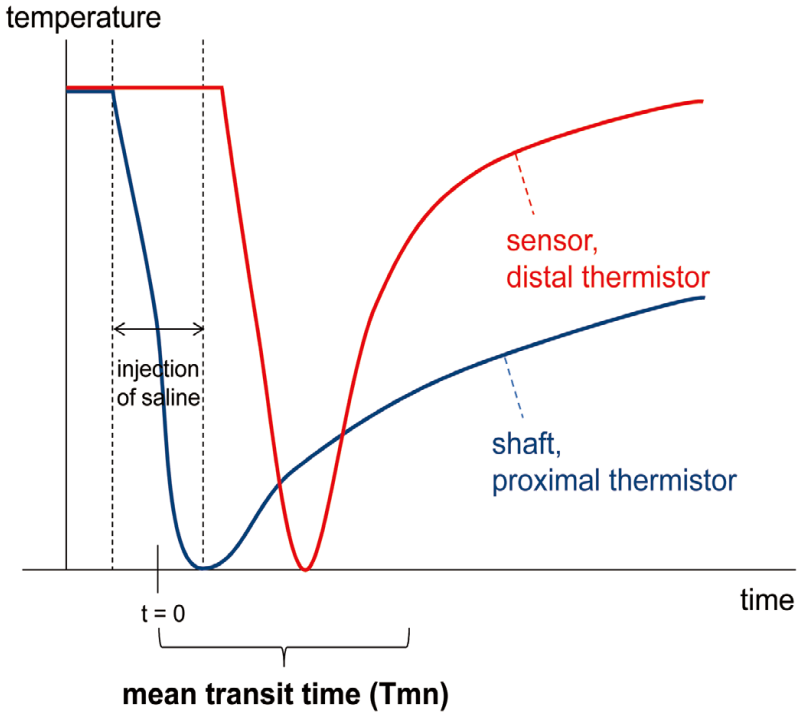


Figure 19 Calculation of mean transit time using thermodilution technique

Blue line indicates the temperature change detected by the shaft of the wire. Red line indicates the temperature change detected at the distal thermistor. Tmn, mean transit time. Invasive Coronary Microcirculation Assessment. Current Status of Index of Microcirculatory Resistance –Yuhei Kobayashi, MD; William F Fearon, MD Circulation Journal

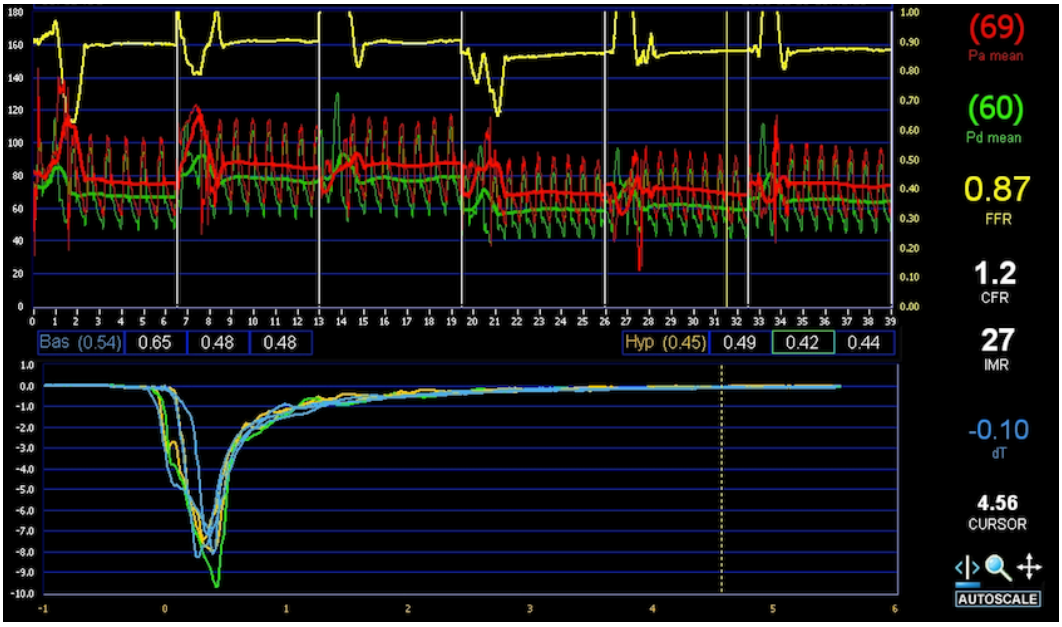


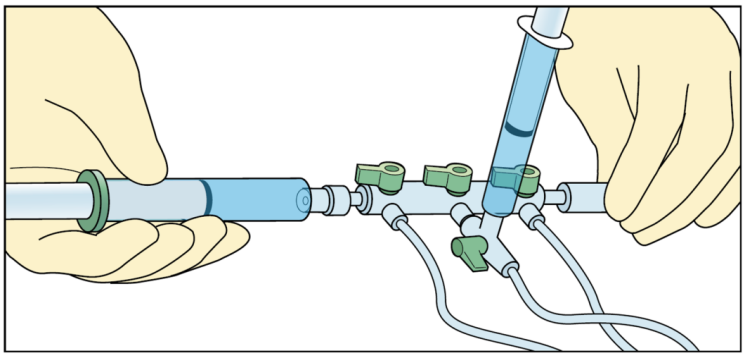
Figure 20 Assessing coronary microcirculation using IMR

IMR= Pd x TmnHyp (hyperemic mean transit time). In this example IMR is 60x0.45= 27

Figure 21 IMR measurement steps

1-Preparation

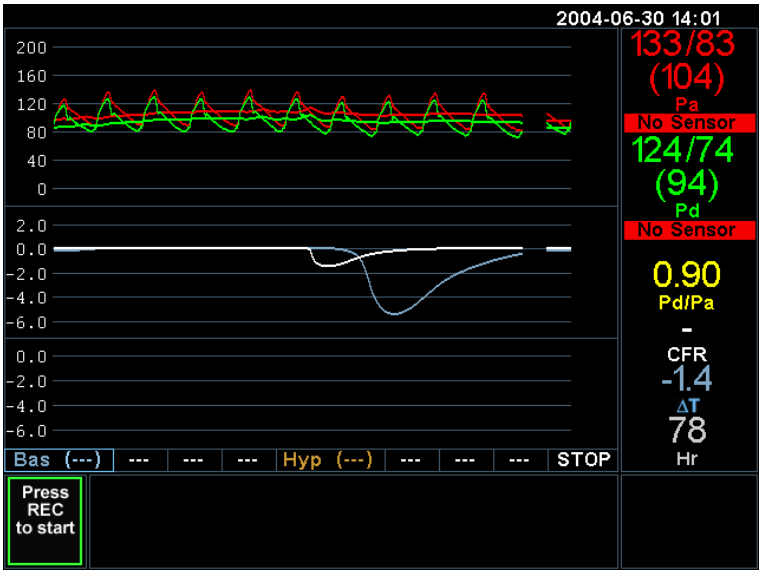
- A 6 F guiding catheter
- A manifold with a 3-way stop-cock (pressure, saline and contrast)
- A syringe with a safety reservoir
- The pressure wire sensor is placed distally in the artery (>5 cm from the catheter opening)



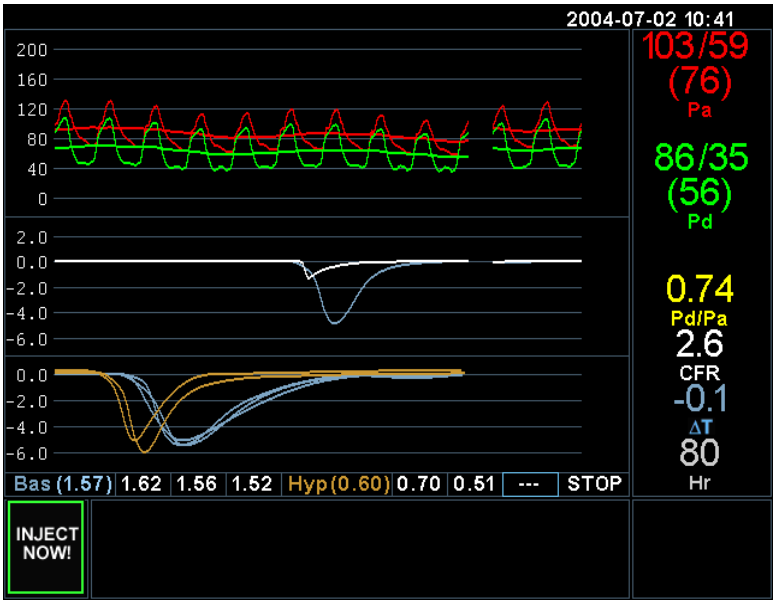
2- Pressure wire is calibrated and CFR Mode is entered



3- Resting injections: The syringe is filled with 3 ml of saline. The saline is then vigorously injected into the guiding catheter 3 times according to screen instructions. A decline in sensor temperature of at least 2 degrees indicates satisfactory injection.



4- Hyperaemic injections: Hyperaemia is induced and saline is vigorously injected into the guiding catheter 3 times according to screen instructions



5- The recording is transferred to RadiView and IMR is reviewed. IMR can be calculated manually on Xpress as Pd x Tmn Hyp (89x0.37=33)

Angiographic Analysis

Measures of coronary reperfusion in the culprit coronary artery were assessed using TFG, TIMI MPG, and cTFC following primary PCI.

Corrected TIMI Frame Count

This technique described by Gibson et al. is a more objective and accurate index of coronary blood flow. It is calculated as the number of cine frames needed for contrast medium to reach a standardised angiographic distal landmark in the infarct-related artery. Its assessment helps predict clinical outcomes. The lower the CTFC, the lower the mortality rate. (202) The relationship of CTFC to clinical outcomes was assessed in 1248 patients with myocardial infarction after thrombolysis. Patients who died in the hospital had a higher CTFC than those who survived (69. 6+/-35.4 [n=53] versus 49.5+/-32.3 [n=1195]; P=0.0003). The 90-minute CTFC was an independent predictor of in-hospital mortality with about 0.7% increase in absolute mortality for every 10-frame rise; P<0.001) and lower 90-minute CTFCs were found to be related to improved in-hospital and 1-month clinical outcomes. (202) Corrected TIMI Frame Count measured 90 minutes following myocardial infarction has been demonstrated to predict left ventricular function at 48 hours following acute myocardial infarction. (203) Also, CTFC measured at 30 days following myocardial infarction correlates well with the infarct-related flow at 12 months. (204)

We measured CTFC 90 minutes after primary PCI with a frame counter on the GE cine-viewer and calculated it as the number of frames needed for contrast to reach a standardised angiographic distal landmark. Coronary angiography was performed in 30 frames per second. The first frame was defined as the frame in which contrast touched both borders of the culprit artery, moved forward and occupied at least 70% of the vessel lumen. The standardised distal landmarks were defined as the first branch of the postero-lateral artery for the right coronary artery, the most distal branch of the obtuse marginal for the circumflex, and the distal bifurcation of the left anterior descending coronary artery. The number of frames from the first frame to the last frame when the contrast entered the standardised distal landmark was counted. We used a correction factor of 1.7 to account for

the increased length of the LAD compared to the right and circumflex arteries and hence giving a cTFC. Improved epicardial flow (TFG 2/3 and low CTFCs) and tissue-level perfusion (MPG 2/3) at 90 minutes following reperfusion therapy are independently associated with improved 2-year survival. (205) Assessing the success of reperfusion therapy using TFG technique is subjective and lacks sensitivity. Corrected TIMI Frame Count and MPG are more sensitive than TFG 3 and provide better prognostic information. These techniques have some limitations, such as their dependence on haemodynamic status and their semi-quantitative nature. Two observers (SU and BW) blinded to clinical and angiographic data, performed all measurements.

ST-segment Elevation Resolution by Electrocardiography

Studies have demonstrated a consistent relationship between the extent of ST-segment elevation resolution (STR) and mortality. (206) ST-segment elevation resolution following primary PCI is an important predictor of patency of the infarct-related artery and effective microcirculatory perfusion. (207) Persistent ST-segment elevation, despite restoration of normal epicardial blood flow, is associated with poor prognosis, larger infarct size, and increased rate of severe cardiovascular adverse events. (208) A 12 lead ECG was recorded before coronary reperfusion and 90-120 minutes following primary PCI. The extent of STR on the ECG performed 90-120 minutes after reperfusion compared to the baseline ECG before reperfusion (3) was assessed and expressed as complete (>70%), incomplete (>30% to < 70%) or none (<30%). (209)

Infarct Size

Initial Infarct Size Assessment by Peak Troponin Level

Troponin is a protein complex comprising three subunits (I, C, and T) that modulates the calcium-mediated interaction between actin and myosin in skeletal and cardiac muscle tissue. Circulating levels of highly sensitive cardiac troponin released in response to myocytes necrosis/damage correlate with infarct size and inversely with ejection fraction. Additionally, it predicts clinical outcomes after STEMI. (210) A study was conducted to investigate the use of peak and serial 6-hour fixed-time highly sensitive cardiac troponin to estimate infarct size expressed as the 48-hour cumulative creatine kinase release, left ventricular function at three months assessed using the echocardiographic wall motion score index and left ventricular ejection fraction using radionuclide ventriculography in 188 patients with STEMI. All-cause death, implantable cardioverter-defibrillator implantation, or hospitalization for heart failure, were recorded at 12 months. The cardiac sensitive troponin value at 24 hours after the onset of symptoms showed the highest correlation ($p < 0.001$ for all) and had the nearest associations with all outcomes. (211)

In this study, we used cardiac troponin T (cTnT) (Roche, Rotkreuz, Switzerland) as a surrogate endpoint for initial infarct size. A blood sample was taken 24-36 hours following primary PCI to determine peak high sensitivity troponin T levels.

Final Infarct Size Assessment by Cardiac Magnetic Resonance Imaging

Cardiac magnetic resonance is increasingly emerging as a gold standard imaging modality and investigation of choice for the assessment of various functional and structural aspects of the heart such as ventricular function, myocardial perfusion at rest and stress, and myocardial viability in patients with acute myocardial infarction. It has a vital role in the assessment of ventricular function over time due to its reproducibility and the high spatial resolution. (212) High spatial resolution and multislice imaging are features of modern CMR scan, which enable accurate tissue characterisation, quantification, and localization of

coronary microvascular obstruction and infarct size. There is a good correlation between coronary microvascular obstruction detected on CMR and microvascular obstruction diagnosed by other means such as myocardial contrast echocardiography and coronary angiography. Cardiac Magnetic Resonance can accurately measure myocardial infarct size, left ventricular volumes, myocardial salvage, microvascular obstruction and myocardial oedema (area at risk). (213-217) Myocardial perfusion can be assessed after administration of gadolinium contrast. Gadolinium is a metallic element (atomic number 64) that can be chelated into paramagnetic agents. It is useful as a magnetic resonance imaging agent because it has seven unpaired electrons and enhances nuclear relaxation rate. Gadolinium molecules reduce spin-lattice relaxation time (T1) of voxels in which they are present. Therefore, they have a brighter signal on T1-weighted images. (218) Gadolinium is an extracellular tracer that can cross the vascular walls into the extracellular space. Within the context of acute myocardial infarction, gadolinium accumulates in the fibrotic interstitium. It also crosses the membrane of the myocardial cells that sustained ischaemic injury. Coronary microvascular obstruction hinders blood flow and perfusion and therefore can be identified on CMR as hypo-enhanced (reduced signal intensity) areas in the central part of the hyper-enhanced area (increased signal intensity), within 1-2 minutes of gadolinium contrast injection (early gadolinium enhancement). (219) Myocardial infarct characteristics such as infarct size and location can be assessed on CMR approximately 10–20 minutes after gadolinium administration (late gadolinium enhancement). (199) CMR can detect the presence of intramural haematoma, which tends to be the consequence of microvascular injury in humans. (220)

CMR Technique

CMR imaging was conducted three months following acute STEMI to assess final infarct size. Patients were studied using a 1.5 Tesla scanner (Philips Ingenia) with a standard 12-channel matrix coil configuration. For cine imaging, steady-state free precession (SSFP) CMR images were acquired during short breath holds in the short axis, two chamber, and four chamber planes; on short-axis images, the left ventricle was assessed from the base to the apex acquiring a total of 10-12 images. Cine SSFP images were obtained using the

following parameters: TR 51.3ms, TE 1.21ms, flip angle 80º, 8mm slice thickness, no inter-slice gap, a matrix of 256x256, a field of view ranging from 340mm to 400mm and a voxel size of 1.7x1.7x8.0mm. For late gadolinium enhancement imaging, a standard inversion recovery gradient echo sequence was adopted. Acquisition planes were acquired at least 10 minutes following administration of 0.1 ml/kg gadobutrol (Gadovist, Bayer Schering Pharma). Typical sequence parameters were obtained using an inversion recovery prepared breath-hold gradient-echo technique. Standard image parameters were TR 700ms, TE 4.33ms; matrix 256×256; flip angle 30°; slice thickness 8.0mm, no inter-slice gap, and voxel size 1.7x1.4x8 mm. The inversion time was progressively optimized to null normal myocardium. Images were acquired on short axis planes covering the entire left ventricle.

Image Analysis

All measurements were performed by two observers (EM and SH, level 3 SCMR) blinded to clinical and angiographic data. Where a discrepancy of >10% was evident between reports, the final figure was reached by consensus. Image analysis for LV volumes and function and LV mass were performed using a semi-automated software (CMR42 Circle Cardiovascular Imaging, Canada, a certified CMR analysis software). Endocardial and epicardial borders were delineated manually and propagated to the end systolic and end diastolic frames. Slices were included as ventricular if at least 50% of the slice contained LV myocardium. Papillary muscles were excluded from the endocardial border and included within the ventricular blood pool. Hypo-intense areas in late gadolinium enhancement images (representing intramyocardial haemorrhage and microvascular obstruction, respectively), when present, were included within the contoured areas. For infarct size analysis, manual contouring was adopted. Infarct size was expressed as a % LV mass. (Figure 21)

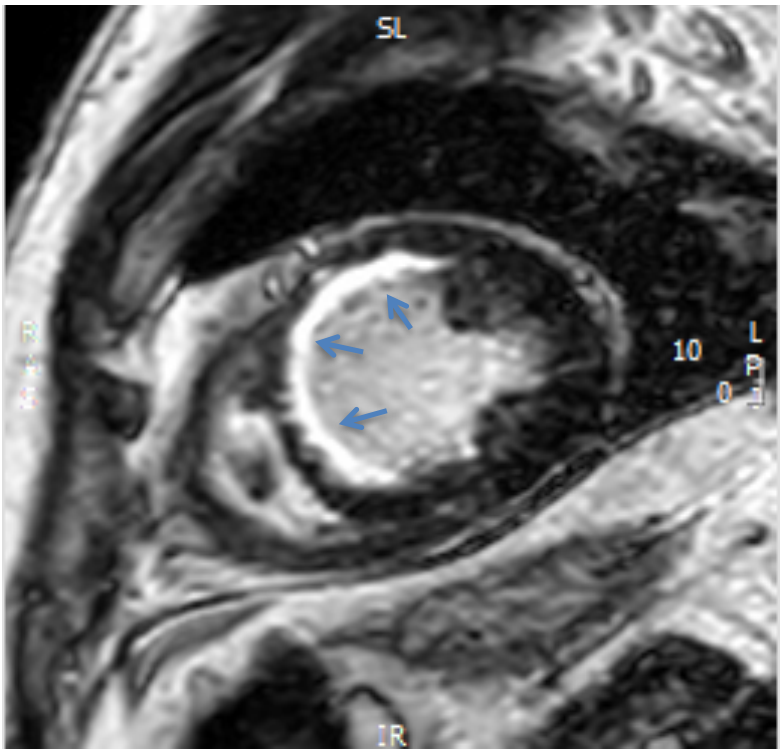


Figure 22 Myocardial infarct on CMR

Myocardial infarct in the left ascending coronary artery territory (arrow) in a study patient with anterior STEMI, after late gadolinium enhancement.

Additional Analysis

Correlation between Coronary Arterial dp/dt measured by Intracoronary Pressure Wire and Final Infarct Size

The pressure wire data (Certus, Abbott Vascular) was re-analysed and recorded as part of a separate study investigating the impact of STEMI on IMR and CFR. The off-line analysis of high fidelity coronary arterial pressure traces was performed using Coroventis (Uppsala, Sweden) software, to allow measurement of peak systolic contractility (dp/dt max) and peak diastolic relaxation (dp/dt min) as well as the time constant of isovolumic relaxation (Tau) within the culprit coronary artery immediately following successful primary PCI at a sampling frequency of 100Hz.

Safety Endpoints

Bleeding events were prospectively assessed using the Bleeding Academic Research Consortium (BARC) criteria during the index admission. (119)

Results

Patients Characteristics

Patients, treatment, and procedure characteristics are described in tables 4 and 5. Two hundred twenty-six patients presenting with first acute STEMI were screened for eligibility between the 21st of July 2016 and 28th of January 2017. One hundred and nine patients did not meet the inclusion criteria and were therefore excluded from enrolment. (Figure 14) Out of 109, 42 patients had already received P2Y12 inhibitor before arrival at the Heart and Lung centre, and 37 patients had previous myocardial infarction. Other reasons for not meeting the inclusion criteria were cardiogenic shock (n=13), being on oral anticoagulation agents (n=8), lacking the capacity to provide consent (n=4), history of significant bleeding (n=3) and renal failure requiring dialysis (n=2). Hundred and seventeen patients were randomised to gain a full treatment cohort of 100. A total of 17 patients were withdrawn from the study due to the use of GPIIb/IIIa inhibitors during PCI (n=11), extreme clinical instability (n=2) or the presence of an alternative diagnosis (myocarditis n=2; Takotsubo cardiomyopathy n=2). Of the 17 withdrawn, one patient died after withdrawal from the study and before hospital discharge. After these exclusions/withdrawals, 100 randomised patients were included for analysis (cangrelor n=50, ticagrelor n=50). All patients received P2Y12 inhibition as per protocol. Of the 100 patients, 90 patients were assessed for microvascular dysfunction using IMR (ticagrelor n=45 cangrelor n=45) with haemodynamic instability precluding measurement in 10 subjects (cangrelor n=5, ticagrelor n=5). The angiographic analysis was performed on all subjects and CMR at three months was performed entirely in 75 subjects (cangrelor n=37, ticagrelor n=38). Reasons for not conducting CMR included contraindication due to renal failure (n=2), lengthy intensive care unit stay (n=1), procedure intolerance/ claustrophobia (n=2) and 20 patients failed to attend booked appointments to have CMR imaging (Figure 14). One patient underwent an emergency in-patient CABG operation and mitral valve replacement necessitating a prolonged admission. Two patients became haemodynamically unstable and needed the

insertion of intra-aortic balloon pump (IABP). Of the full analysis set, all 100 patients survived to discharge.

Table 4 Patients characteristics

	Cangrelor	Ticagrelor	P value
Characteristic	(n = 50)	(n = 50)	
Demographics			
Age, years	61.2 ±13.9	63.4 ± 12.9	0.41
Males	39 (78)	33 (66)	1.00
BMI, kg/m^2	28.5 ± 6.3	28.1 ± 5.7	0.73
Smoking status			
Current smoker	26 (52)	24 (48)	0.84
Former smoker	7 (14)	9 (18)	0.53
Medical history			
CVD Family history	18 (36)	13 (26)	0.42
Diabetes	10 (20)	9 (18)	0.79
Hypertension	20 (40)	24 (48)	0.65
Hyperlipidaemia	13 (26)	7 (14)	0.15
Previous MI	0	0	0
Previous CABG	0	0	0

Previous TIA/CVA	0	0	0
Previous PCI	1 (2)	3 (6)	0.27
Pre-infarct angina	3 (6)	1 (2)	0.027
Admission blood tests			
Haemoglobin, g/L	138.1 ± 16.6	138.4± 19.4	0.93
Neutrophils, 10^9 g/L	9.6 ± 3.7	9.2 ± 3.5	0.57
Platelet count, 10^9 g/L	245.8 ± 66.0	255.5 ± 66.1	0.46
WCC, 10^9 g/L	12.4 ± 4.4	12.0 ± 3.8	0.62
Angiographic variables			
MI Localisation			
Anterior	13 (26)	18 (36)	0.42
Inferior	32 (64)	29 (58)	0.80
Infero-lateral	0	1 (2)	
Lateral	4 (8)	1 (2)	0.011
Posterior	1 (2)	1 (2)	1.00
Culprit Vessel			

LMS	0	1 (2)	
LAD	15 (30)	16 (32)	0.87
LCX	8 (16)	6 (12)	0.48
INT	0	1 (2)	
RCA	27 (54)	26 (52)	0.92
Number of vessels diseased			
0	0	1 (2)	
1	11 (22)	17 (34)	0.29
2	16 (32)	14 (28)	0.74
3	23 (46)	18 (36)	0.54
Number of vessels treated			
0	1 (2)	1 (2)	1.00
1	42 (84)	47 (94)	0.77
2	7 (14)	2 (4)	0.017

Values are mean ± SD, n (%) or median (IQR) as appropriate. IQR = interquartile range; SD = standard deviation; BMI = body mass index; CABG = coronary artery bypass graft; CVA = cerebrovascular accident; CVD = cardiovascular disease; LMS = Left main stem; LAD = left anterior descending coronary artery; LCX = left circumflex artery; INT = Intermediate coronary artery; RCA = right coronary artery; MI = Myocardial Infarction; PCI = percutaneous coronary intervention; TIA = transient ischaemic attack; WCC = white cell count.

Table 5 Treatment and procedure characteristics

	All	Cangrelor	Ticagrelor	P value
Characteristic	(n = 100)	(n = 50)	(n = 50)	
Call to balloon time, minutes	121 [102, 140]	116 [100, 136]	126 [106, 150]	0.16
Door to balloon time, minutes	57 [45, 71]	53 [45, 71]	59 [44, 70]	0.83
Treatment duration, minutes	28 [20, 37]	24 [12, 30]	33 [23, 48]	<0.001
Ischaemia duration, minutes	192 [143, 289]	164 [133, 233]	195 [148, 345]	0.26
Morphine given	77 (77)	37 (34)	40 (80)	0.48
Total Heparin, units	8000 [6500, 10000]	8500 [5000, 10000]	8000 [7000, 10000]	0.83
Total length of stent, mm	42.1 ± 22.1	43.2 ± 22.9	40.8 ± 21.4	0.59
Thrombectomy	14 (14)	7 (14)	7 (14)	1.00

Values are mean ± SD, n (%) or median [IQR] as appropriate, IQR = interquartile range. P-values are from Two-sample t test, Wilcoxon sum rank test or Chi-squared test as appropriate.

Primary Endpoints

Platelet Inhibition

$P2Y_{12}$ inhibition by VerifyNow Assay

Compared to ticagrelor, cangrelor produced more significant P2Y12 inhibition at balloon inflation time during primary PCI. This difference was no longer apparent at 4 and 24-36 hours after study drug administration. (Table 6 and Figures 23-26)

While there was a slight numerical increasing PRU in patients within the cangrelor group after transitioning to ticagrelor (cangrelor 145.2 ± 50.6 to 158.1±92.1), this was not statistically significant. At balloon inflation time, 45 out of 50 (90%) cangrelor-treated patients achieved an optimal PRU (<208 units). Only 11 out of 50 (22%) ticagrelor treated subjects were in range (P= <0.0001). At 4 hours following drug dosing, 15 out of 50 ticagrelor-treated patients (30%) and 35 out of 50 cangrelor-treated patients (70%) showed high-on treatment platelet reactivity. At 4 hours following drug dosing, 15 out of 50 ticagrelor-treated patients (30%) and 20 out of 50 cangrelor-treated patients (40%) had PRU values above 208, indicating high-on treatment platelet reactivity (HPR).

Correlation between PRI and PRU Values

No significant correlation was seen between PRI and PRU values as measured by VerifyNow and VASPFix assays at the three-time points. (Table 8)

Timings

The time of treatment initiation (study drug loading) to coronary balloon inflation time was measured in both cangrelor and ticagrelor groups. The preparation time of IV cangrelor was longer than that for oral ticagrelor; this resulted in a shorter cangrelor initiation-coronary balloon inflation time and a longer ticagrelor initiation-coronary balloon inflation time (23.0±12.8 minutes for cangrelor vs 36.3±16.9 for ticagrelor; P= <0.0001).

Table 6 Platelet inhibition

Characteristic	All (n = 100)	Cangrelor (n = 50)	Ticagrelor (n = 50)	P value
PRU at balloon inflation time	196.7 ± 73.8	145.2 ± 50.6	248.3 ± 55.1	<0.001
PRU at 4 hours	144.8 ± 93.0	158.1 ± 92.1	131.2 ± 92.9	0.15
PRU at 24-36 hours	60.5 ± 52.9	61.0 ± 50.0	60.1 ± 56.3	0.93

Values are mean ± SD. P-values are from Two-sample t-test. PRU= $P2Y_{12}$ reaction units; SD = standard deviation.

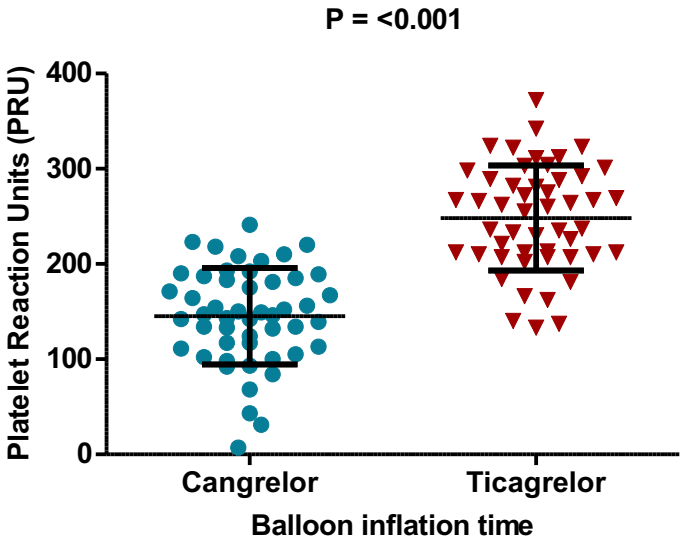


Figure 23 Degree of P2Y12 inhibition at coronary balloon inflation time

PRU=Platelet Reaction Units.

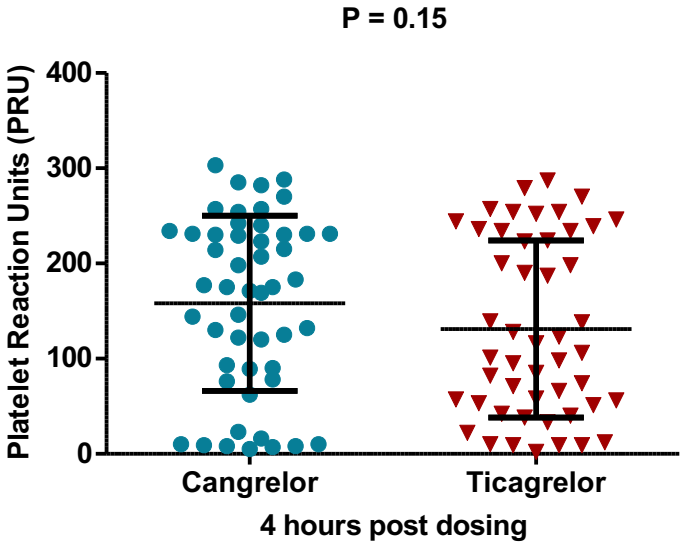


Figure 24 Degree of P2Y12 inhibition 4 hours post study drug dosing

PRU=Platelet Reaction Units.

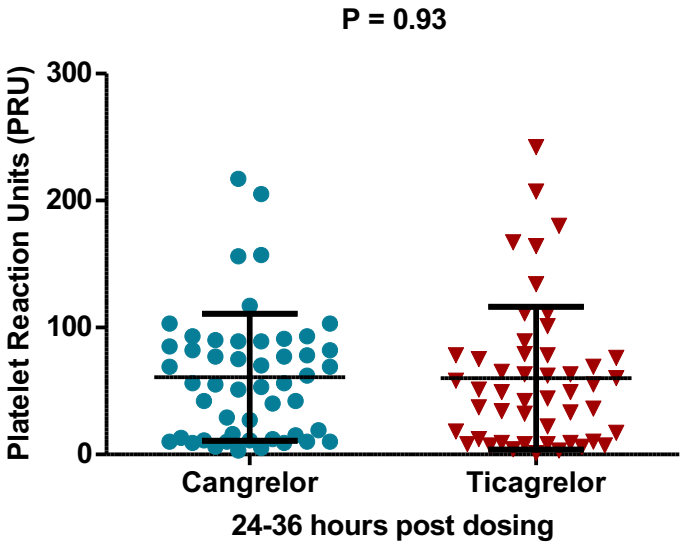


Figure 25 Degree of P2Y12 inhibition 24-36 hours post study drug dosing

PRU=Platelet Reaction Units.

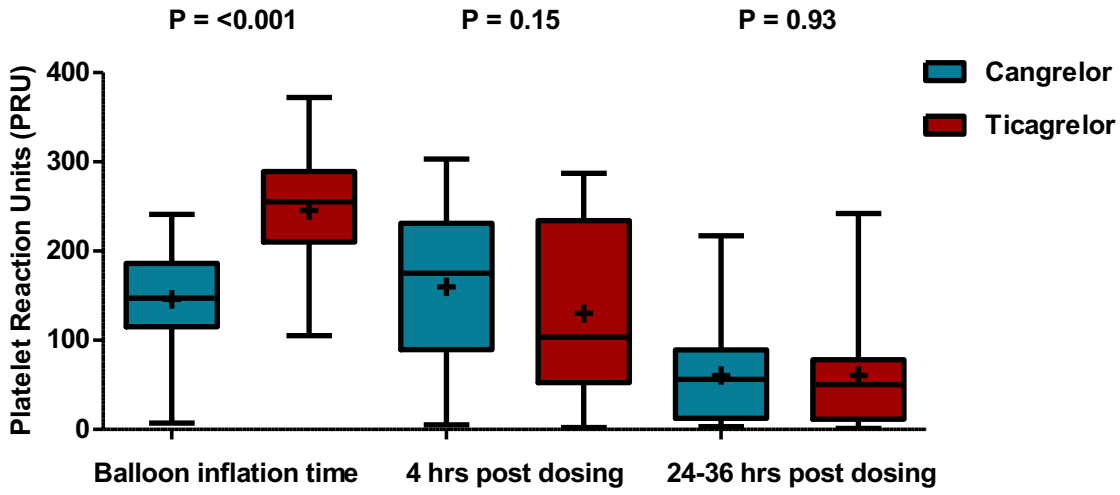


Figure 26 Degree of P2Y12 inhibition at 3 time points

PRU=Platelet Reaction Units.

Table 7 VASPFix PRI

Characteristic	Statistic	All (n = 100)	Cangrelor (n = 50)	Ticagrelor (n = 50)	P value
PRI at balloon inflation time	N	21 (79)	13 (37)	8 (42)	0.0082
	Median [IQR]	54 [32, 73]	34 [18, 56]	78 [62, 92]	
PRI at 4 hours	N	22 (78)	16 (34)	6 (44)	0.49
	Median [IQR]	47 [25, 71]	44 [22, 66]	52 [35, 73]	
PRI at 24-36 hours	N	19 (81)	9 (41)	10 (40)	1.00
	Median [IQR]	14 [9, 22]	15 [10, 19]	13 [8, 28]	

P-values are from Wilcoxon sum rank test. PRI = platelet reactivity index; IQR = interquartile range.

Table 8 Correlation between PRI and PRU values

Characteristic	All (n = 100)			Cangrelor (n = 50)			Ticagrelor (n = 50)		
	N	Correlation coefficient	P value	N	Correlation coefficient	P value	N	Correlation coefficient	P value
PRI at balloon inflation time	21	0.357	0.11	13	-0.489	0.090	8	0.071	0.87
PRI at 4 hours	22	0.386	0.076	16	0.601	0.014	6	-0.143	0.79
PRI at 24-36 hours	18	0.029	0.91	9	-0.603	0.086	9	0.109	0.78

Values are from Spearman's correlations. PRI = platelet reactivity index; PRU = $P2Y_{12}$ reaction units; IQR = interquartile range.

Secondary Surrogate Endpoints

Index of Microvascular Resistance and Coronary Flow Reserve

At the end of primary PCI, invasive coronary physiology parameters were measured in 90 infarct-related arteries (cangrelor n=45/50, ticagrelor n=45/50) with haemodynamic instability precluding measurement in 10 patients. The mean time from the loading of study drugs to IMR measurement was 82 minutes in the cangrelor arm and 96 minutes in ticagrelor arm. Intravenous cangrelor was not superior to oral ticagrelor in improving microcirculatory reperfusion in patients presenting with STEMI undergoing primary PCI as assessed by IMR. The mean IMR was 29±21, and CFR was 1.71±0.90. There was statistically no significant difference between cangrelor and ticagrelor in mean IMR values (30±22 for cangrelor vs 28±21 for ticagrelor; p= 0.52). This difference in effect remained insignificant when a cut off value of ≤ 40 or > 40 was applied. No significant difference was observed in mean CFR values (1.57±0.66 for cangrelor vs 1.85±1.08 for ticagrelor; p= 0.14) between the two treatment groups. Also, there was no significant difference in mean RRR, mean FFR and mean Pd/Pa values between the two treatment arms. (Table 7, Figure 27).

Table 9 Coronary physiology and angiographic parameters

Characteristic	All	Cangrelor	Ticagrelor	P value
	(n = 90)	(n = 45)	(n = 45)	
IMR	29 [21,51]	30 [22, 58]	28 [21, 40]	0.52
IMR				0.11
≤ 40	61 (68)	27 (60)	34 (76)	
> 40	29 (32)	18 (40)	11 (24)	
CFR	1.71 (0.90)	1.57 (0.66)	1.85 (1.08)	0.14
CFR				0.24
≤2.0	65 (72)	35 (78)	30 (67)	
> 2.0	25 (28)	10 (22)	15 (33)	
RRR	2.00 (1.01)	1.82 (0.72)	2.18 (1.22)	0.092
FFR	0.92 (0.09)	0.94 (0.09)	0.90 (0.09)	0.10
Pd/Pa	0.96 (0.07)	0.97 (0.07)	0.95 (0.07)	0.22
	(n = 99)	(n = 50)	(n = 49)	
Corrected TIMI frame count	21.5 (12.3)	21.7 (14.2)	21.4 (10.2)	0.93

TIMI flow grade				0.27*
1	2 (2)	2 (4)	0 (0)	
2	17 (17)	10 (20)	7 (14)	
3	80 (81)	38 (76)	42 (86)	
Myocardial perfusion grade				0.54*
0	3 (3)	2 (4)	1 (2)	
1	8 (8)	6 (12)	2 (4)	
2	24 (24)	11 (22)	13 (27)	
3	63 (64)	31 (62)	32 (67)	

Values are mean ± SD, n (%) or median [IQR] as appropriate. P-values are from Two-sample t-test, Wilcoxon sum rank test or Chi-squared test as appropriate. * Fisher Exact Test. CFR= coronary flow reserve; FFR= fractional flow reserve; IMR= index of microvascular resistance; IQR= interquartile range; PD/PA = distal pressure/aortic pressure; RRR= resistance reserve ratio.

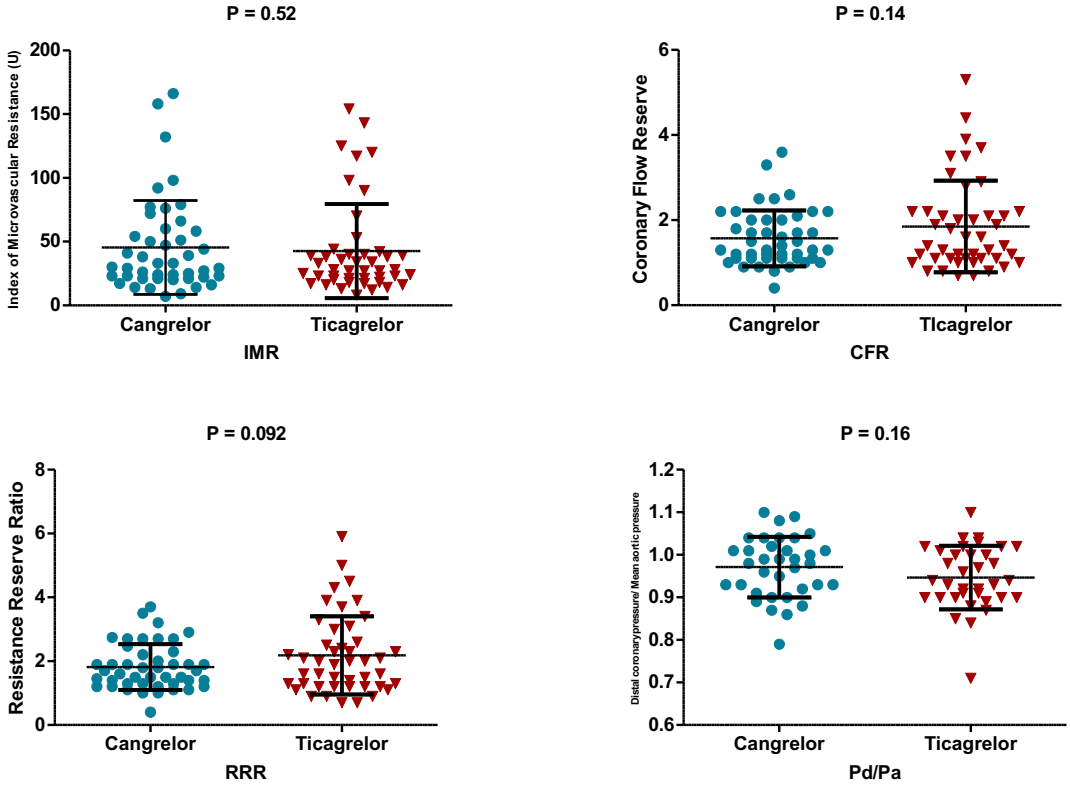


Figure 27 Coronary physiology parameters

Angiographic Analysis

Following PCI, angiographic and ECG analysis was conducted in all 100 patients. There was no significant difference in the incidence of MPG 3 (cangrelor n=31/50 vs ticagrelor n=32/50; p=0.54) and TFG 3 (cangrelor n=38/50 vs ticagrelor n=42/50; p=0.27) between the cangrelor and ticagrelor treatment groups. Likewise, there was no difference in the occurrence of CTFC (21.7±14.2 for cangrelor vs 21.4±10.2 for ticagrelor; p=0.93). (Figure 28)

ECG Analysis

There were no significant differences in ST-segment resolution between cangrelor and ticagrelor groups, measured 90-120 minutes following PCI (Table 8, Figure 29).

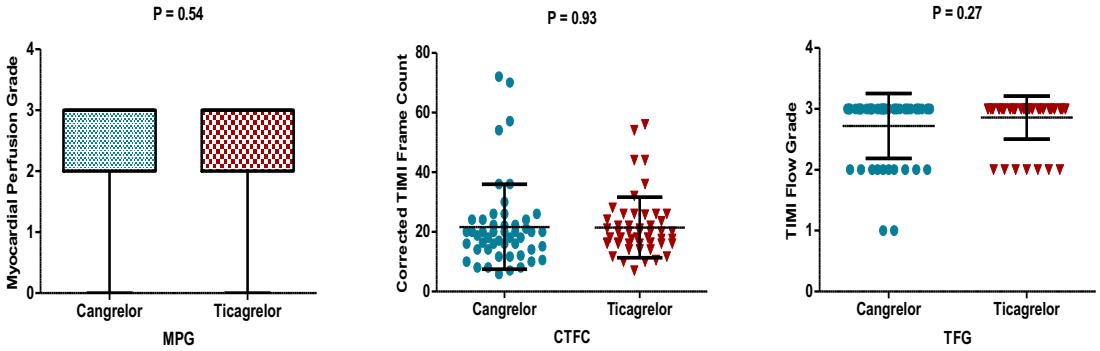


Figure 28 Angiographic parameters

Table 10 Degree of ST-segment resolution

ST-segment resolution %	Cangrelor %	Ticagrelor %
Complete (C)	32	36
Partial (P)	11	7
None (N)	7	7

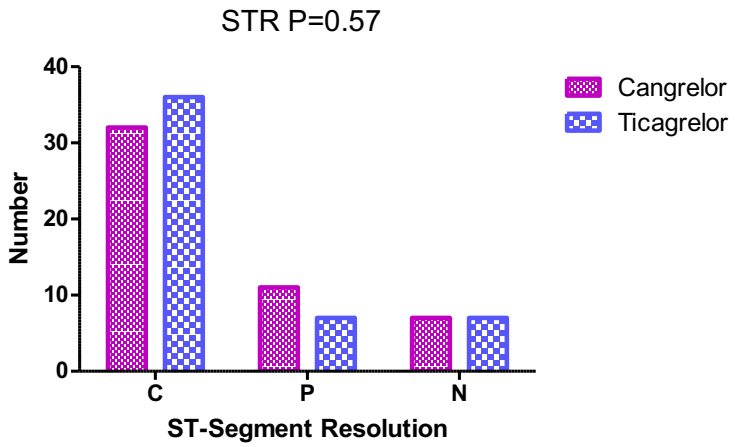


Figure 29 ST-segment resolution

Myocardial Infarct size

Infarct Size by Peak Troponin

Peak troponin levels at 24-36 hours following study drug administration did not differ significantly between the two treatment groups indicating no clear benefit of cangrelor over ticagrelor in relation to infarct size by peak troponin levels (Table 9 and Figure 30).

Infarct Size by Cardiac Magnetic Resonance

Cardiac magnetic resonance results are displayed in table 9 and figure 30. Cardiac magnetic resonance was performed at a median of 13 weeks after primary PCI in both groups. Infarct was demonstrated on late gadolinium enhancement in 68 out of 75 (90.6%) patients who had CMR imaging. Out of 68 patients, infarct was shown in 31 patients (45.5%) and 37 patients (54.4%) in the cangrelor and ticagrelor groups, respectively. No significant difference was observed in the total infarct size as a percentage of LV mass between the two treatment groups (cangrelor 13.7 (7.7,17.5), ticagrelor 10.9 (6.6,17.5), Median, (IQR); p=0.61). Similarly, left ventricular ejection fraction was not different (cangrelor 56.50 (47.50,59.25), ticagrelor 55 (44.50,61.50) median, (IQR); p=0.96).

Table 11 Infarct size by CMR and peak troponin levels

	All	Cangrelor	Ticagrelor	P value
Characteristic	(n = 64)	(n = 29)	(n = 25)	
Infarct size (CMR)	12.4 [7.0,17.8]	13.5 [6.6, 16.4]	12.3 [7.4, 24.2]	0.32
Infarct size by territory				0.50
LAD	26 (41)	10 (34)	16 (46)	
LCX	6 (9)	4 (14)	2 (6)	
RCD	32 (50)	15 (52)	17 (49)	
Infarct size (Peak Troponin, ng/L)	29556 [13879, 58988]	37169 [14230, 56740]	23896 [13663, 66565]	0.84

Values are median [IQR] or n (%). P-values are from Two-sample t-test or Fisher Exact test. IQR = interquartile range; SD = standard deviation; LAD = left anterior descending artery; LCX = left circumflex artery; RCA = right coronary artery;

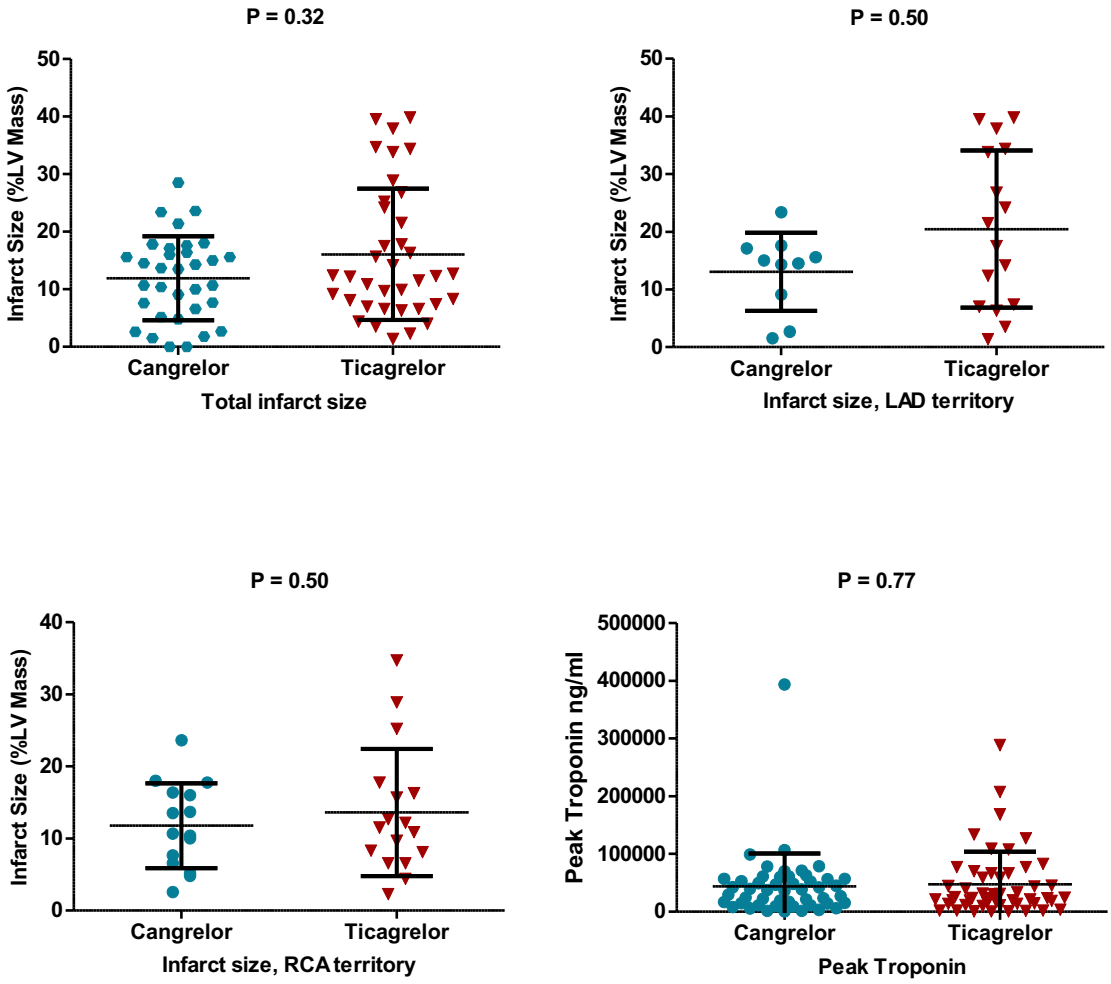


Figure 30 Infarct size by CMR and peak troponin levels

Additional Analysis

Effect of Morphine administration on $P2Y_{12}$ Inhibition

Morphine for pain relief was administered to 37 out of 50 (74%) cangrelor-treated patients, at an average dose of 9.7mg, and to 40 out of 50 (80%) ticagrelor-treated patients, at an average dose of 8.5mg. The mean time from morphine administration to study drug loading was 60 minutes in the cangrelor arm and 55 minutes in the ticagrelor arm. The administration of morphine did not influence the degree of P2Y12 inhibition at the time of coronary reperfusion in either of the treatment groups. (Table 10)

Effect of $P2Y_{12}$ Inhibition on Microcirculation and Infarct Size

Potent early P2Y12 inhibition achieved with cangrelor was not associated with improved microvascular function as measured by IMR or reduced infarct size as assessed by CMR at three months**.** (Tables 11 and 12)

Correlation between IMR values and Infarct Size at 3 months

There was no correlation between coronary microcirculation determined by IMR and infarct size assessed by CMR at three months in the context of primary PCI. (Table 13)

Correlation between Coronary Arterial dp/dt measured by Intracoronary Pressure Wire and Final Infarct Size

The result of this analysis is described in figure 31. Fifty-six STEMI naïve patients (12 female), mean age 61, undergoing primary PCI were assessed. The culprit vessel was LMS in one patient, LAD in twenty-three, LCx in seven and RCA in twenty-five. No patients had significant aortic stenosis. Following primary PCI, pressure wire generated dp/dt traces were obtained from the pressure sensor in the distal coronary artery before IMR and CMR estimation. Four consecutive cardiac cycles were analysed for dp/dt max, dp/dt min and Tau and the mean used for analysis. The diastolic parameter, dp/dt min was positively correlated with final infarct size on

CMR at three months (R=0.35, P=0.008), whereas the systolic parameter dp/dt max was not (R=0.18, P=NS). Tau similarly was associated with final infarct size (R=0.08, P=NS). Interestingly, the measured pressure wire derived parameters were not correlated to the CMR determined left ventricular ejection fraction (LVEF).

In this study, it is likely that the coronary pressure traces reflect central aortic pressure and thereby left ventricular contractility. Caution should be used; however, as a number of confounding variables are added between the left ventricular pressure development, aortic trace and then coronary arterial trace. There is evidence however that peripheral arterial dP/dt parameters mirror left ventricular contractility indices well, and our data suggest that this might be the case, given the association seen between infarct size and dP/dt min. Previous studies have shown that left ventricular ischaemia has an earlier and more profound impact on left ventricular diastolic function than systolic contraction, a finding that appears to be borne out by this study. Left ventricular ejection fraction is influenced by several parameters including loading conditions, infarct size and left ventricular remodelling, and therefore the finding that dP/dt min correlated with final infarct size (indicating the size of the initial ischaemic insult) but not LVEF could be in part related to these factors.

Table 12 Comparison of morphine administration, by treatment group and overall

Variable	Statistic	Cangrelor (n =50)	Ticagrelor (n =50)	All (n=100)	P-value*
Morphine given	N (Missing)	50 (0)	50 (0)	100 (0)	0.48
	Yes	37 (74.00%)	40 (80.00%)	77 (77.00%)	
	No	13 (26.00%)	10 (20.00%)	23 (23.00%)	

*P-value from chi-squared test

Table 13 Comparison of PRU1 and IMR values

Variable	Statistic	IMR <=40	IMR > 40	All IMR	P-value*
PRU1	N (Missing)	61 (0)	29 (0)	90 (0)	0.27
	<=180	22 (36.07%)	14 (48.28%)	36 (36.00%)	
	>180	39 (63.93%)	15 (51.72%)	54 (54.00%)	
PRU1	N (Missing)	61 (0)	29 (0)	90 (0)	0.038
	<=208	30 (49.18%)	21 (72.41%)	51 (51.00%)	
	>208	31 (50.82%)	8 (27.59%)	39 (39.00%)	
PRU1	N (Missing)	61 (0)	29 (0)	90 (0)	0.041
	<=235	40 (65.57%)	25 (86.21%)	65 (65.00%)	
	>235	21 (34.43%)	4 (13.79%)	25 (25.00%)	

*P-value from chi-squared test

Table 14 Comparison of PRU1 and infarct size by CMR at 3 months

	All	PRU ≤ 208	PRU > 208	P value
Characteristic	(n = 70)	(n = 40)	(n = 30)	
Infarct Size	12.4 [7.0, 17.8]	13.6 [6.6, 17.4]	12.2 [7.4, 21.5]	0.66

Values are median IQR. IQR = interquartile range. P-values are from Wilcoxon sum rank test.

Table 15 Correlation between IMR values and infarct size at 3 months

	All	IMR ≤ 40	IMR > 40	P value
Characteristic	(n = 68)	(n = 47/45)	(n = 21/45)	
Infarct Size	12.4 [6.8, 17.8]	10.9 [4.8, 17.8]	15.6 [10.4, 17.6]	0.090

Values are median IQR. IQR = interquartile range. P-values are from Wilcoxon sum rank test.

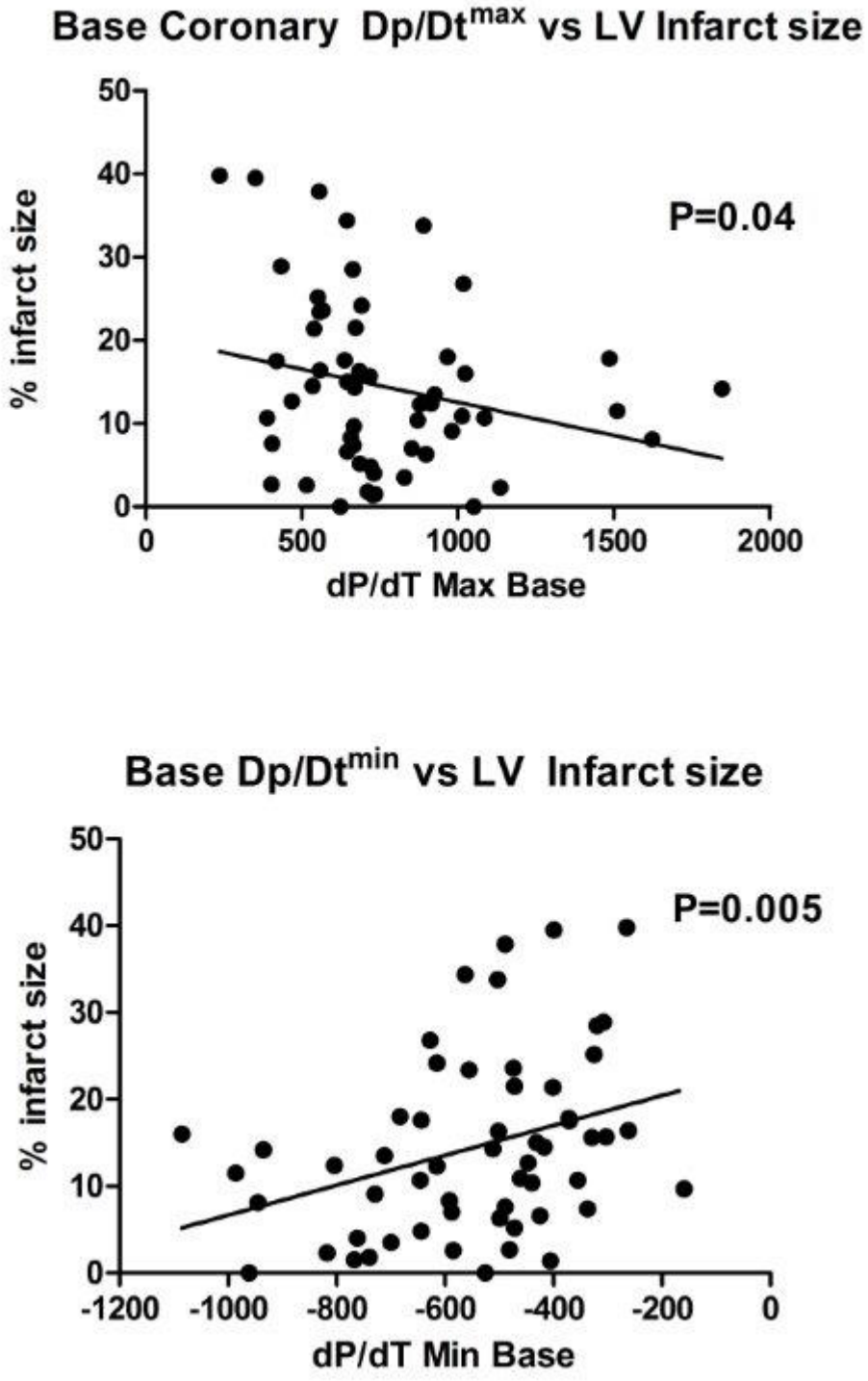


Figure 31 Correlation between coronary dp/dt and left ventricular infarct size

Safety Endpoints

There were no study-related complications from drug administration or pressure wire studies. Two out of 50 cangrelor-treated patients and 3 out of 50 ticagrelor-treated patients developed haematoma at the radial access site around 20-50 minutes following primary PCI (Type 2 BARC). This was managed conservatively and required no surgical intervention in either of the treatment arms. One patient in the ticagrelor arm developed limiting shortness of breath two days after initiation necessitating replacement with clopidogrel, which resulted in complete resolution of symptoms.

Discussion

This is the first prospective randomised controlled trial assessing the effect of IV cangrelor compared to oral ticagrelor on P2Y12 inhibition, coronary microcirculation and infarct size in patients with acute STEMI undergoing primary PCI. The main findings are the following:

Firstly, cangrelor, compared to ticagrelor, produced a more significant platelet inhibition at the time of infarct-related coronary artery reperfusion during primary PCI. Coronary reperfusion by primary PCI in over three-quarters of study participants randomised to oral ticagrelor was performed with suboptimal antiplatelet effect at that time.

Secondly, IV cangrelor was not superior to oral ticagrelor in improving coronary microcirculatory reperfusion in patients presenting with STEMI undergoing primary PCI as assessed by IMR and CFR. No difference was detected between the two treatment groups in terms of angiographic markers of coronary reperfusion and STR. Also, no significant difference was seen between the two treatment groups in infarct size assessed by peak troponin levels and CMR 3 months following MI. Similarly, left ventricular ejection fraction was not significantly different.

These results support our hypothesis that IV cangrelor, when compared with oral ticagrelor, will yield more significant platelet inhibition at the time of coronary reperfusion by primary PCI. Our study results, however, are not consistent with the hypothesis that more significant early platelet inhibition will lead to improved microcirculatory function/perfusion, and result in reduced myocardial infarct size.

ST-segment elevation myocardial infarction is associated with a high degree of intrinsic platelet activation, the level of which is associated with the magnitude of both subsequent antiplatelet therapy effect and clinical outcomes. (221) Furthermore, PPCI is the coronary interventional procedure associated with the highest frequency of severe thrombotic complications and therefore, rapid, and consistent platelet inhibition is a crucial objective in STEMI management. (222)

Prasugrel and ticagrelor are potent and rapidly acting P2Y12 antagonists that reduce adverse ischaemic events in STEMI patients when compared with clopidogrel. (86)

However, there are several limitations associated with oral P2Y12 receptor antagonists such as the need for hepatic biotransformation, delayed onset of action, suboptimal platelet inhibition, and irreversible inhibition.

The therapeutic effect of the more potent oral P2Y12 antagonists, prasugrel and ticagrelor, is significantly delayed in the context of STEMI, and so PPCI is likely to be performed in the setting of sub-optimal P2Y12 inhibition. (86) We showed that over three-quarters of study participants randomised to oral ticagrelor have a suboptimal level of P2Y12 inhibition at the time of first coronary balloon inflation.

In the STEMI sub-analyses of both the TRITON-TIMI 38 (prasugrel) and PLATO (ticagrelor) trials, the incidence of early stent thrombosis (in the first 24 hours) was similar between groups, possibly implicating delayed onset of action for these oral agents. Sub-optimal early P2Y12 inhibition may also be implicated in the PLATO STEMI sub-set finding that ticagrelor did not improve post-PPCI STR and also that no increase in the incidence of post-procedural TIMI 3 flow was seen with this agent. (85, 223)

Attempts to circumvent this limitation of the oral route include upstream administration, (88) dose modification (224) and changes in the formulation such as crushing tablets before administration. (225)

Cangrelor, the rapidly acting potent intravenous P2Y12 inhibitor, might mitigate against the perceived failings of oral P2Y12 inhibition in the context of STEMI. It has been studied in three major clinical trials, each using clopidogrel as the comparator. CHAMPION PCI and CHAMPION PLATFORM both failed to meet their primary objective, whereas in the later CHAMPION PHOENIX trial, randomising 10,942 subjects with stable angina and ACS, cangrelor reduced the primary endpoint (a composite of death, MI, ischaemia driven revascularisation and stent thrombosis).

In a pooled analysis of patient-level data, cangrelor was superior to clopidogrel in reducing the primary endpoint of all-cause death, MI and ischaemia-driven

revascularisation at 48 hours (odds ratio [OR], 0.81, 95% confidence interval [CI], 0.71–0.91, p. 0.0007). Results in the 2,891 subjects treated for STEMI were consistent with this but did not reach significance (OR, 0.84, 95% CI, 0.55–1.27, p. 0.41).

Of interest, clopidogrel was given before PPCI in only 55.7% of subjects in this analysis. The rate of intraprocedural stent thrombosis in clopidogrel-treated patients was markedly higher than in the cangrelor-treated patients. (98) Many have questioned whether the early antiplatelet advantage seen with cangrelor versus clopidogrel would be seen if a more rapidly acting and a potent oral agent was used as the comparator and the results of our study inform this debate and add to our knowledge regarding the utility of early P2Y12 inhibition in the setting of PPCI.

The principal finding of our study—that cangrelor leads to more potent P2Y12 inhibition at the time of coronary balloon inflation during PPCI than oral ticagrelor lends support to its use for STEMI patients undergoing PPCI if an oral agent cannot be administered. Such circumstances are relatively common; examples include intubated patients having suffered out of hospital cardiac arrest, those with severe nausea and patients in whom the diagnosis is uncertain prior to angiography who might need early surgical intervention.

Despite the impressive pharmacodynamic results achieved with cangrelor in our study, the clinical importance of potent platelet inhibition in the early course of STEMI treatment with PPCI remains to be determined and requires further investigation in larger scale clinical trials.

Majority of patients receiving cangrelor will be eventually switched to an oral P2Y12 inhibitor. Two recently published studies have investigated the pharmacodynamic effect of cangrelor compared with different ticagrelor loading regimens during PPCI. In the first, 30 patients received ticagrelor loading prior to angiography and then were randomised in the catheter laboratory to either cangrelor or no additional antiplatelet treatment. (226) It showed significantly more potent P2Y12 inhibition (PRU) 15 minutes following loading in subjects treated with cangrelor. There was a suggestion, in this trial, of an increase in platelet reactivity following cessation of the cangrelor infusion with 4 out of 15 patients exhibiting an increase in PRU at 2 to 4 hours.

The loading regimen used was in contrast to our current study, where cangrelor was administered as monotherapy before angiography and during PPCI in the cangrelor arm, and the transition to ticagrelor occurred following PPCI, with the oral agent being given 30 minutes before cangrelor cessation.

In the CANTIC trial whereby 50 subjects received ticagrelor loading as crushed tablets at the time of randomisation to cangrelor or placebo, once again more potent P2Y12 inhibition was demonstrated in the cangrelor-treated patients, particularly at the primary endpoint time of 30 minutes. (227) Interestingly, in this study with assessment of P2Y12 inhibition at eight-time points, no increase in PRU was seen after the cangrelor infusion was stopped, suggesting no rebound in platelet activity and no drug-drug interaction. These findings are in line with our study of 100 STEMI patients in which no significant increase in P2Y12 inhibition was evident in the cangrelor-treated subjects, when measured at 4 hours after randomisation, following the transition from cangrelor to ticagrelor.

Despite the previously demonstrated drug-drug interaction between clopidogrel and cangrelor, prasugrel (and also ticagrelor) administered at the onset of the cangrelor infusion yielded high levels of P2Y12 inhibition soon after cangrelor cessation seemingly preventing a clinically relevant gap in platelet inhibition. (228)

We were unable to demonstrate a significant difference in the tested surrogate measures of STEMI outcome or in terms of final infarct size. The lack of apparent effect of early and potent P2Y12 inhibition during primary PCI on coronary microcirculation and infarct size could potentially have several explanations;

1-The effect of platelet inhibition could be small, and the study was not powered to detect benefit. The current study was not adequately powered for the secondary surrogate endpoints assessing PPCI success. These secondary outcome findings should be regarded as hypothesis generating, therefore.

2-The effect of IV cangrelor compared to ticagrelor could have been theoretically more prominent in high-risk patients such as those who have had STEMI complicated by cardiogenic shock or those with impaired conscious level. The 100 patients

included for full analyses were relatively stable and not in cardiogenic shock, limiting information on the comparative efficacy of cangrelor in this group of patients. This concept could be supported by the fact that benefits of pretreatment with GPIIb/IIIa inhibitors were observed in high-risk patients in early clinical trials.

3- Cangrelor compared to GPIIb/IIIa inhibitors might not appear to have benefits in patients with STEMI likely because GPIIb/IIIa inhibitors are more potent antiplatelet agents than cangrelor. GPIIb/IIIa inhibitors inhibit the final pathway of platelet aggregation by competing with von Willebrand factor and fibrinogen for GPIIb/IIIa receptor binding and thus inhibit platelets response to all agonists whereas cangrelor inhibit P2Y12 receptor only. Furthermore, whether GPIIb/IIIa inhibitors or cangrelor provide significant clinical antithrombotic effects in patients with marked platelet inhibition remains to be determined.

4-Effective platelet inhibition at the time of primary PCI might not be as vital as it has been perceived, and other mechanisms might be more critical. Independent of platelet inhibition, pathophysiological changes that occur during prolonged myocardial ischaemia and reperfusion injury might mask the benefits of potent early P2Y12 inhibition achieved by cangrelor. Furthermore, inflammation is being increasingly recognised as an essential player in the pathophysiology of STEMI. The recent CANTOS trial supports this. (229) This trial showed that canakinumab (a monoclonal antibody targeting interleukin-1β) at a dose of 150mg was superior to placebo at preventing adverse cardiac events in patients with a history of myocardial infarction and elevated high-sensitivity C-reactive protein.

5-Cangrelor could cause unrecognised effects that might mask the benefit of effective platelet inhibition.

The results of our study need to be taken with the context of three major cangrelor trials, CHAMPION PCI, CHAMPION PLATFORM, and CHAMPION PHOENIX studies. Cangrelor can help explore whether the lack of efficacy of pretreatment with oral P2Y12 inhibitors is due to poor absorption or lack of effect on P2Y12 pathway.

Clinical Implications

The more significant and rapid P2Y12 inhibition produced at the time of infarct-artery reperfusion demonstrated in our study supports the periprocedural administration of cangrelor in the setting of primary PCI as a bridging IV antiplatelet therapy until the full antiplatelet effect is achieved with oral P2Y12 receptor inhibitors. This approach would help overcome the main issue encountered with oral P2Y12 inhibitors in the setting of primary PCI, which is their delayed onset of action. Additionally, cangrelor can be an attractive strategy in other circumstances where achieving an immediate and potent antiplatelet is required, or the administration of oral P2Y12 inhibitors is not feasible. The following are examples of circumstances where cangrelor can be considered a reasonable alternative to oral P2Y12 inhibitors: haemodynamically unstable, intubated or sedated patients, patients with STEMI complicated by cardiogenic shock where intestinal absorption is reduced or when nausea or vomiting occur because of morphine administration. Cangrelor could also be beneficial during PCI-related complications. Additionally, it could be used as a bridging therapy if an emergency CABG operation is needed.

Conclusions

The currently available oral antiplatelet agents given in the setting of primary PCI have several limitations, delayed onset of action being the main issue. Cangrelor is an appealing treatment strategy that could achieve immediate and potent platelet inhibition during primary PCI. The results of our study, which is the first, randomised controlled trial comparing IV cangrelor with oral ticagrelor in patients with STEMI undergoing primary PCI, supports the periprocedural administration of cangrelor as a bridging IV antiplatelet therapy until the full antiplatelet effect is achieved with oral P2Y12 receptor inhibitors.

Despite the impressive pharmacodynamic results achieved with cangrelor in our study, the clinical significance of potent P2Y12 inhibition in the early course of STEMI treatment with primary PCI remains to be determined and requires further investigation in larger scale clinical trials.

We were unable to demonstrate a significant difference in the tested surrogate measures of STEMI outcome or in terms of final infarct size; therefore no definitive conclusions could be drawn from the results of surrogate endpoints, and they should be interpreted as hypothesis generating and can form the basis for larger future studies.

Study Limitations

The principal limitation of this study is its sample size. While adequately powered for the P2Y12 related endpoints, the results of surrogate endpoints do not provide convincing data and should be interpreted as hypothesis generating. Lack of baseline PRU values, prior to study drugs initiation is another study limitation, at an average of 36 minutes following ticagrelor loading, this result might not necessarily imply ticagrelor action and could represent baseline PRU values before ticagrelor initiation. The nature of the trial enrollment meant that the 100 patients included for full analysis were relatively stable, with no deaths seen in the analysed cohort, limiting the applicability of these findings to more unstable STEMI patients including those who present with cardiogenic shock or with impaired conscious level. The trial was an open-label randomised trial and therefore, subject to the risk of bias. To mitigate this risk, all surrogate endpoints were analysed by researchers blinded to the treatment allocation.

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Results P2Y12 inhibition at first balloon inflation time was significantly greater in cangrelor-treated patients (cangrelor P2Y12 reaction unit [PRU] 145.2 ± 50.6 vs. ticagrelor 248.3 ± 55.1). There was no difference in mean PRU at 4 and 24 to 36 hours post-dosing. IMR, final infarct size, angiographic and electrocardiographic measures of reperfusion were all similar between groups.

Conclusion Cangrelor produces more potent P2Y12 inhibition at the time of first coronary balloon inflation time compared with ticagrelor. Despite this enhanced P2Y12 inhibition, coronary microvascular function and final infarct size did not differ between groups.

Introduction

Coronary artery disease is the primary cause of premature mortality in the developed world and ST-elevation myocardial infarction (STEMI) is its most lethal acute manifestation.[1] Anti-platelet therapy with potent oral P2Y12 receptor antagonists improves outcomes in STEMI with both ticagrelor and prasugrel showing superior efficacy to clopidogrel.[2] One important limitation of all orally administered P2Y12 inhibitors is delayed anti-platelet effect, which can take several hours to achieve in the setting of STEMI.[3]

Cangrelor, an intravenous adenosine triphosphate analogue, has an onset of action of 1 to 3 minutes and does not require metabolic transformation to become fully active. It induces marked platelet inhibition very rapidly and has a plasma half-life of just 3 to 6 minutes. Three large randomized trials have compared its use to oral clopidogrel.[2] The CHAMPION PHOENIX showed cangrelor reduced the combined endpoint of death, myocardial infarction (MI), ischemia driven re-vascularization or stent thrombosis at 48 hours when compared with clopidogrel. The notion that earlier more potent P2Y12 inhibition will benefit patients undergoing primary percutaneous coronary intervention (PPCI) is biologically plausible and is supported by the current American College of Cardiology/American Heart Association/European Society of Cardiology guidelines for acute coronary syndrome (ACS), which give a class 1 recommendation for early treatment with a P2Y12 inhibitor.[2]

A recent randomized pharmacodynamics study has assessed the anti-platelet effect of cangrelor versus ticagrelor plus cangrelor at the time of PPCI in 30 patients, showing enhanced early P2Y12 inhibition in patients treated with both ticagrelor and cangrelor. Intriguingly, in this study, a proportion of the patients receiving both agents exhibited an increase in platelet reactivity after stopping the cangrelor infusion.[4]

In the recently published CANTIC study, 50 patients undergoing PPCI received crushed ticagrelor and were then randomized to be treated with simultaneous cangrelor or matching placebo. Cangrelor reduced the P2Y12 reaction unit (PRU) throughout the infusion, compared with placebo and consequently therefore high on-treatment platelet reactivity (HPR) rates were reduced in the cangrelor arm. After stopping the infusion, no rebound increase in platelet activity occurred, suggesting no drug–drug interaction.[5] The clinical importance of optimal P2Y12 inhibition at the time of PPCI remains incompletely explored, and in addition, safe transition from intravenous therapy to an oral P2Y12 inhibitor is an important issue.[6]

Even with timely PPCI, up to half of patients have limited microvascular perfusion despite restoration of normal epicardial flow.[7] These patients have larger infarcts[8] and are at higher risk of adverse events.[9] Recent studies highlight the role of platelets in contributing to microvascular dysfunction in the context of acute STEMI through various mechanisms including ischemia, reperfusion injury and distal embolization.[10]

We set out to determine the differential effect of cangrelor versus ticagrelor on P2Y12 inhibition at the time of first balloon inflation in the culprit coronary artery, and following PCI in a cohort of patients undergoing PPCI for STEMI. Additionally, we studied the impact of these two treatment strategies on a variety of measures of microvascular function and infarct size following PPCI.

Study Endpoints

The co-primary endpoints for this trial were the between-group difference in P2Y12 inhibition at the time of first intracoronary balloon inflation, 4 and 24 hours following initial dosing. Secondary surrogate outcome measures were the assessment of microcirculatory and epicardial reperfusion in addition to myocardial infarct size.

Materials and Methods

Study Population and STEMI Management

This is an open label, prospective, randomized controlled trial enrolling patients with acute STEMI undergoing PPCI. Acute STEMI was defined as chest pain lasting for > 30 minutes associated with ST-segment elevation > 2 mm in 2 contiguous chest leads or 1 mm in 2 contiguous limb leads. Following informed consent, subjects were randomized 1:1 to routine care (aspirin and ticagrelor) or aspirin and intravenous cangrelor immediately prior to PPCI. Patients were eligible if they had an indication for PPCI, were able to give informed consent, were P2Y12 receptor inhibitor naive and had no contraindication to ticagrelor or cangrelor. Exclusion criteria included significant active bleeding, current oral anticoagulation therapy, established cardiogenic shock, previous MI and contraindications to cardiac magnetic resonance (CMR) imaging. Patients treated with glycoprotein (GP) IIb/IIIa receptor antagonist therapy during PPCI were withdrawn from the analysis. All

patients provided written consent and continued in the study for 3 months. The study was approved by the U.K. National Research Ethics Service (reference 16/EM/0094).

Drug Therapy

A total of 100 subjects were enrolled, 50 in the cangrelor arm and 50 in the ticagrelor arm. All patients received aspirin 300 mg loading at the time of first medical contact, prior to randomization. Patients allocated to ticagrelor received a loading dose of 180 mg of the drug orally immediately following randomization and prior to admission to the catheter suite followed by a dose of 90 mg twice daily for 12 months. Patients in the cangrelor arm were treated with a bolus of 30 mcg/kg then 4 mcg/kg/min intravenous infusion immediately following randomization and then transferred to the cardiac catheter suite to undergo PPCI. Cangrelor infusion was continued for 2 hours or for duration of the procedure; whichever was longer. Ticagrelor 180 mg was given 30 minutes prior to stopping the infusion, as per manufacturer's instructions, followed by a dose of 90 mg twice daily for 12 months. Use of morphine was recorded prospectively.

Primary Endpoint Measures

Platelet Function Testing

P2Y12 inhibition was measured using VerifyNow (ACCRIVA Diagnostics, San Diego, California, United States) rapid platelet function analyser at the time of infarct vessel balloon inflation, 4 hours following study drug loading and at 24 to 36 hours.

Results are expressed as PRU, indicating the degree of adenosine diphosphate-mediated aggregation specific to the P2Y12 receptor. PRU values of $\geq$208 are indicative of a sub-optimal response and are associated with poor clinical outcomes including death, MI and stroke at 1 year.[11]

Surrogate Endpoint Measures

Index of Microvascular Resistance and Coronary Flow Reserve

Index of microvascular resistance (IMR) and coronary flow reserve (CFR) were measured in the culprit coronary at the end of the PPCI procedure. IMR, a combined pressure-/temperature-tipped guidewire-based quantitative assessment of coronary microvasculature function, is defined as the distal coronary pressure multiplied by the mean transit time of a 3-mL bolus of saline at room temperature measured simultaneously during maximal coronary hyperemia (Certus, St. Jude medical, St. Paul, Minnesota, United States).[12] Maximal coronary hyperemia was induced with intravenous adenosine at a dose of 140 mcg/kg/min. The dose was increased at operator's discretion if there was a sub-optimal symptomatic or hemodynamic response at the standard dose.

We set out to assess the absolute IMR values in each group and the proportion of subjects in each group with an IMR $>$ 40. CFR is calculated as the ratio of maximal blood flow during maximal coronary hyperemia to resting flow. It is influenced by both epicardial arterial and microvascular function. A CFR $<$ 2.0 is considered abnormal and is associated with cardiovascular disease states. We report the mean CFR in each group.[13]

All physiological metrics were independently assessed by two experienced cardiologists at the University of Glasgow Physiology Core Laboratory (T.F. and C.B.) blinded to treatment group assignment.

Angiographic Analysis

Thrombolysis in myocardial infarction (TIMI) flow grade (TFG), corrected TIMI frame count (*c*TFC) and TIMI myocardial perfusion grade (MPG) were measured using standard techniques and a frame counter.[14]

ST-Segment Resolution

A 12-lead electrocardiogram (EKG) was recorded before coronary reperfusion and 90 to 120 minutes following PPCI to assess ST-segment resolution (STR). This variable was expressed as complete ($>$ 70%), incomplete ($>$ 30% to $<$ 70%) or none ($<$ 30%).[15]

Initial Infarct Size Estimation by Peak Troponin Level

High sensitivity cardiac troponin T was measured at 24 to 36 hours following PPCI (Roche, Rotkreuz, Switzerland).

Final Infarct Size Assessment by CMR Imaging

Patients were studied at 3 months post-presentation using a 1.5 Tesla scanner (Philips Ingenia) with a standard 12-channel matrix coil configuration.[16]

All measurements were performed by two observers (E.M. and S.H., Level 3 Society for Cardiovascular Magnetic Resonance qualified) blinded to clinical and angiographic data. Where a discrepancy of $>$ 10% was evident between reports, the final figure was reached by consensus. Image analysis for left ventricular (LV) volumes, LV function and LV mass were performed using semi-automated software (CMR42 Circle Cardiovascular Imaging, Canada). Infarct size was expressed as a percentage of LV mass.

Safety Endpoints

Bleeding events were prospectively assessed using the Bleeding Academic Research Consortium (BARC) criteria during the index admission.[17]

Statistics and Data Analysis

Categorical variables are reported as number and percentage (n [%]). Continuous variables are summarized by mean and standard deviation (SD) if normally distributed and median and interquartile range (IQR) if non-normally distributed. Continuous outcome measures were compared between groups with two-sample t-tests or Wilcoxon rank sum tests. Categorical outcome measures were compared using chi-squared tests or Fisher's exact tests. All p-values are two-sided and statistical significant was considered as $p\leq$ 0.05. Data were analyzed by the Robertson Centre for Biostatistics, University of Glasgow using SAS for windows v9.3 (SAS Institute Inc., Cary, North Carolina, United States). Graphs were produced using the Prism software (GraphPad Prism version 5.0, La Jolla, California,

Table 1 Patient characteristics

Characteristic	All ($n = 100$)	Cangrelor ($n = 50$)	Ticagrelor ($n = 50$)
Demographics			
Age, years	62.3 ± 13.4	61.2 ± 13.9	63.4 ± 12.9
Males	72 (72)	39 (78)	33 (66)
BMI, kg/m^2	28.3 ± 6.0	28.5 ± 6.3	28.1 ± 5.7
Smoking status			
Current smoker	50 (50)	26 (52)	24 (48)
Former smoker	16 (16)	7 (14)	9 (18)
Medical history			
CVD family history	31 (31)	18 (36)	13 (26)
Diabetes	19 (19)	10 (20)	9 (18)
Hypertension	44 (44)	20 (40)	24 (48)
Hyperlipidemia	20 (20)	13 (26)	7 (14)
Previous MI	0	0	0
Previous CABG	0	0	0
Previous TIA/CVA	0	0	0
Previous PCI	4 (4)	1 (2)	3 (6)
Pre-infarct angina	4 (4)	3 (6)	1 (2)
Admission blood tests			
Hemoglobin, g/L	138.2 ± 18.0	138.1 ± 16.6	138.4 ± 19.4
Neutrophils, 10^9 g/L	9.4 ± 3.6	9.6 ± 3.7	9.2 ± 3.5
Platelet count, 10^9 g/L	250.6 ± 65.9	245.8 ± 66.0	255.5 ± 66.1
WCC, 10^9 g/L	12.2 ± 4.1	12.4 ± 4.4	12.0 ± 3.8
Angiographic variables			
MI localization			
Anterior	31 (31)	13 (26)	18 (36)
Inferior	61 (61)	32 (64)	29 (58)
Inferolateral	1 (1)	0	1 (2)
Lateral	5 (5)	4 (8)	1 (2)
Posterior	2 (2)	1 (2)	1 (2)
Culprit vessel			
LMS	1 (1)	0	1 (2)
LAD	31 (31)	15 (30)	16 (32)
LCX	14 (14)	8 (16)	6 (12)
INT	1 (1)	0	1 (2)
RCA	53 (53)	27 (54)	26 (52)
Number of vessels diseased			
0	1 (1)	0	1 (2)
1	28 (28)	11 (22)	17 (34)
2	30 (30)	16 (32)	14 (28)
3	41 (41)	23 (46)	18 (36)
Number of vessels treated			
0	2 (2)	1 (2)	1 (2)
1	89 (89)	42 (84)	47 (94)
2	9 (9)	7 (14)	2 (4)

Abbreviations: BMI, body mass index; CABG, coronary artery bypass graft; CVA, cerebrovascular accident; CVD, cardiovascular disease; INT, intermediate artery; IQR, interquartile range; LAD, left anterior descending artery; LCX, left circumflex artery; LMS, left main stem; MI, myocardial infarction; PCI, percutaneous coronary intervention; RCA, right coronary artery; SD, standard deviation; TIA, transient ischemic attack; WCC, white cell count.
Note: Values are mean ± SD, *n* (%) or median (IQR) as appropriate.

Table 2 Treatment and procedure characteristics

Characteristic	All ($n = 100$)	Cangrelor ($n = 50$)	Ticagrelor ($n = 50$)	*p*-Value
Call to balloon time, minutes	121 [102, 140]	116 [100, 136]	126 [106, 150]	0.16
Door to balloon time, minutes	57 [45, 71]	53 [45, 71]	59 [44, 70]	0.83
Treatment duration, minutes	28 [20, 37]	24 [12, 30]	33 [23, 48]	< 0.001
Ischemia duration, minutes	192 [143, 289]	164 [133, 233]	195 [148, 345]	0.26
Morphine given	77 (77)	37 (74)	40 (80)	0.48
Total heparin, units	8,000 [6,500, 10,000]	8,500 [5,000, 10,000]	8,000 [7,000, 10,000]	0.83
Total length of stent, mm	42.1 $\pm$ 22.1	43.2 $\pm$ 22.9	40.8 $\pm$ 21.4	0.59
Thrombectomy	14 (14)	7 (14)	7 (14)	1.00

Note: Values are mean $\pm$ SD, *n* (%) or median [IQR] as appropriate. *p*-Values are from two-sample *t*-test, Wilcoxon sum rank test or chi-squared test as appropriate.

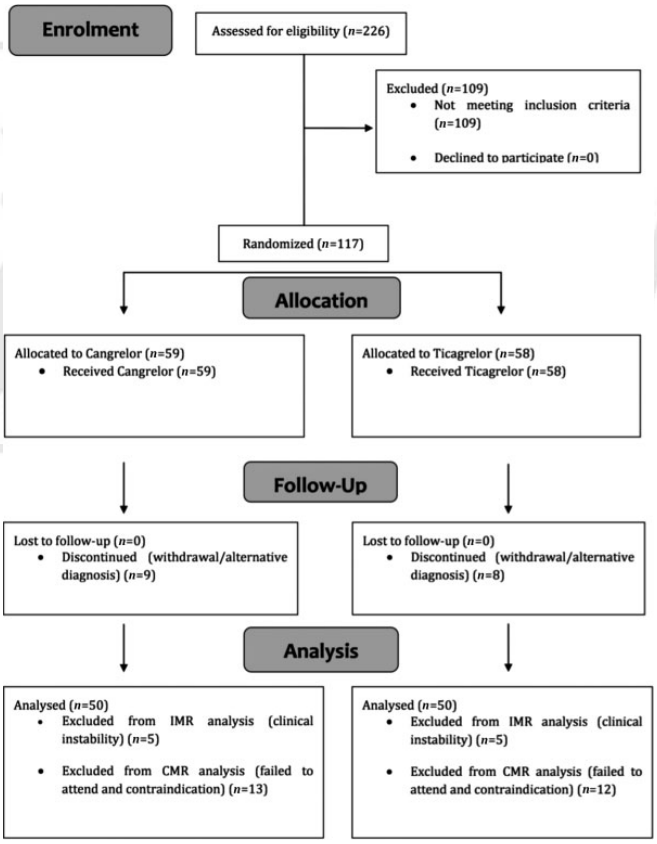


Fig. 1 Study flow diagram.

United States). A sample size calculation was performed using preliminary data on a prior study of 15 patients with a mean (SD) for PRU of 257 (61.1). A sample size of 50 in each group would have 80% power to detect an effect size of 0.566 using a two-group *t*-test with a 5% two-sided significance level. This is equivalent to a difference of 34.6 units of PRU between the cangrelor and ticagrelor groups. Post hoc analysis shows that 50 patients per group provides 95% power to demonstrate a 30% reduction in PRU at first coronary balloon inflation time in the cangrelor group compared with the ticagrelor group.

Table 3 Comparison of P2Y12 reaction units (PRU) at coronary reperfusion (balloon inflation) time, by treatment group

Characteristic	Category	Cangrelor ($n = 50$)	Ticagrelor ($n = 50$)	*p*-Value
PRU units at balloon inflation	≤208	45 (90%)	11 (22%)	< 0.0001
	> 208	5 (10%)	39 (78%)	

Note: *p*-Values from chi-squared test.

Results

Study Population

Patient, treatment and procedure characteristics are described in ►**Tables 1** and **2**. Two hundred twenty-six patients presenting with STEMI were screened, 117 randomized. Of the 109 excluded patients, 42 had previously received a P2Y12 inhibitor and 37 had suffered previous MI. Other exclusions included cardiogenic shock ($n = 13$), oral anticoagulant therapy ($n = 8$), lacking capacity for consent ($n = 4$), history of bleeding ($n = 3$) and renal failure requiring dialysis ($n = 2$) (►**Fig. 1**). Seventeen subjects were withdrawn from the study after randomization due to either the use of GPIIb/IIIa inhibitors (cangrelor, $n = 6$; ticagrelor, $n = 5$; total, $n = 11$; 9.4%), extreme clinical instability ($n = 2$) or the presence of an alternative diagnosis (myocarditis, $n = 2$; takotsubo cardiomyopathy, $n = 2$). Of the 117 randomized patients, 1 died after withdrawal from the study. After these exclusions/withdrawals, 100 randomized patients were included for analysis (cangrelor, $n = 50$; ticagrelor, $n = 50$). All patients received P2Y12 inhibitors as per protocol. Of the 100 patients, 90 were assessed for microvascular function using IMR (ticagrelor, $n = 45$; cangrelor, $n = 45$) with hemodynamic instability precluding measurement in 10 subjects. Angiographic analysis was performed on all subjects and CMR at 3 months was performed in 75 (cangrelor, $n = 37$; ticagrelor, $n = 38$). Reasons for not undertaking CMR included renal failure ($n = 2$), lengthy intensive care unit stay ($n = 1$), procedure intolerance/claustrophobia ($n = 2$) and 20 patients declined. Morphine for pain relief was administered to 37 out of 50 (74%) cangrelor-treated patients, at an average dose of 9.7 mg, and to 40 out of 50 (80%) ticagrelor-treated patients, at an average dose of 8.5 mg. The mean time from morphine administration to study drug loading was 60 minutes in the cangrelor arm and 55 minutes in the ticagrelor arm.

All 100 patients survived to discharge. One patient underwent in-patient coronary artery bypass operation necessitating a prolonged intensive care unit stay. Two patients became hemodynamically unstable during PPCI and needed the insertion of intra-aortic balloon pump.

Primary Endpoints

Platelet Inhibition

At the time of initial coronary balloon inflation, cangrelor produced significantly greater P2Y12 inhibition (cangrelor 145.2 ± 50.6 vs. ticagrelor 248.3 ± 55.1; $p < 0.001$, mean, SD). This difference was no longer apparent at 4 hours (cangrelor 158.1 ± 92.1 vs. ticagrelor 131.2 ± 92.9; $p = 0.15$) and 24 to 36 hours after study drug administration (cangrelor 61.0 ± 50.0 vs. ticagrelor 60.1 ± 56.3; $p = 0.93$) (►**Fig. 2**). While there was a slight numerical increasing PRU in patients within the cangrelor group after transitioning to ticagrelor (cangrelor 145.2 ± 50.6–158.1 ± 92.1), this was not statistically significant, indicating that this transitioning

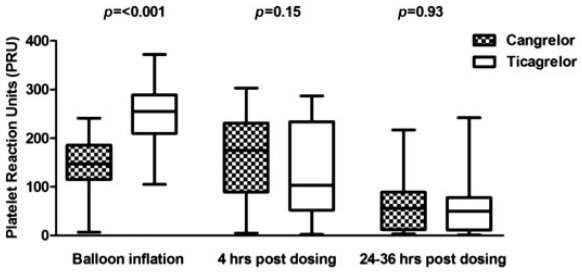


Fig. 2 Box and whiskers plots showing comparison of the degree of P2Y12 inhibition measured by platelet reaction units at balloon inflation (vessel opening) time, 4 and 24 to 36 hours post-anti-platelet drugs administration. Group data shown (median, IQR range). IQR, interquartile range; PRU, platelet reaction units.

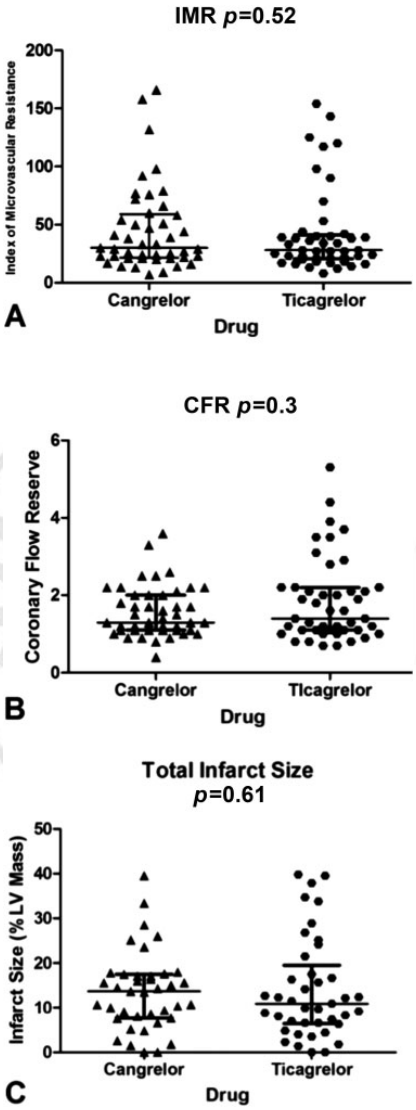


Fig. 3 Graphs comparing the effect of cangrelor and ticagrelor on IMR (**A**) and CFR (**B**) immediately post-index PPCI procedure, and total infarct size (**C**) by CMR imaging at 3 months' follow-up. Group (median and interquartile range) and individual data shown (▲ indicates cangrelor while • indicates ticagrelor). CFR, coronary reserve flow; CMR, cardiac magnetic resonance; IMR, index of microvascular resistance; PPCI, primary percutaneous coronary intervention.

Table 4 Infarct size by CMR and peak troponin levels

Characteristic	All ($n = 64$)	Cangrelor ($n = 29$)	Ticagrelor ($n = 25$)	*p*-Value
Infarct size (CMR, %)	11.8 [6.8, 17.5]	13.7 [7.7, 17.5]	10.9 [6.6, 17.5]	0.61
Infarct size (peak troponin, ng/L)	29,556 [13,879, 58,988]	37,169 [14,230, 56,740]	23,896 [13,663, 66,565]	0.84

Abbreviation: CMR, cardiac magnetic resonance.
Note: Values are median [IQR]. *p*-Values are from Wilcoxon sum rank test.

period is safe in the context of STEMI. With the randomization and treatment allocation in the emergency setting, both drugs were given as soon as practicable after randomization, before PPCI. The preparation time of intravenous cangrelor was longer than that for administering ticagrelor; this translated into a longer ticagrelor initiation-balloon inflation time than cangrelor initiation-balloon inflation time (23.0 ± 12.8 minutes for cangrelor vs. 36.3 ± 16.9 for ticagrelor; $p < 0.0001$). At balloon inflation, 45 out of 50 (90%) cangrelor-treated patients achieved an optimal PRU (< 208 units). Only 11 out of 50 (22%) ticagrelor-treated subjects were in range ($p < 0.0001$) (►**Table 3**). At 4 hours post-initial drug dosing, 15 out of 50 ticagrelor-treated patients (30%) and 20 out of 50 cangrelor-treated patients (40%) had PRU values above 208, indicating HPR. In the cangrelor-treated group, this measure was taken 2.5 hours following transitional ticagrelor loading and 2 hours after the cangrelor infusion had ended. The administration of morphine did not influence the degree of P2Y12 inhibition at the time of coronary balloon inflation in either of the treatment groups ($p = 0.48$).

Surrogate Endpoints

Index of Microvascular Resistance and Coronary Flow Reserve

The mean time from administration of study drugs to IMR measurement was 88 minutes. Following PPCI, IMR was similar in each group (►**Fig. 3A**), (cangrelor 30 [22, 58], ticagrelor 28 [21, 40], median [IQR]; $p = 0.52$). Similarly, the proportion of patients with IMR greater than 40 (cangrelor 18 [40%] vs. ticagrelor 11 [24%]; $p = 0.11$) was not different. CFR results were also similar between groups (►**Fig. 3B**) (cangrelor median [18], 1.3 [19], ticagrelor 1.4 [20]; $p = 0.30$).

Angiographic Analysis

There was no significant difference in the occurrence of post-PCI MPG 3 (cangrelor, $n = 31$ vs. ticagrelor, $n = 32$; $p = 0.54$) and TFG 3 (cangrelor, $n = 38$ vs. ticagrelor, $n = 42$; $p = 0.27$) between treatment groups. Likewise, there was no difference in the mean cTFC (21.7 ± 14.2 for cangrelor vs. 21.4 ± 10.2 for ticagrelor; $p = 0.93$). Suboptimal TFGs (1 and 2) were present at the end of the PPCI procedure in 12 cangrelor-treated patients and 7 ticagrelor-treated patients.

EKG Analysis

At 90 to 120 minutes following PPCI, no difference was seen in STR between the cangrelor and ticagrelor groups (complete = 32%, partial = 11%, none = 7% for cangrelor vs. complete = 36%, partial = 7%, none = 7% for ticagrelor; $p = 0.57$).

Myocardial Infarct Size

CMR was performed at a median of 13 weeks after PPCI in both the groups (►**Fig. 3C**; ►**Table 4**). Infarct scar was revealed on late gadolinium enhancement in 68 out of 75 (90.6%) patients who had CMR performed (cangrelor 31, ticagrelor 37 patients). There was no difference in infarct size as a percentage of LV mass between groups (cangrelor 13.7 [7.7, 17.5], ticagrelor 10.9 [6.6, 17.5], median [IQR]; $p = 0.61$). Similarly, LV ejection fraction was not different (cangrelor 56.50 [47.50, 59.25], ticagrelor 55 [44.50, 61.50], median [IQR]; $p = 0.96$). Peak troponin levels at 24 to 36 hours post-drug administration did not differ significantly between the treatment groups (►**Table 4**).

Safety Endpoints

Two out of 50 cangrelor-treated patients and 3 out of 50 ticagrelor-treated patients developed hematoma at the radial access site around 20 to 50 minutes following PPCI (Type 2 BARC). This was managed conservatively and required no surgical intervention in either of the treatment arms. One patient in the ticagrelor arm developed limiting shortness of breath 2 days after initiation necessitating replacement with clopidogrel, which resulted in complete resolution of symptoms.

Discussion

This randomized controlled and assessor-blinded study assesses the effect of a strategy of intravenous cangrelor transitioning to ticagrelor, compared with ticagrelor standard therapy on P2Y12 inhibition; coronary microcirculation and infarct size in a STEMI population treated with PPCI. The main findings are as follows.

First, intravenous cangrelor, compared with oral ticagrelor, produced a markedly greater P2Y12 inhibition at the time of infarct-related artery balloon inflation during PPCI.

Second, intravenous cangrelor was not shown to be superior to oral ticagrelor in improving coronary microcirculatory reperfusion as assessed by IMR and CFR and no difference was seen in terms of the angiographic markers of coronary reperfusion and STR. Similarly, no significant difference was seen between groups in the initial infarct size assessed by peak troponin and the final infarct size assessed by CMR at 3 months.

These results support our hypothesis that intravenous cangrelor when compared with oral ticagrelor will yield greater P2Y12 inhibition at the time of coronary balloon inflation by PPCI. This greater early P2Y12 inhibition did not appear to lead to improved microcirculatory function/perfusion, or result in a reduced myocardial infarct size.

If the degree of peri-interventional P2Y12 inhibition in STEMI treatment is of significant importance, strategies to both provide strong inhibition and also limit the possible negative effect of transitioning to an oral agent might be valuable. Two recently published studies have investigated the pharmacodynamic effect of cangrelor compared with different ticagrelor loading regimens during PPCI.

In the first, 30 patients received ticagrelor loading prior to angiography and then were randomized in the catheter laboratory to either cangrelor or no additional anti-platelet treatment.[4] It showed markedly more potent P2Y12 inhibition (PRU) 15 minutes following loading in subjects treated with cangrelor. There was a suggestion, in this trial, of an increase in platelet reactivity following cessation of the cangrelor infusion with 4 out of 15 patients exhibiting an increase in PRU at 2 to 4 hours. The loading regimen used was in contrast to our current study, where cangrelor was administered as monotherapy before angiography and during PPCI in the cangrelor arm, and the transition to ticagrelor occurred following PPCI, with the oral agent being given 30 minutes prior to cangrelor cessation.

In the CANTIC trial whereby 50 subjects received ticagrelor loading as crushed tablets at the time of randomization to cangrelor or placebo, once again more potent P2Y12 inhibition was demonstrated in the cangrelor-treated patients, particularly at the primary endpoint time of 30 minutes.[5] Interestingly, in this study with assessment of P2Y12 inhibition at eight time points, no increase in PRU was seen after the cangrelor infusion was stopped, suggesting no rebound in platelet activity and no drug–drug interaction. These finding are in line with our study of 100 STEMI patients in which no significant increase in P2Y12 inhibition was evident in the cangrelor-treated subjects, when measured at 4 hours after randomization, following the transition from cangrelor to ticagrelor. This issue of transition from cangrelor to an oral agent was elegantly studied in more stable patients undergoing PCI in the ExcelsiorLOAD2 trial. Despite the previously demonstrated drug–drug interaction between clopidogrel and cangrelor, prasugrel (and also ticagrelor) administered at the onset of the cangrelor infusion yielded very good levels of P2Y12 inhibition soon after cangrelor cessation seemingly preventing a clinically relevant gap in platelet inhibition cangrelor.[18]

The Paradigm of Potent Antiplatelet Agents to Improve STEMI PPCI Outcomes and Reduce Myocardial Infarct Size

STEMI is associated with a high degree of intrinsic platelet activation, the level of which is associated with the magnitude of both subsequent anti-platelet therapy effect and clinical outcomes.[19] Furthermore, PPCI is the coronary interventional procedure associated with the highest frequency of severe thrombotic complications and therefore rapid and consistent platelet inhibition is a key objective in STEMI management.[20] Prasugrel and ticagrelor are potent and rapidly acting P2Y12 inhibitors that reduce adverse ischemic events in STEMI patients when compared with clopidogrel.[3] The therapeutic effect of prasugrel and ticagrelor is markedly delayed in the context of STEMI,[3] and so PPCI is likely to be

What is known about this topic?

- Anti-platelet therapy with potent oral P2Y12 receptor antagonists improves outcomes in STEMI with both ticagrelor and prasugrel showing superior efficacy to clopidogrel.
- One important limitation of all orally administered P2Y12 inhibitors is delayed anti-platelet effect, which can take several hours to achieve in the setting of STEMI. Therefore, PPCI is likely to be performed in the context of sub-optimal P2Y12 inhibition.
- Cangrelor being a direct reversible P2Y12 inhibitor with rapid onset and offset of action overcomes many of the limitations associated with oral P2Y12 inhibitors, making its use in the setting of acute STEMI undergoing primary PCI where prompt anti-platelet inhibition is required, appealing.

What does this paper add?

- This study confirms that cangrelor produces early, potent P2Y12 inhibition in patients treated with PPCI.
- It supports the peri-procedural administration of cangrelor in the setting of primary PCI as a potential bridging intravenous anti-platelet therapy until the full anti-platelet effect is achieved with oral P2Y12 receptor inhibitors. This approach would help overcome the main issue encountered with oral P2Y12 inhibitors in the setting of primary PCI, which is their delayed onset of action.
- In our cohort, acceptable levels of P2Y12 inhibition were achieved with oral ticagrelor by 4 hours following loading, and in cangrelor-treated patients, the post-PPCI transition to ticagrelor did not appear to lead to a significant rebound in platelet activity.

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Conflict of Interest
J.M.C. has received consultancy fees and travel support from Astra Zeneca. C.B. is employed by the University of Glasgow, which holds consultancy and research agreements with companies that have commercial interests in the diagnosis and treatment of angina. The companies include Abbott Vascular, AstraZeneca, Boehringer Ingelheim, HeartFlow, Menarini Pharmaceuticals, Opsens and Siemens Healthcare. None of these companies have had any involvement with this study. None of the other authors have any potential conflicts of interest.

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THIEME