CARDIAC SURGERY AND ANTIPLATELET THERAPY

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ABSTRACT

Background

Dual antiplatelet therapy (DAPT) with acetylsalicylic acid (ASA) and a $P2Y_{12}$ inhibitor improves outcome in acute coronary syndrome (ACS). In the subset of ACS patients undergoing urgent cardiac surgery, ongoing or recently discontinued DAPT is associated with increased risk of bleeding. Postoperative DAPT in ACS patients after coronary artery bypass grafting (CABG) may improve graft patency and short-term survival. The aim of this project was to study ACS patients undergoing cardiac surgery and how DAPT with ASA and ticagrelor influences perioperative bleeding risks, how bleeding can be treated, and to investigate if survival after CABG is influenced by antiplatelet therapy.

Methods

In paper I, recovery of platelet function after discontinuation of ticagrelor was investigated using multiple-electrode aggregometry (MEA) in ACS patients awaiting CABG. The effect of platelet concentrate at different discontinuation times was also studied. Paper II was a prospective observational study of patients undergoing cardiac surgery with ongoing or recently discontinued ticagrelor treatment. The relationship between preoperative MEA and postoperative bleeding was investigated. In paper III, MEA was used to investigate the effect of aprotinin and tranexamic acid on platelet function in ACS patients with ongoing DAPT using ASA and ticagrelor. Paper IV was a nationwide study of all ACS patients undergoing isolated CABG surgery during a four-year period. The influence of postoperative antiplatelet therapy on one-year mortality was investigated using propensity score matching.

Results

Mean platelet ADP-induced aggregation increased gradually after ticagrelor discontinuation and reached normal values after 72–96 hours. There was a large inter-individual variability. Platelet concentrate did not improve ADP-induced aggregation at any time, but markedly increased arachidonic acid-

induced aggregation at all time points. Preoperative ADP-induced aggregation predicted severe bleeding complications, with an optimal cut-off of 22 aggregation units. Aprotinin, but not tranexamic acid increased ADP-induced aggregation in patients with ongoing DAPT using ASA and ticagrelor. Postoperative treatment with ASA + ticagrelor was associated with a reduced one-year mortality compared to ASA only (hazard ratio 0.42, p=0.020).

Conclusions

Platelet function testing improved the assessment of the operative risk in ticagrelor treated patients. Platelet transfusion have no or limited effect in treating bleeding in patients with recent ticagrelor therapy. From a platelet function perspective, aprotinin may be preferred over TA in ticagrelor treated patients. Survival after CABG in ACS patients is likely influenced by postoperative antiplatelet therapy, with improved outcome associated with ticagrelor treatment.

Keywords: Cardiac surgery, Platelets, Acute Coronary Syndrome

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SAMMANFATTNING PÅ SVENSKA

Syrebrist i hjärtmuskeln är den vanligaste dödsorsaken i Sverige och världen. Den orsakas vanligen av blodproppar som bildas vid åderförkalkning i hjärtats kranskärl. Sprickor i blodkärlens innersta skikt orsakar aktivering av trombocyter (blodplättar) som klumpar ihop sig och bildar blodproppar. När blodet inte kan passera drabbas patienten av ett akut koronart syndrom (AKS) som kan leda till hjärtinfarkt och i värsta fall hjärtstopp. Prognosen hos patienter med AKS förbättras om man hämmar trombocytfunktionen. Ticagrelor är en relativt ny trombocythämmare och används i Sverige som tillägg till acetylsalicylsyra (ASA) hos de flesta patienter med AKS.

De flesta patienter med AKS behandlas med ballongsprängning eller bara läkemedel, men en av tio patienter behöver genomgå en öppen hjärtoperation, oftast i form av en bypassoperation. Om operationen sker kort tid efter utsättning av ticagrelor är patienterna mer benägna att blöda. Vid allvarlig blödning ökar risken för andra komplikationer och död. Denna risk måste vägas mot risken för ny infarkt om man skjuter upp operationen.

I första delarbetet undersöktes återhämtningen av trombocytfunktionen efter utsättning av ticagrelor hos patienter som väntade på bypassoperation. Mätningar genomföres efter 12, 24, 48, 72 och 96 timmar. I genomsnitt sågs en återhämtning efter tre dygn, men det fanns en stor variation mellan olika patienter. Vissa hade dålig trombocytfunktion fyra dygn efter utsättning, medan andra återhämtat funktionen redan efter 24–48 timmar. Försök att motverka ticagrelors hämning genom tillsats av nya trombocyter var inte framgångsrikt.

I det andra delarbetet undersöktes om mätning av trombocytfunktion före operation kunde användas för att avgöra risken för allvarlig blödning. Alla undersökta patienter hade behandlats med ticagrelor inom 5 dagar från operation. Med hjälp av testresultaten kunde risken för allvarlig blödning bestämmas med rimlig visshet. Ett tröskelvärde kunde fastställas när risken för allvarlig blödning var hög.

I syfte att minska blödning får patienter som hjärtopereras rutinmässigt läkemedel som hämmar nedbrytningen av blodproppar (s.k. fibrinolyshämmare), vanligen i form av tranexamsyra. En annan fibrinolyshämmare, aprotinin, kan ibland påverka trombocyterna. I

delstudie tre testades trombocytfunktionen hos patienter med pågående ticagrelor behandling. Efter tillsats av aprotinin förbättrades trombocytfunktionen signifikant, medan tranexamsyra inte hade någon sådan effekt. Detta talar för att aprotinin kan vara att föredra hos patienter som behandlats med ticagrelor.

För att förhindra nya proppar efter en bypassoperation rekommenderas åter trombocythämning med två olika läkemedel, men övertygande bevis om vinsten med dubbel behandling efter operation saknas, och majoriteten av alla patienter får bara ASA. I det fjärde delarbetet undersöktes alla patienter med AKS som genomgått bypassoperation i Sverige under 2012–2015 med syftet att undersöka om trombocythämning påverkade överlevnad efter operation. Hos dem som fick ticagrelor var risken för död mer än halverad under första året.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. Hansson EC, Malm CJ, Hesse C, Hornestam B, Dellborg M, Rexius H, Jeppsson A. Platelet function recovery after ticagrelor withdrawal in patients awaiting urgent coronary surgery. Eur J Cardiothor Surg, 2016; doi:10.1093/ejcts/ezw373.

II. Malm CJ, Hansson EC, Åkesson J, Andersson M, Hesse C, Shams Hakimi C, Jeppsson A. Preoperative platelet function predicts perioperative bleeding complications in ticagrelor-treated cardiac surgery patients: a prospective observational study. Br J Anaesth. 2016 Sep;117(3):309-15.

III. Malm CJ, Singh S, Hesse C, Jeppsson A. Aprotinin but not tranexamic acid improves in vitro platelet function in blood samples from ticagrelor and aspirin treated patients. *Submitted*

IV. Malm CJ, Björklund E, Hansson EC, Wessman C, Rexius H, Nozohoor S, Nielsen S, Jeppsson A. Platelet inhibition and survival after coronary artery bypass grafting in patients with acute coronary syndrome: A nationwide study from the SWEDEHEART registry. *Submitted*

CONTENT

ABBREVIATIONS

AA	Arachidonic acid
ACS	Acute coronary syndrome
ADP	Adenosine diphosphate
ASA	Acetylsalicylic acid
AUC	Area under the curve
CABG	Coronary artery bypass grafting
CI	Confidence interval
COX	Cyclo-oxygenase
CPB	Cardiopulmonary bypass
CTO	Chest tube output
CYP	Cytochrome P450
DAPT	Dual antiplatelet therapy
EACA	Epsilon amino caproic acid
ENT	Equilibrative nucleotide transporter
FDP	Fibrin degradation products
FFP	Fresh frozen plasma
Hb	Haemoglobin
HPR	High on-treatment platelet reactivity
HR	Hazard ratio
IHD	Ischaemic heart disease
IQR	Interquartile range
LTA	Light transmission aggregometry
MEA	Multi-electrode impedance aggregometry
MI	Myocardial infarction
NPV	Negative predictive value
nSTEMI	Non ST-segment elevation myocardial infarction

PCI	Percutaneous coronary intervention
PFT	Platelet function test
POC	Point-of-care
PPV	Positive predictive value
PS	Propensity score
PTCA	Percutaneous transluminal coronary angioplatsy
RBC	Red blood cell
RCT	Randomized controlled trial
RR	Relative risk
ROC	Receiver operating characteristic
SD	Standard deviation
SEM	Standard error of the mean
STEMI	ST-segment elevation myocardial infarction
TA	Tranexamic Acid
TF	Tissue factor
tPA	Tissue plasminogen activator
UA	Unstable angina
UDPB	Universal definition of peri-operative bleeding
uPA	Urokinase-type plasminogen activator
vWF	von Willebrand Factor

1 INTRODUCTION

1.1 Ischaemic heart disease

Coronary thrombosis was recognized as a cause of death during the nineteenth century, but it was long regarded as a medical curiosity[1]. Today, ischaemic heart disease (IHD) is the leading cause of death, both worldwide and in Sweden. Despite improvements in treatment and outcome in recent years, more than seven million persons succumb to the disease every year[2]. In Sweden, more than 13% of deaths are caused by IHD, although there are large regional variations, with annual death rates ranging from 76–173 deaths per 100,000 in different counties[3].

IHD can be classified as chronic, stable angina or as an acute coronary syndrome (ACS). The former patients have stable symptoms and no evidence of acute myocardial infarction. ACS is characterized by a sudden worsening of symptoms, usually due to formation of an intra-coronary thrombus. ACS is further categorized depending on the degree of coronary obstruction and associated myocardial ischaemia. A partially occlusive thrombus is the usual cause of the closely related syndromes unstable angina (UA) and non-ST-segment elevated myocardial infarction (nSTEMI), the difference being that UA patients do not have myocardial necrosis. When the thrombus completely obstructs the epicardial coronary artery, an ST-segment elevation myocardial infarction (STEMI) ensues, in which the ischaemia and resulting myocardial necrosis is more severe.

At the beginning of the twentieth century, some insight was gained into the causal relationship between coronary sclerosis, coronary thrombosis, and myocardial necrosis[4]. Today, we understand that intra-coronary thrombi may form when blood comes in contact with areas of blood vessels where the endothelial lining is disrupted due to underlying atherosclerotic plaques. This leads to the activation of platelets and coagulation systems, which form thrombi that reduce blood flow and may cause distal embolization[5]. This pathophysiology provides the basis for the anti-thrombotic and fibrinolytic therapies that have become so prevalent in the management of these patients in modern times.

1.2 Platelets

Platelets were first described in the nineteenth century after the invention of the twin-lens microscope[6]. An understanding of the importance of platelets in the haemostasis of humans was gained from animal studies and clinical experience during the early twentieth century[7]. In a case reported from 1910, a young patient suffered from uncontrollable epistaxis. Coagulation time was normal, but the platelet count was markedly reduced (6,000/μL). After whole-blood transfusion, the bleeding ceased and the platelet count simultaneously rose dramatically. This illustrated the importance of adequate platelet function in bleeding patients, and also that normal coagulation tests do not ensure acceptable haemostasis in the presence of thrombocytopenia[8].

Between 1,000 and 3,000 platelets are formed as subcellular, disk-shaped fragments (2–5 μm in diameter, 0.5 μm in thickness) from a mega-karyocyte residing in the bone marrow[9]. After release into the bloodstream, platelets circulate for 7–10 days at a normal concentration of 150–400 × 10^9/L. Platelets have no nucleus, but they do contain mRNA and a translation apparatus, and can therefore synthesize certain proteins[10]. They also have a cytoskeleton that enables changes in shape, mitochondria for generation of energy, and a large number of granules that contain protein receptors and signalling molecules. Old platelets are cleared from the circulation through phagocytosis in the spleen and liver.

Due to their shape and small size, circulating platelets are pushed towards the vessel wall by the larger erythrocytes and leukocytes. This peripheral position is optimal for rapidly detecting and responding to vascular injury. Interactions with the vessel wall are also facilitated by the laminar blood flow being slower adjacent to the vessel wall[11]. Membrane receptors serve as contacts between platelets and their environment, and a wide range of agonists and adhesive proteins trigger platelet reactivity. Collagen, von Willebrand factor (vWF), thrombin, thromboxane A2, and adenosine diphosphate (ADP) are the most important platelet activators[12].

1.3 Haemostasis

Haemostasis is the normal process for causing bleeding to stop[13]. It involves the coordinated effect of platelets, coagulation proteins in the blood, and endothelial and sub-endothelial tissue where expression of initiators of coagulation is found. Together, these systems interact to maintain the integrity of the blood circulation by creating a stable haemostatic thrombus plug. This blood clot is the result of both primary and secondary haemostasis, which are dependent on each other and can be regarded as concurrent.

Primary haemostasis is initiated after circulating blood is exposed to an injury in the vessel wall. Platelets are captured from the blood through interaction with vWF, which is ubiquitously expressed in sub-endothelial tissue. Stable adhesion requires additional interactions with collagen. This triggers an activation of the platelets with a rapid morphological change and release of granule contents, including coagulation factors, pro-inflammatory mediators, platelet activators, and vasoconstrictors. Expression of the surface receptor glycoprotein GP IIb/IIIa, with a high affinity for circulating fibrinogen, causes platelet-platelet aggregation. In addition, activation exposes the anionic phospholipid phosphatidylserine at the outer platelet surface; this facilitates the interaction of pro-coagulant proteins, leading to a burst of thrombin, fibrin formation and further platelet activation[14].

Secondary haemostasis, also referred to as coagulation, is a process that results in insoluble cross-linked fibrin strands that stabilize the primary platelet plug (Figure 1). Coagulation is activated by extravascular tissue factor (TF), a ligand that is expressed in the tunica media of the vessel wall[15]. Factor VII and TF form a complex, which, through a cascade of reactions, amplifies the production of thrombin (activated factor II). Thrombin cleaves fibrino-peptides from fibrinogen, creating fibrin monomers which rapidly polymerize into insoluble fibrin strands.

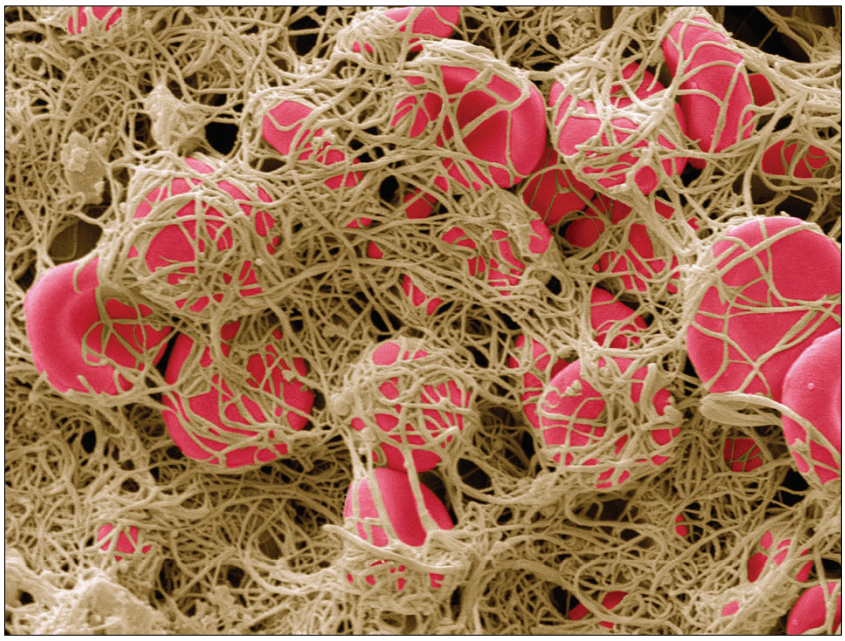

Figure 1. Scanning electron microscopy of red blood cells trapped in fibrin strands interconnected by attached platelets. Magnification 5000x. Science Photo Library / Alamy Stock Photo, with permission.

The breakdown of an established thrombus is called fibrinolysis. This process is also important in preventing excessive thrombus formation after activation of the coagulation system. It is mediated through the action of plasmin, which is generated from the zymogen plasminogen by either tissue plasminogen activator (tPA) or urokinase (uPA)[16].

Plasmin is a serine protease that, among other functions, cleaves fibrin. Plasmin has a lysine-binding site to which it can attach and split the fibrin strand, resulting in the release of soluble fibrin degradation products (FDPs) such as d-dimer. The coagulation and fibrinolytic systems are highly regulated and interrelated, to ensure balanced haemostasis[16].

1.4 Antithrombotic treatment

An antithrombotic agent is a drug that reduces the formation of blood clots. Antiplatelet drugs limit the adhesion, aggregation, and activation of platelets. Anticoagulants limit the coagulation system and the formation of thrombin and fibrin strands. Thrombolytic drugs activate fibrinolysis to dissolve clots after they have formed.

Antithrombotic drugs can reduce myocardial injury in patients with ACS. More potent drugs or a combination of multiple treatments may have an incremental effect in reducing coronary thrombus formation, but there is an increased risk of bleeding complications. Finding the optimal balance between reduced thrombus formation and increased bleeding risk requires accurate assessment of the respective risks, and can be a challenging in the clinical setting.

1.4.1 Acetylsalicylic acid

Acetylsalicylic acid (ASA) was originally developed in the nineteenth century as an analgesic and antipyretic medication. Its antithrombotic properties were observed much later –gastrointestinal bleeding was linked to ASA in 1938[17], and prolongation of the bleeding time by ASA was first demonstrated in the 1950s. In 1967, after noting his own increased bleeding from razor nicks when using ASA, Weiss demonstrated that ASA inhibits thrombus formation through inhibition of platelet aggregation initiated by connective tissue[18].

ASA irreversibly inhibits the enzyme cyclo-oxygenase (COX), which converts arachidonic acid to prostaglandin; this is in turn required for the synthesis of thromboxane A2[19]. Platelets normally synthesize thromboxane A2 to create a positive feedback by stimulating and recruiting more platelets to the primary haemostatic plug[20].

In ACS, ASA was shown to be effective in pioneering trials, which demonstrated a relative risk reduction of approximately 25% regarding

vascular mortality[21]. The efficacy and safety of ASA has now been documented in over 70 randomized clinical trials, which have included more than 115,000 patients at variable risk of thrombotic complications of atherosclerosis[22].

Various doses of ASA have been tested in different clinical settings and over the whole spectrum of athero-thrombosis –from apparently healthy, low-risk individuals to patients presenting with an acute myocardial infarction. Life-long ASA is now recommended for all patients with ischaemic heart disease, including ACS patients without contraindications[23].

1.4.2 Dual antiplatelet therapy

During the 1970s, in an effort to find new anti-inflammatory drugs, a number of compounds belonging to the thienopyridine group were synthesized and screened in animal models mimicking human pathologies. None of the thienopyridine compounds had anti-inflammatory effects, but some displayed unexpected antiplatelet and antithrombotic effects after oral administration in rats.

One of the most active compounds, ticlopidine, was evaluated further in clinical settings where platelet interactions with artificial surfaces could lead to thrombotic complications, such as mechanical circulatory support and haemodialysis. It was launched on the market in 1978 for these restricted indications[24].

During the 1990s, intra-coronary stenting after percutaneous transluminal coronary angioplasty (PTCA) was limited by the risk of thrombotic occlusion of the stents. ASA and anticoagulation therapy were used to improve the outcome, but resulted in an increased risk of haemorrhagic complications[25]. In these patients, increased platelet activation was found to be an independent predictor of stent occlusion, suggesting that platelets played a more central role in stent thrombosis than the coagulation system[26]. This triggered an increased interest in antiplatelet therapy, leading to a number of trials on the effect of dual antiplatelet therapy (DAPT)[27]. A marked decrease in the occurrence of stent thrombosis was demonstrated using ticlopidine in combination with ASA (ASA: 3.6%; DAPT: 0.5%; $p = 0.001$)[28].

Due to adverse side effects of ticlopidine[29], a continued search for new drugs with a more favourable pharmacological profile was undertaken. More than a thousand ticlopidine analogues were synthesized and

tested in animals for their antiplatelet and antithrombotic effects. Eight of them were developed up to phase-1 studies in healthy volunteers and only the last one, PCR4099, proved to be clearly more active and better tolerated than ticlopidine[24]. After ten years of development and large clinical studies, PCR4099, or clopidogrel, was launched in 1998.

Ticlopidine and clopidogrel require hepatic metabolism through a cytochrome P450- (CYP-) dependent pathway to form active metabolites. It is the metabolites that are active and irreversibly inhibit the ADP-dependent $P2Y_{12}$ receptor of the platelets[30].

In the large randomized Clopidogrel in Unstable Angina to Prevent Recurrent Events (CURE) trial, DAPT with ASA + clopidogrel was compared to ASA only in 12,562 patients with nSTEMI ACS. The patients were treated over a mean length of time of 9 months. DAPT resulted in almost a 20% lower relative risk of the combined primary endpoint cardiovascular death, non-fatal myocardial infarction, or stroke ($p < 0.001$)[27].

During the study, 2,072 patients in the CURE trial (16.5%) underwent coronary artery bypass grafting (CABG), either at the initial hospitalization (49%) or later during the study period (51%). There was a non-significant reduction in the primary outcome in DAPT patients (14.5% vs. 16.2%) without any significantly higher incidence of major bleeding (9.6% in DAPT patients, 7.5% in placebo patients).

Clopidogrel became a huge success, and during the last year before the patent expired, clopidogrel was the second-best selling drug –with global sales reaching $9.4 billion[24].

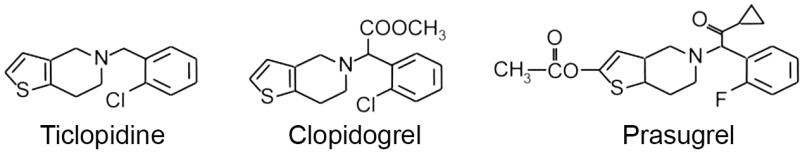

Figure 2. Molecular structures of different thyienopyridines used as anti-platelet drugs.

1.4.3 Third generation $P2Y_{12}$ inhibitors

Despite the success of clopidogrel, patients continued to suffer ischaemic events, albeit to a lesser degree. Also, 15–30% of patients did not respond to clopidogrel treatment[31], in part due to genetic polymorphism of enzymes involved in hepatic metabolism[32]. Also, the relatively slow onset of clopidogrel could be problematic in acute settings[33].

New $P2Y_{12}$ inhibitors continued to be tested in ACS patients, including the thienopyridine prasugrel[34] and also ticagrelor[35], which belongs to a different class of drugs (triazolpyrimidines) (Figure 3).

Prasugrel, like clopidogrel, is a pro-drug that requires conversion to an active metabolite before binding irreversibly to the $P2Y_{12}$ receptor. Compared to clopidogrel, the onset is faster, with maximal effect after 30 minutes. This is due to a simpler metabolism, which occurs both in the liver and intestine. Prasugrel also has a higher and more consistent degree of platelet inhibition, probably related to its simpler metabolism, more rapid conversion to the active metabolite, and the lack of non-responders[36].

DAPT with ASA and prasugrel was compared to DAPT with ASA and clopidogrel in the Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition with Prasugrel–Thrombolysis in Myocardial Infarction (TRITON-TIMI) 38 involving 13,608 ACS patients scheduled for percutaneous coronary intervention (PCI) treatment[34]. A significant reduction in the combined primary endpoint death from cardiovascular causes, non-fatal myocardial infarction (MI), or non-fatal stroke was observed (HR 0.81, 95% CI 0.73–0.90), but with an increased risk of bleeding complications – including fatal bleeding.

One of the inclusion criteria for patients in the TRITON study was a scheduled PCI. Despite this, 448 of 13,608 patients underwent CABG surgery during the study period. A sub-analysis of these patients showed a significant reduction in all-cause death in prasugrel-treated patients (HR 0.26, 95% CI 0.09–0.77), despite there being an increased risk of CABG-related bleeding (13.4% vs. 3.2%; $p < 0.001$)[37].

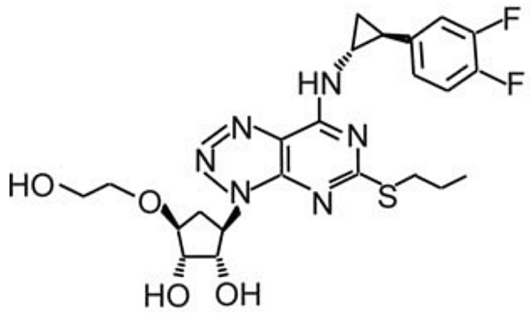

Figure 3. The molecular structure of ticagrelor.

Ticagrelor, yet another inhibitor of the $P2Y_{12}$ receptor, was introduced to the European market in 2010 and the US market in 2011. In contrast to thienopyridines, ticagrelor has a $P2Y_{12}$ receptor binding site that is different from that of ADP, making it an allosteric antagonist, and the blockage is reversible[38].

Ticagrelor directly antagonizes binding of ADP to the $P2Y_{12}$ receptor without the need for any metabolic activation, although at least one metabolite is active, with pharmacological characteristics similar to those of the parent drug. Maximum platelet inhibition is reached after 1–3 hours of oral intake. As ticagrelor binds reversibly, individual platelets may regain their ability to activate through this signalling pathway. Recovery of platelet function is dependent on elimination of the drug and metabolites via hepatic metabolism. The half-life of ticagrelor in plasma is 6–13 hours[36].

Compared to clopidogrel, ticagrelor provides a higher degree of (and much less variable) $P2Y_{12}$ inhibition. This is also true of prasugrel[36]. In addition to platelet inhibition, ticagrelor has other $P2Y_{12}$-independent effects, including inhibition of cellular uptake of adenosine via the membrane-bound protein Equilibrative Nucleotide Transporter (ENT)-1[39]. This increases the circulating levels of adenosine in ticagrelor-treated patients, which may contribute to favourable effects including increased coronary blood flow, improved endothelial function, and reduced ischaemia/reperfusion injury, and may reduce the inflammatory responses[40].

In the Study of Platelet Inhibition and Patient Outcomes (PLATO) from 2009, DAPT with ticagrelor was compared to DAPT with clopidogrel in 18,624 patients with ACS. Again, in patients with more potent anti-thrombotic treatment, the outcome was superior with lower risk of the combined endpoint death from vascular causes, MI, or stroke[35]. Also, 1,899 (10.4%) of the ACS patients included underwent CABG surgery during the study period. In patients who underwent CABG within 7 days after discontinuation of study treatment, all-cause mortality was reduced from 9.7% to 4.7% (HR 0.49, 95% CI 0.32–0.77)[41].

1.4.4 Triple antiplatelet therapy

One study compared the use of vorapaxar, a platelet thrombin receptor antagonist, in addition to standard therapy in ACS patients without STEMI. Standard therapy included DAPT with ASA and a thienopyridine in the vast majority of patients, so most patients with active treatment received triple antiplatelet therapy. The combined primary endpoint death from cardiovascular causes, myocardial infarction, stroke, recurrent ischaemia with re-hospitalization, or urgent coronary revascularization was not significantly better in patients with vorapaxar (HR 0.92, 95% CI 0.85–1.01), despite there being a lower risk of myocardial infarction (HR 0.89, 95% CI 0.81–0.98). The negative results were mainly due to an increased risk of bleeding, including haemorrhagic stroke[42].

1.5 Testing of platelet function

Early in the twentieth century, assessment of platelet function started with the determination of in vivo bleeding time. The technique involves inflicting a small skin wound on the forearm or ear lobe and recording the time required for a clot to form and the bleeding to stop. This technique is simple and there is no need for specialized laboratory equipment or expertise, but the specificity of platelet function and the clinical significance of bleeding time have been questioned, and the method has been replaced by other less invasive techniques.

During the 1950s, accurate platelet counts and description of platelet morphology gained wider usage. Since then, different tests have been developed to assess different aspects of normal platelet function, including tests of platelet-platelet aggregation, detection of activation-dependent changes in the platelet surface, assessment of activation-

dependent release from platelets, and testing of shear-induced platelet adhesion and aggregation[43].

Platelet function tests (PFTs) are used to diagnose both inherited and acquired platelet dysfunction. As the clinical use of antiplatelet drugs has increased, platelet function tests are also used to assess the efficacy of treatment. Theoretically, these tests may be used to identify both patients who have an increased risk of bleeding and patients with sub-optimal drug response and high on-treatment platelet reactivity (HPR).

Widely available viscoelastic methods (such as thromboelastography) that are used to test blood clotting are unable to detect the effect of antiplatelet medications on platelet function[43].

Aggregometry is based on the principle that blood platelets are non-thrombogenic in their resting state, but that upon activation, surface receptors are exposed –enabling adhesion to sites of vascular injury, aggregation of platelets through interaction with fibrinogen, and attachment to artificial surfaces.

Light transmission aggregometry (LTA), or turbidometric aggregometry, was the first laboratory test for platelet-platelet aggregation[44]. It is still regarded as the golden standard of platelet function testing. The test is performed using platelet-rich plasma, which is obtained by centrifuging anticoagulated blood. Platelet-rich plasma is naturally turbid and absorbs light. After addition of platelet agonists to the sample, the platelets aggregate and the transmission of light through the sample increases. The test is calibrated so that 100% corresponds to light levels transmitted through platelet-poor plasma.

LTA is time-consuming and requires a specialized laboratory for complex sample preparation, which makes the technique unsuitable for many clinical situations. Point-of-care (POC) platelet function tests overcome these limitations and allow rapid assessment of platelet function. POC methods that use platelet-platelet aggregation methods include impedance aggregometry, VerifyNow®, and Plateletworks®.

The multiple-electrode impedance aggregometry analyser (Multiplate®; Roche Diagnostics, Basel, Switzerland) uses whole-blood samples. Sample and platelet-stimulating reagent are added to test cells, which incorporates two sets of paired sensor wires and a coated stirring magnet. Platelets adhere to the sensor wires and increase the electrical

resistance between them. The increase in impedance is measured in aggregation units (AU) and plotted against time. The area under the curve (AUC) is a measure of platelet aggregation, and measured in (AU × min), which is then converted to units (U) for simplicity (Figure 4).

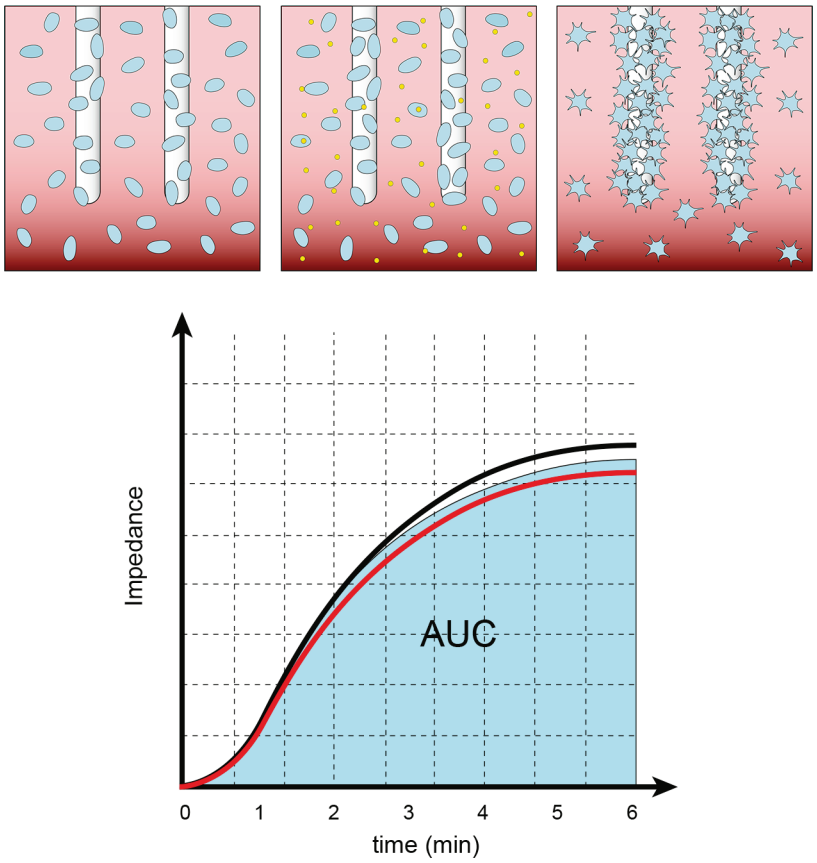

Figure 4. Principles of Multiplate® platelet aggregation testing. Platelets are shown in the blood samples with an electrode pair (top left). After addition of an agonist (top middle), platelets are activated and aggregate at the electrodes (top right). The aggregation of platelets increases the electrical resistance between electrodes. Two electrode pairs continuously record two seperate aggregation curves (bottom). The average aggregation curve is used to calculate the area under the curve (AUC) wich is an estimate of platelet reactivity.

Several specific tests are available for stimulation of different receptors or signal transduction pathways, including:

- ASPI test: arachidonic acid (AA) is added for formation of the potent platelet agonist thromboxane A2 via the COX enzyme. If the COX enzyme is inactivated (i.e. by ASA or anti-inflammatory drugs) platelets will not be activated.
- ADP test: ADP activates platelets by stimulation of platelet ADP receptors, including the $P2Y_{12}$ receptor.
- TRAP test: thrombin receptor-activating peptide-6 stimulates the thrombin receptors PAR-1 and PAR-4 on the platelet surface. This signalling is not blocked by ASA or $P2Y_{12}$ inhibitors, thus allowing TRAP tests to detect the effect of GpIIb/IIIa receptor inhibitors in blood samples from patients treated with ASA or $P2Y_{12}$ inhibitors.

Impedance aggregometry may predict stent thrombosis and bleeding after PCI treatment[45 46], platelet transfusion in adult cardiac surgery patients[47], and postoperative bleeding in patients undergoing cardiac surgery with preoperative thienopyridine treatment[48].

1.6 Cardiac surgery

Surgery on the heart was first described in case reports of traumatic stab wounds. The first successful repair is attributed to Dr Ludwig Rehn of Frankfurt, Germany. In 1896, a 22-year-old patient suffered a penetrating trauma to the right ventricle and presented with severe shock. During surgery, a 1.5-cm stab wound was identified on the right ventricle and three sutures were placed. Full recovery followed, and Dr Rehn concluded his remarks with the following: *"I hope this will lead to more investigation regarding surgery of the heart. This may save many lives."*[49]

After the combined efforts of the medical and engineering professions, the first successful operation with the aid of a heart-lung machine was performed by John Gibbon on 6 May 1953, on an 18-year-old woman with heart failure due to atrial septal defect[50]. Dismayed by subsequent failed operations, largely due to imprecise preoperative diagnoses, Gibbon soon stopped performing cardiac operations, but other

surgeons continued to refine the heart-lung machine and to use it to operate on patients with various cardiac pathologies.

With increasing insight that ischaemic heart disease and angina pectoris were conditions associated with reduced myocardial blood flow, novel methods were developed to restore myocardial perfusion. Claude Beck, a Cleveland surgeon, developed methods to restore blood flow to animal hearts by attaching adjacent tissue, such as pectoral muscles and the omentum. He also experimented with grafting of the internal mammary artery to the coronary venous system[51]. Implantation of the internal mammary artery into a tunnel of ischaemic left ventricle myocardium was performed in several cases by the Canadian surgeon Arthur Vineberg[52]. After the development of angiography, patent grafts were amazingly demonstrated with communications to the coronary system, and case reports have demonstrated patent grafts up to 30 years after surgery[53].

With the advent of coronary angiography, it became possible to plan coronary grafting and assess the results postoperatively[54]. Endarterectomy and patching of coronary arteries with pericardium or saphenous veins were tested, and sporadic cases of aorto-coronary grafting were reported during the 1960s as an alternative or bail-out method after difficulties with these procedures. It was Favaloro and Effler at the Cleveland Clinic who developed the CABG operation similar to what is used today[55]. They performed their first operation using saphenous vein graft conduit in 1967. Three years later, the internal mammary artery was being used to graft the left anterior descending artery (Figure 5).

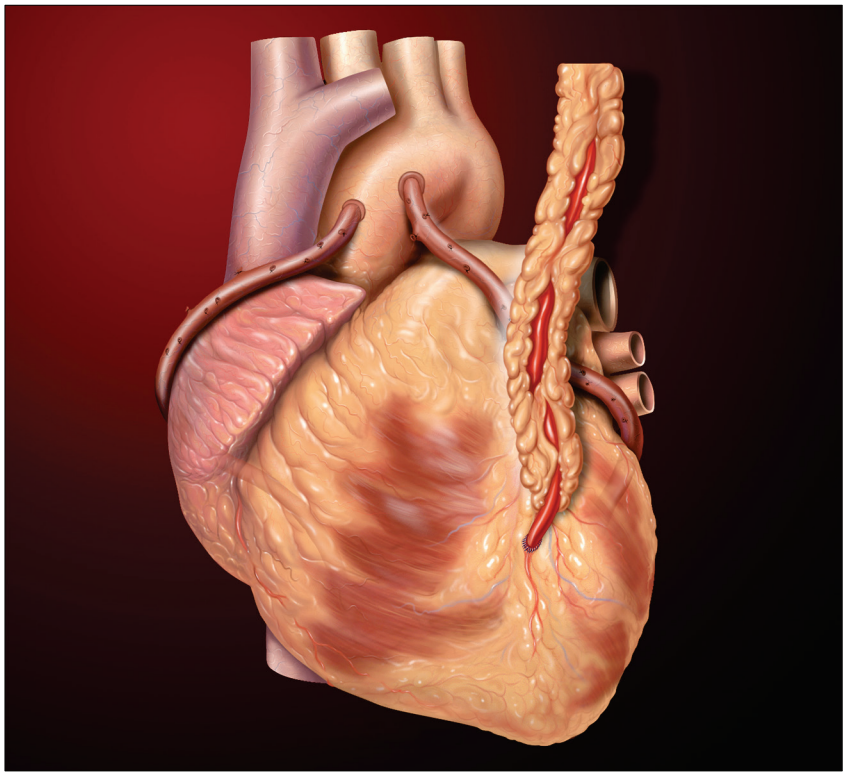

Figure 5. Coronary artery bypass grafting with internal mammary artery and saphenous vein grafts. Illustration by Patrick J. Lynch, medical illustrator; C. Carl Jaffe, MD, cardiologist.

Preoperative diagnostics, surgical techniques, perioperative care, and pharmacological treatments have evolved during the last five decades[51], with improved survival and lower complication rates –despite the fact that the patients' age and risk factors have increased in recent years[56].

1.6.1 Bleeding after cardiac surgery

All open-heart surgery is associated with some degree of perioperative bleeding, due to both surgical trauma and impaired haemostasis. Several factors are associated with impaired haemostasis, including the preoperative use of antithrombotic drugs[57], haemodilution caused by prime volume in the cardiopulmonary bypass (CPB) circuit[58], reduced platelet count and dysfunction after CPB, enhanced fibirinolysis, and hypothermia during surgery[59].

Excessive bleeding is widely recognized as being a risk factor after cardiac surgery[60 61]. This has been reported using a number of different variables, including postoperative chest-tube output[62], transfusion of blood products[63], and re-operation for bleeding or tamponade[64].

In addition to using a single variable to estimate bleeding, a number of clinical studies have defined composite endpoints, as exemplified in Table 1. Although definitions for many other complications after cardiac surgery exist[65], no standardized definition of perioperative bleeding has been established, making the interpretation and comparison of clinical trials more difficult.

Of these composite endpoints, only the UDPB class has been externally evaluated for prediction of mortality[65]. Using a single-centre cohort of 1,144 adult patients, mortality was assessed after adjusting for other relevant risk factors. Bleeding class remained an independent predictor of 30-day mortality, with an eightfold increase in risk in patients with severe bleeding compared to patients with insignificant bleeding.

BART[66]	BARC[67]	PLATO CABG[35]	UDPB[65]
CTO > 1.5 liter (any 8-h period)	CTO ≥ 2 L/24 h	Drop in Hb ≥ 5.0 g/dL	CTO > 1 L/12 h
> 10 RBCs/24 h	≥ 5 U whole blood/RBCs/48 h	≥ 4 RBCs	≥ 5 RBCs/24 h ≥ 5 FFP/24 h
Re-operation for bleeding	Re-operation for bleeding	Re-operation for bleeding	Re-operation for bleeding
Fatal bleeding	Intracranial bleeding	Fatal bleeding	Delayed sternal closure
			Use of rFVIIa

Table 1. Definitions of major bleeding. CTO = chest tube output; Hb = haemoglobin RBC = red blood cell; FFP = fresh frozen plasma; rVIIa = recombinant activated factor VII (NovoSeven®).

1.7 Preoperative APT and risk of bleeding

Patients undergoing cardiac surgery with ongoing or recently discontinued DAPT have an increased risk of bleeding[68], which is associated with poor clinical outcome[65]. Despite the risk of bleeding, patients with ongoing ischaemia or haemodynamic instability should be

operated without delay, regardless of the discontinuation time of the DAPT[69].

In stable patients, $P2Y_{12}$ inhibitors should be discontinued for an adequately long period to allow recovery of platelet function. Current guidelines recommend five days for clopidogrel, seven days for prasugrel, and five days for ticagrelor[69 70]. These recommendations are based on the pharmacological properties of the $P2Y_{12}$ inhibitors[36], the recovery of platelet function after drug discontinuation in patients with stable angina[71], and experience from CABG subgroups in randomized trials[37 41 72]. A nationwide Swedish study showed that discontinuation of the platelet inhibitor three days before surgery, as opposed to five days, did not increase the incidence of major bleeding complications in ticagrelor-treated patients undergoing CABG[73], supporting the implementation of a shorter discontinuation time in these patients.

In semi-elective and urgent cases with increased risk of thrombosis (i.e. patients with critical coronary anatomy or previous recurrent episodes of ischaemia), case-by-case decisions should be made to balance the risk of thrombosis and the risk of bleeding. There are no clear recommendations on how to assess the risk of recurrent ischaemia in the stabilized patient with an ACS, although discontinuation of $P2Y_{12}$ inhibitor treatment might be associated with an increased risk of death and myocardial infarction[74].

Time since discontinuation may be used to assess bleeding risk, but there is individual variation in the magnitude and duration of the anti-platelet effect of $P2Y_{12}$ inhibitors[36 71]. An individualized assessment based on platelet function shortly before operation might therefore be preferable to the discontinuation time for prediction of the risk of bleeding complications. This approach is endorsed in the European re-vascularization guidelines, which state that platelet function testing should be used to guide interruption of antiplatelet therapy, rather than using an arbitrary specified period of delay in patients undergoing CABG surgery[69]. However, this statement is based on limited data[75 76], as indicated by a C level of evidence. Evidence in support of this approach has so far been published only for thienopyridine-treated CABG patients[48 77], with no data being available for patients treated with the newer and more efficient platelet inhibitor ticagrelor.

1.8 Antiplatelet therapy after CABG surgery

Excessive bleeding –not thromboembolic complications– has been the major haemostatic concern during the early postoperative period. However, thrombotic complications are not uncommon after cardiac surgery and may, at least in part, be avoided with optimal perioperative care[78].

Early restart of ASA after CABG surgery is associated with reduced 30-day mortality and ischaemic complications of the heart, brain, kidneys, and intestines[79]. During the first postoperative year, vein graft patency is improved[80]. Today, low-dose ASA is recommended and used in the absence of contraindications in nearly all CABG patients[69 81].

Whether more intense antiplatelet therapy, such as DAPT, benefits the subset of ACS patients who undergo CABG surgery (approximately 10%[82]) has not been adequately studied, but a number of indications support the hypothesis. For instance, platelet and coagulation systems are activated in ACS patients[83]; ACS patients often have multiple vulnerable coronary artery plaques in addition to the culprit lesion[84]; and vein graft conduits are at risk of failure through thrombosis, intimal hyperplasia, and accelerated atherosclerosis[85], associated with worse outcome[86].

There have been no large randomized controlled trials, but two meta-analyses have reported the outcome when intensified antiplatelet therapy was used after CABG. The first meta-analysis included a total of 25,728 patients from both randomized and observational studies[87] comparing DAPT with ASA + clopidogrel to single therapy with only ASA. DAPT was associated with better early vein graft patency (RR 0.59, 95% CI 0.43–0.82; $p = 0.02$) and also lower 30-day mortality (0.8% vs. 1.9%; $p < 0.0001$). Long-term survival was not reported.

The second meta-analysis studied the effect of intensified antiplatelet therapy after CABG, using somewhat overlapping data compared to the previous meta-analysis. Altogether, 4,829 patients from (1) five randomized controlled trials on elective CABG patients (a total of 979 patients) and (2) four sub-studies of CABG-treated patients from large randomized controlled trials (RCTs) of ACS (a total of 3,850 patients) were included in the meta-analysis. In patients with more intense antiplatelet therapy, there was a trend of lower all-cause mortality (RR

0.68, 95% CI 0.43–1.08), despite an increased risk of major bleeding (RR 1.31, 95% CI 0.81–2.10)[88].

One additional study, not included in either of the meta-analyses, compared DAPT with ASA + ticagrelor to ASA only during the first 3 months after isolated CABG[89]. Primary outcome was graft occlusion on CT angiography (CTA), performed three months after surgery. The study was underpowered, due to premature termination because of slow recruitment. Nevertheless, due to larger benefit than expected on graft patency, ticagrelor treatment was associated with a significant reduction in graft occlusion (7/25 patients vs. 17/31; p = 0.044). The study was not powered to compare clinical endpoints of cardiovascular or bleeding events.

Current guidelines recommend starting DAPT for ACS patients undergoing CABG "as soon as considered safe" after surgery, with a recommended duration of 12 months (class 2A, level C recommendation)[69 90]. The reported adherence to the recommendation has been low, with the use of DAPT in only 20–45% of patients after CABG[91-93].

1.9 Fibrinolysis and cardiac surgery

The use of CPB results in increased plasma levels of tPA[94], with subsequent generation of plasmin[59] and increased fibrinolysis.

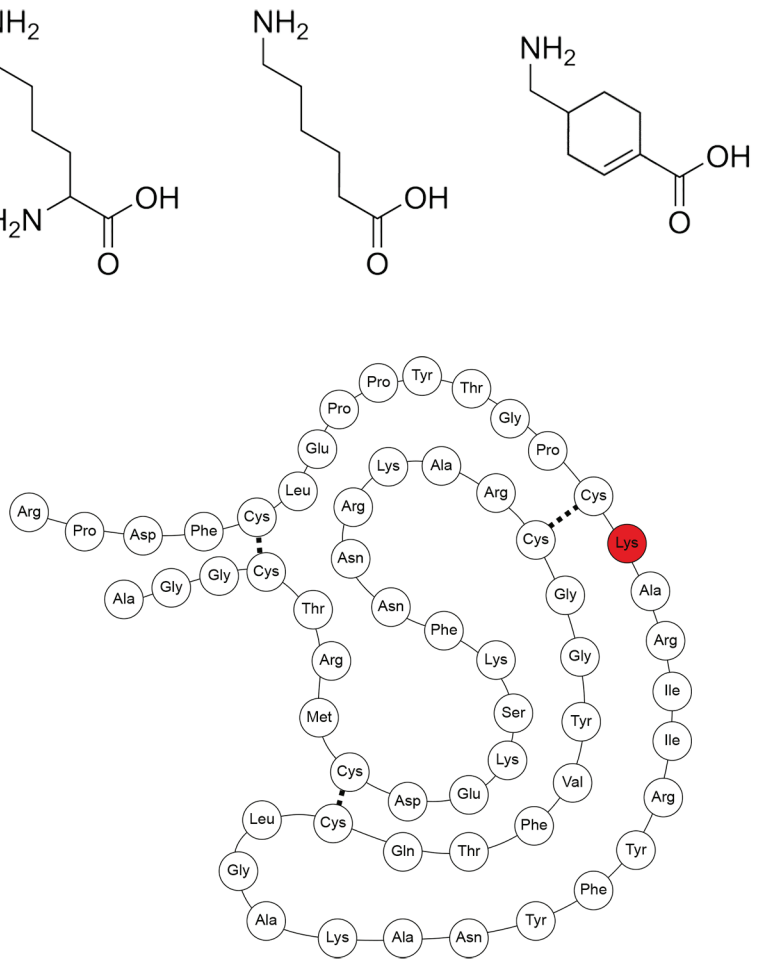

Figure 6. The chemical structure of lysine (top left), epsilon amino caproic acid (top middle), and tranexamic acid (top right). The 58 amino acids of the more complex polypeptide aprotininl are also shown (bottom). The inhibition of plasmin is associated with the 15th amino acid lysine (highlighted in red), which acts as a binding site for most of the serine proteases that it inhibits.

The antifibrinolytic drugs tranexamic acid (TA), epsilon amino caproic acid (EACA), and aprotinin can reduce hyper-fibrinolysis. TA and EACA are small molecules with structures similar to lysine, while aprotinin is a more complex molecule consisting of 58 amino acids (Figure 6). TA, EACA, and aprotinin (through a lysine amino acid) all bind to the lysine-binding site of plasmin, thereby hindering the association and subsequent degradation of fibrin (Figure 7).

Aprotinin has additional biological effects by inhibition of other serine proteases[95]. It has also been reported that aprotinin improves platelet function in different settings, including the restoration of platelet function deficits induced by long-term storage or CPB[96], attenuation of eptifibatide-induced platelet inhibition[97], and improvement of the ADP-dependent platelet activation in CABG surgery patients[98].

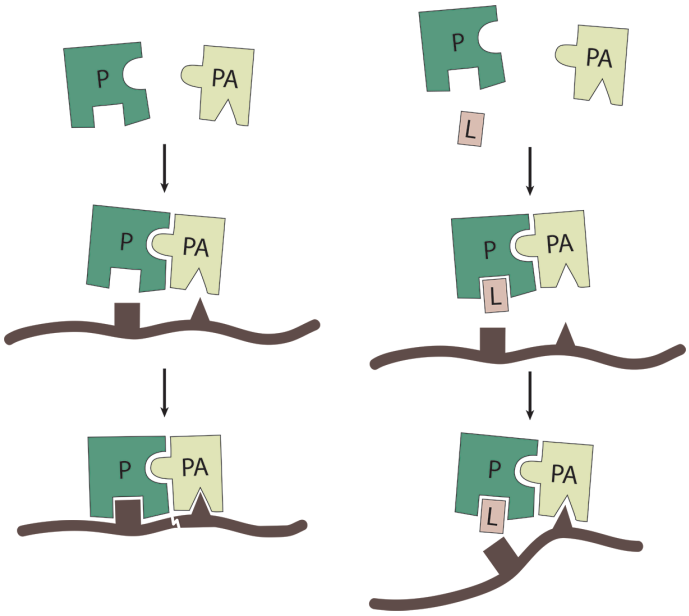

Figure 7. The mechanism of normal fibrinolysis (left) and inhibition with anti-fibrinolytic drug (right). P = plasminogen; PA = plasminogen activator; L = lysine analogue.

The use of antifibrinolytic drugs in cardiac surgery dates back to the early 1960s[99]. In 1989, a placebo-controlled study on a series of 350 patients found that prophylactic use of EACA decreased postoperative chest-tube drainage and transfusion without increasing thrombotic complications[100]. Since then, there have been other, similar reports[101]. Of the three antifibrinolytic drugs, aprotinin has the strongest effect on blood loss and transfusions[102].

The routine use of antifibrinolytics is debated, especially in low-risk patients[103 104]. Despite aprotinin's greater ability to reduce blood loss, a large, randomized controlled trial comparing the three drugs was terminated early because of an increase in mortality in those treated with aprotinin[66]. This led to the withdrawal of aprotinin from the market in 2008. Later reviews revealed problems with the BART study[105 106], and in Europe and Canada, the suspension of aprotinin was subsequently lifted for CABG patients.

Today, it is unclear which cardiac surgery patients would benefit from aprotinin treatment, but patients with ongoing or recently stopped DAPT are one possible subgroup.

2 AIM

The goal of this project was to increase the understanding of how patients with antiplatelet therapy considered for CABG are best managed, and also investigate if different postoperative antiplatelet treatment strategies affect postoperative survival after CABG in ACS patients.

Seven specific aims have been defined:

1. To describe the recovery of platelet function after withdrawal of dual antiplatelet therapy with ASA and ticagrelor treatment in patients with ACS (paper I)
2. To investigate the efficacy of platelet transfusion at consecutive time points after withdrawal of ticagrelor in patients with ACS (paper I)
3. To investigate if preoperative PFT using impedance aggregometry can predict the risk for bleeding complications in patients with recently discontinued ticagrelor treatment (paper II)
4. To investigate if aprotinin or tranexamic acid improves platelet function in patients with ongoing DAPT with ticagrelor and ASA (paper III)
5. To describe the prevalence of DAPT in ACS patients after CABG in Sweden (paper IV)
6. To investigate if there is a difference in one-year all-cause mortality in patients with ACS undergoing CABG discharged with DAPT using ASA and clopidogrel or ticagrelor compared to ASA alone (paper IV)
7. To investigate if there is a difference in one-year all-cause mortality in patients with ACS undergoing CABG discharged with DAPT using ASA and ticagrelor compared to ASA alone (paper IV)

3 PATIENTS AND METHODS

3.1 Patients

All studies were conducted in accordance with the Declaration of Helsinki, and were approved by the regional ethics review board at the University of Gothenburg. The ethics review board waived the need for informed consent in study II and study IV. In study I and III the patients were included after obtaining written informed consent.

All patients in the studies were hospitalized for ACS. In papers I, II and IV, all patients underwent cardiac surgery as treatment for the ACS, while patients in study III had different treatment strategies including medical, percutaneous coronary intervention (PCI), and surgery. Patient characteristics are summarized in Table 2.

	Paper I	Paper II	Paper III	Paper IV
n	25	90	30	5183
Age (years)	68 ± 9	68 ± 9	65 ± 9	67 ± 9
Female gender	2 (8%)	18 (20%)	4 (20%)	1061 (20%)
Study period	Jan 2013 – May 2015	Oct 2012 – Apr 2015	Mar 2014 – Mar 2016	Jan 2012 – Dec 2015
Hb (g/L)	139 ±14	137 ± 15	142 ± 3	137 ± 15
PLT ($x10^9$/L)	236 ± 63	246 ± 64	271 ± 26	NA
s-Cr (µmol/L)	92 ± 18	92 ± 32	92 ± 6.8	91 ± 56
Diabetes	3 (12%)	25 (28%)	8 (27%)	1415 (27%)
ASA + clopidogrel	0	0	0	447 (9%)*
ASA + ticagrelor	25 (100%)	90 (100%)	30 (100%)	896 (17%)*

*Table 2. Patient characteristics. Number with percentage or mean ± SD; Hb = Haemoglobinb; PLT = Platelet count; s-Cr = Serum-creatinine; ASA = Acetylsalicylic acid; * treatment at discharge*

3.2 Methods

3.2.1 Dual antiplatelet therapy

Patients with DAPT were studied in all four papers. In papers I, II and III, only patients with DAPT using ASA and ticagrelor were included. In

paper IV, patients treated with both ASA and ticagrelor and ASA and clopidogrel were included.

3.2.2 Platelet function testing

Multi-electrode impedance aggregometry (MEA; Multiplate®; Roche Diagnostics, Basel, Switzerland) was used to test platelet function in papers I, II and III. Blood was sampled from peripheral veins and collected in test tubes with hirudin anticoagulation. Three different tests were used to activate platelets: the ADP-HS (high sensitivity) test, which uses ADP in combination with prostaglandin E1 to assess $P2Y_{12}$-dependent aggregation (papers I, II, III), the ASPI test (papers I, II, III) and the TRAP test (papers I, II).

The manufacturer's normal range when using hirudin anticoagulated blood is 43–100 U for the ADP-HS test, 71–115 U for the ASPI test and 84–128 U for the TRAP test. If the difference between the two electrode pairs was greater than 20% the analysis was considered flawed and repeated.

3.2.3 Platelet supplementation

Ex vivo supplementation with platelets was done in study I and III using ABO-compatible apheresis platelets from the local blood bank. Supplemented doses were adjusted to correspond to clinical relevant in vivo doses (2–4 units of apheresis platelets transfused to a 70 kg patient).

3.2.4 Paper I

Paper I is divided in two sub-studies. In sub-study I the recovery of platelet function was studied in 25 ACS patients with DAPT awaiting CABG surgery. Ticagrelor treatment was stopped upon acceptance for CABG, and MEA was subsequently done at five time points: after 12, 24, 48, 72 and 96 hours.

In sub-study II, samples from 15 of the 25 patients were supplemented with either a low or high dose of platelet concentrates corresponding to 2 or 4 units of transfused apheresis platelets. Platelet supplementation and testing was performed at the same time points as in sub-study I.

3.2.5 Paper II

Ninety ACS patients with DAPT requiring acute or urgent cardiac surgery were included in a prospective observational study. All patients had ongoing or recently (< 5 days) discontinued ticagrelor treatment and ongoing ASA treatment at the time of surgery. The decision to operate despite the ticagrelor discontinuation time being shorter than recommended was made by a heart team, including a senior cardiac surgeon, the treating cardiologist and a and a senior cardiac interventionist.

Blood samples were collected immediately before surgery and analysed with MEA using ADP-HS, ASPI and TRAP tests. Operative data, chest tube output, incidence of re-exploration for bleeding, transfusion of blood products and the use of pro-haemostatic drugs was collected from hospital records. Severe bleeding according to UDPB criteria (one or more of the following: chest tube output > 1 L during the first 12 hours; transfusion of ≥ 5 units of red blood cells or ≥ 5 units of fresh frozen plasm during the first 24 hours; re-operation for bleeding within 24 hours; delayed sternal closure; or use of recombinant factor VII) was compared for different preoperative values of the aggregation tests, and the accuracy for predicting severe bleeding was explored using receiver operating characteristic (ROC) curves.

3.2.6 Paper III

Blood samples were obtained from 30 patients hospitalized due to ACS. All patients had ongoing DAPT and had received at least two 90 mg doses of ticagrelor at the time of blood sampling.

Each sample was divided in five portions. First, a portion for baseline platelet function was secured. Two portions were supplemented with either a low or high dose of aprotinin, corresponding to the clinical use of half or full Hammersmith regimen[107]. The last two portions were supplemented with different doses of TA, corresponding to intravenous bolus doses of either 1 g or 2 g. ADP- and AA-induced aggregation was assessed for all portions.

In a subset of 20 patients, a part of each portion was also supplemented with platelet concentrates corresponding to 3 units of transfused apheresis platelets, after which the same analyses were performed.

3.2.7 Paper IV

Data from the SWEDEHEART registry was collected for all patients undergoing isolated CABG surgery in Sweden during 2012 – 2015. Data included > 100 patient variables such as preoperative risk factors, operative variables, postoperative complications, and medications at discharge. Due to incomplete data of medications at discharge from four centres (Karlskrona, Lund, Umeå, Örebro) manual retrieval of this information was done from hospital records.

6,020 patients with ACS undergoing isolated CABG was identified during the study period. Patients with in-hospital mortality, patients discharged with anticoagulation or rare combinations of antiplatelet therapy and foreign patients without mortality follow-up data were excluded. Thirteen patients underwent two isolated CABG procedures during the study period, and their first procedure was excluded from the analysis. The remaining 5,183 patients were divided in three groups according to their antiplatelet medication at discharge: ASA only (n=3,840), DAPT using ASA and clopidogrel (n=447), and DAPT using ASA and ticagrelor (n=896) (Figure 8).

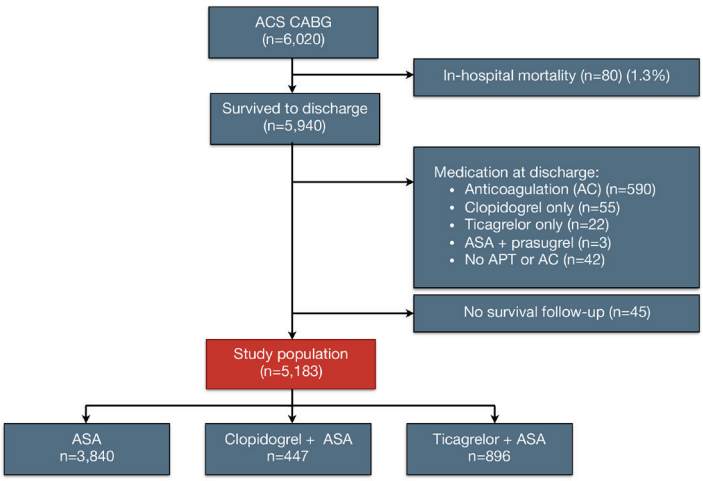

Figure 8. Flow chart for paper IV.

Mortality data during the first year after surgery was derived from the Swedish national population registry, which is 100% complete (excluding 45 patients that were not Swedish citizens). Mortality was

then compared between the different treatment groups using both crude and propensity score matched data.

3.3 Statistical analyses

For all studies, the data was presented as mean with standard deviation (SD), mean with standard error of the mean (SEM), median with range or interquartile range (IQR), or frequency with percent. Normality of data was tested with the Shapiro–Wilk test (papers I – III). Statistical significance was assumed when the two-sided p-value was < 0.05.

All statistical analyses were made with computer software (IBM SPSS Statistics software ver. 22 – 23, IBM Corp. Armonk, NY, USA; Prism 6.0, GraphPad Software Inc., La Jolla, CA, USA; SAS Software version 9.4, SAS institute, Cary, NC, USA; R software, version 3.0.3, http://www.R-project.org/; and Stata 13, StataCorp, College Station, TX, USA)

3.3.1 Paper I

Sub-study I was regarded as a descriptive pilot study. For sub-study II, 15 pairs of samples gave 80% power with a two-sided test to detect a difference of 10 U in ADP-induced platelet aggregation when platelet concentrate was added, at $\alpha=0.05$ and a standard deviation of 12 U.

Change in platelet aggregation over time compared with baseline was tested with a general linear model for repeated measurements. A mixed effects model for repeated measurements was used to analyse efficacy of platelet concentrate addition and interaction between time point and dose of platelets. No formal adjustment for multiplicity was assumed necessary.

3.3.2 Paper II

Categorical variables were compared using Fisher's exact test. Continuous variables were compared with Student's unpaired t-test (normally distributed data) or the Mann–Whitney U-test (non-parametric data).

The accuracy of aggregability tests for predicting severe bleeding was explored using a receiver operating characteristic (ROC) curve, which yielded an area under the curve (AUC) with its accompanying 95% CI. Youden's index, J = (sensitivity + specificity – 1), was defined for all

points on the ROC curve. The maximal Youden's index value was used as a criterion for selecting the optimal cut-off point.

Positive predictive value (PPV) and negative predictive value (NPV) were calculated according to standard methods. The association between the preoperative ADP HS test value and the probability of severe bleeding was explored with logistic regression analysis.

3.3.3 Paper III

Twenty pairs of samples gave a power of 84% with a 2-sided test to detect a difference of 25% in ADP-induced platelet aggregation (baseline aggregation=13 U), at α=0.05 and a standard deviation of 3.5.

The aggregometry results after addition of different supplements were compared to baseline results using Student's paired t-test.

3.3.4 Paper IV

All-cause mortality was compared with univariable and multivariable Cox proportional hazards regression models applied to unmatched and propensity score (PS) matched data. The PS matching was based on a 27 pre- and perioperative covariates and performed separately for each pairwise comparisons. The covariates included 18 variables used in Euroscore II risk model[108]. Data imputation was performed for two of these variables, postoperative circulatory support and pulmonary hypertension, where "0" was imputed when data were missing. In addition, preoperative haemoglobin, type of acute coronary syndrome (UA, nSTEMI or STEMI) and the following postoperative complications were included as covariates: postoperative circulatory support, new dialysis, new stroke, reoperation for bleeding, reoperation for mediastinitis, prolonged mechanical ventilation (> 48 h), and postoperative atrial fibrillation.

The PS matching was performed with the MatchIt package in R 3.03. Greedy matching method was used with up to three control patients matched to each treated patient and a calliper of 0.2 (i.e. the difference in PS between two matched patients is at most 0.2 SD of the PS)[109].

Ten variables were included in the multivariable Cox proportional hazard regression analysis: antiplatelet treatment, log Euroscore II, type of ACS, postoperative circulatory support, new dialysis, new stroke,

reoperation for bleeding, reoperation for mediastinitis, prolonged mechanical ventilation (> 48 h), and new atrial fibrillation.

4 RESULTS

4.1 Platelet function after ticagrelor discontinuation

In the first sub-study of paper I, mean ADP-induced platelet aggregation increased gradually after the discontinuation of ticagrelor treatment. After 96 hours, the mean level of aggregation was 55 ± 31 U compared to 10 ± 8 U at 12 hours after discontinuation ($p < 0.001$, Figure 9 A). There was a high degree of inter-individual variability in recovery of platelet function (Figure 9 B). AA- and TRAP-induced platelet aggregation also increased significantly with time, albeit to lesser extent (Figure 9 A).

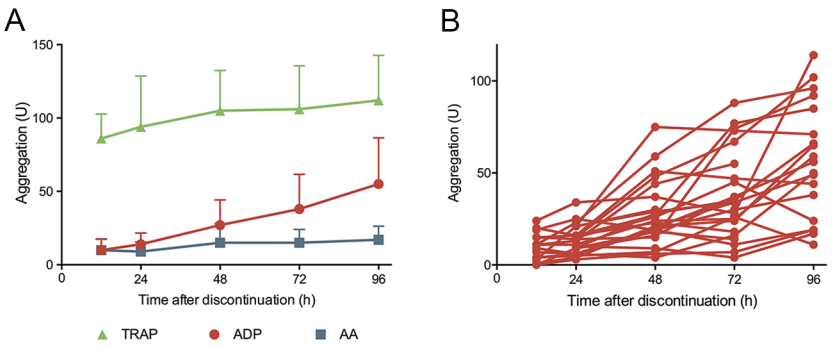

Figure 9. A. Mean ADP-, AA- and TRAP-induced platelet aggregation at consecutive sampling time points after ticagrelor discontinuation. B. ADP-induced aggregation of individual patients at consecutive sampling time points after ticagrelor discontinuation.

In paper II, there was a weak linear correlation between the time since last dose of ticagrelor and ADP-induced platelet aggregation (R^2=0.30, P<0.001). Individual aggregation values had a wide spread around the linear regression line (Figure 10 B).

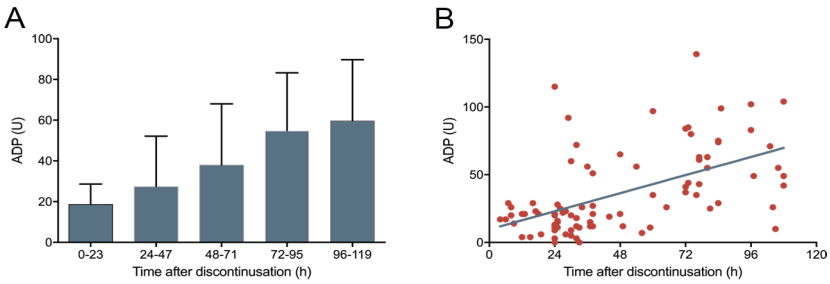

Figure 10. A. Preoperative ADP-induced aggregation grouped in five intervals of different discontinuation times. B. Scatter plot of ADP-induced aggregation and time after discontinuation of ticagrelor. There was a weak linear correlation between the plotted variables (R^2=0.30, P<0.001).

4.2 Platelet supplementation after ticagrelor discontinuation

In the second sub-study of paper I, supplementation with low or high dose of platelet concentrate did not increase the ADP-induced platelet aggregation at any time point (Figure 11 A). In contrast, AA-induced aggregation was markedly increased at all time points after supplementation using the low dose of platelets (p<0.001), with an even higher increase using the higher dose (+21 U, p = 0.0013) (Figure 11 B). TRAP-induced platelet aggregation was significantly increased after addition of the higher dose of platelet concentrate (11 U; p=0.0058), but not with the lower dose (p=0.59) (Figure 11 C).

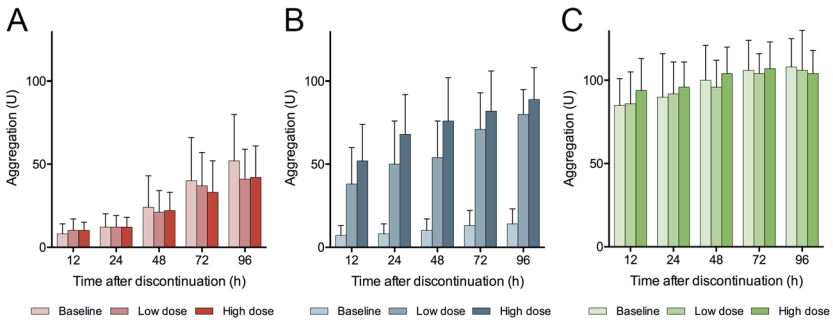

Figure 11. Mean platelet aggregation at consecutive time points after ticagrelor discontinuation at baseline and after supplementation of low and high dose platelet concentrate. A. ADP-induced platelet aggregation. B. AA-induced platelet aggregation. C. TRAP-induced platelet aggregation. Whiskers denote upper standard deviation.

4.3 Risk of bleeding in ticagrelor-treated patients

In paper II, the median time between last dose of ticagrelor and start of surgery was 35 h (4-108 h). Thirty-two of 90 (36 %) patients suffered severe bleeding according to the UDPB criteria.

Patients with severe bleeding had a lower median preoperative ADP-induced platelet aggregation compared with non-bleeders (17 vs 32 U, $p < 0.001$), but no significant difference in AA- and TRAP-induced aggregation.

The accuracy of platelet function tests to predict severe bleeding was highest for the ADP-HS test, with an area under the ROC curve of 0.73 (95% CI 0.63–0.84). Corresponding values for the TRAP- and ASPI-tests were significantly lower (TRAP 0.61, 95% CI 0.49–0.74; ASPI 0.53, 95% CI 0.40–0.66).

The maximal Youden's index for ADP-induced aggregation was 22 U. Using this cut-off value, the sensitivity was 75% and specificity 76% in our cohort. Patients with an ADP-test below the cut-off (<22 U) had greater median postoperative bleeding volume (660 vs 470 ml, $p = 0.004$), higher incidence of re-exploration for bleeding (16 vs 4 %, $p = 0.066$), and were more likely to receive transfusions of RBCs (76 vs 58

%, p = 0.076), plasma (66 vs 21 %, p < 0.001), and platelets (76 vs 27%, p < 0.001). The distribution of the patients preoperative ADP-induced aggregation and the individual values of patients with severe bleeding is shown in Figure 12.

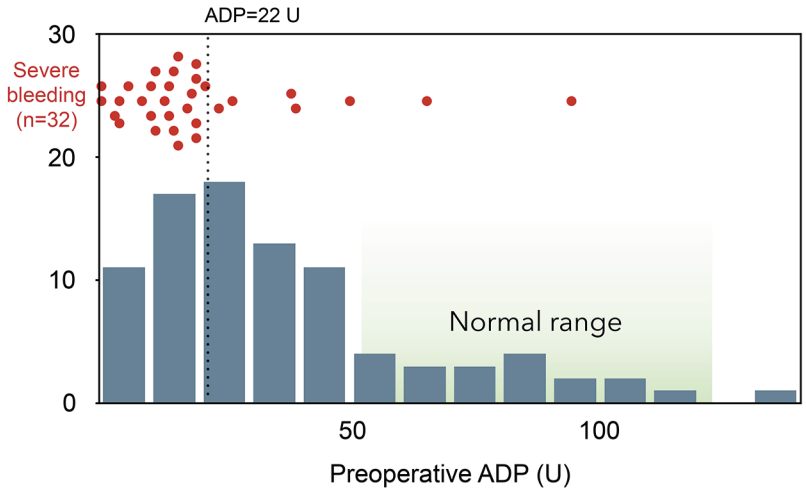

Figure 12. The distribution of the ADP- induced aggregation values. The upper portion of the figure shows a scatter plot of the ADP-induced aggregation of the subjects with severe bleeding. The normal range of ADP-induced aggregation (43–100 U) is marked by a green background.

Median closure time (time from weaning off CPB to skin closure) was 88 min in subjects with ADP-induced aggregation < 22 U, compared with 52 min in subjects with ADP ≥ 22 U (p < 0.001). Using univariable logistic regression analysis, a model was fitted to predict the risk for severe bleeding complications depending on the preoperative ADP-induced aggregation. Low preoperative ADP-induced aggregation was associated with a markedly increased risk of bleeding (Figure 13).

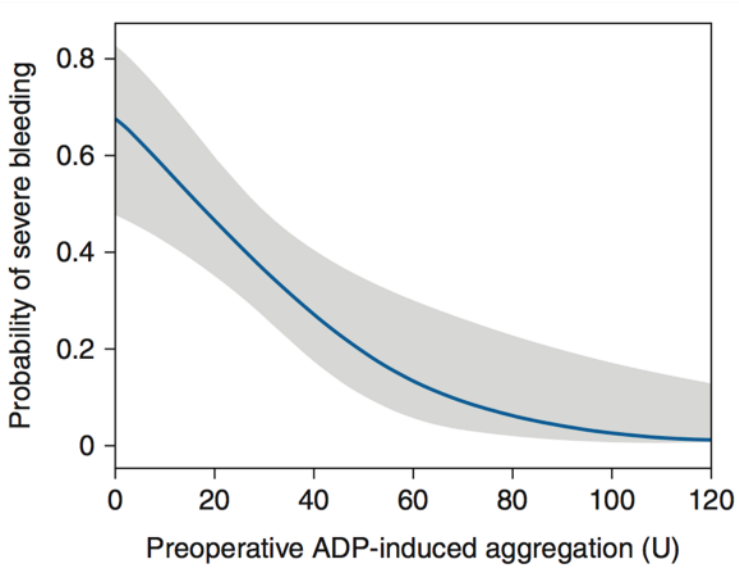

Figure 13. The probability of severe bleeding in relation to the preoperative ADP-induced aggregation. The grey area corresponds to the 95% confidence interval.

4.4 Influence of aprotinin or tranexamic acid on platelet function

In paper III, the addition of aprotinin increased the ADP-induced aggregation compared to baseline levels in whole blood samples from ACS patients with ongoing ASA and ticagrelor treatment. Low dose aprotinin increase aggregation with 20.4 ± 6.0 % ($p = 0.004$), and high dose increase with 22.6 ± 5.4 % ($p < 0.001$). The addition of TA did not alter ADP-induced aggregation (low dose +3.2 ± 7.5 %, $p = 0.55$; high dose –5.3 ± 6.3 %, $p = 0.50$) (Figure 14).

Figure 14. Changes from baseline in ADP-dependent platelet aggregation after addition of low or high dose aprotinin and tranexamic acid. Apr = aprotinin; TA = tranexamic acid.

Similar to the results from paper I, the addition of platelets did not significantly change ADP-induced aggregation (+11.8 ± 5.0 %, p = 0.12). The addition of both platelets and aprotinin increased ADP-induced aggregation from baseline to a similar degree as when only aprotinin was used: platelets and low dose aprotinin +23.5 ± 7.3 % (p = 0.003); platelets and high dose aprotinin +26.0 ± 9.4 % (p = 0.016).

The combination of TA and platelets did not have any effect on ADP-induced aggregation (low dose +8.6 ± 7.6 %, p = 0.41; high dose +1.6 ± 5.3 %, p = 0.79).

AA-induced aggregation did not significantly change after addition of aprotinin compared to baseline (low dose +44.6 ± 22.4 %, p = 0.066; high dose +30.2 ± 17.5 %, p = 0.32). The addition of TA slightly decreased AA-induced aggregation compared to baseline in both low dose (–4.7 ± 12.6 %, p = 0.010) and high dose (–18.6 ± 10.3 %, p = 0.002).

In line with the results in paper I, the addition of platelets, alone or in combination with either antifibrinolytic agent markedly increased AA-induced aggregation compared to baseline (figure 15).

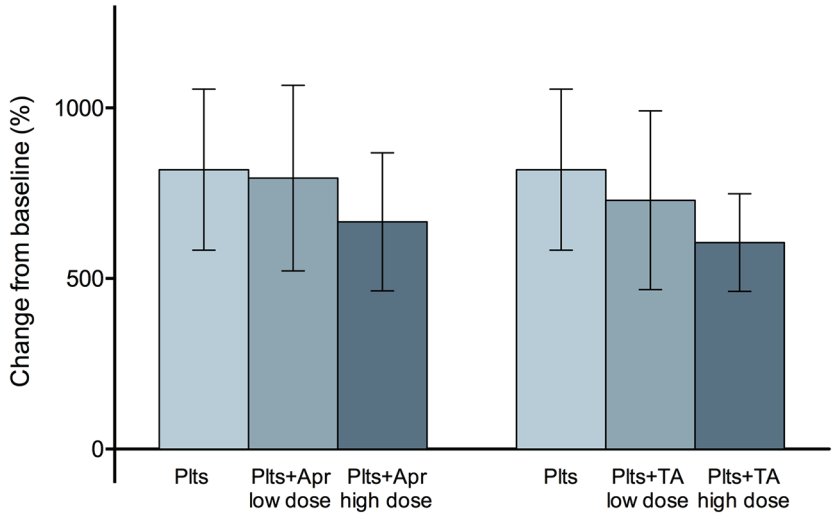

Figure 15. Changes from baseline in AA-dependent platelet aggregation after addition of platelets in combination with low or high dose aprotinin or in combination with low or high dose TA. Plts = platelets; Apr = aprotinin; TA = tranexamic acid.

4.5 Prevalence of DAPT after CABG surgery

After exclusion of patients with anticoagulation, the antiplatelet treatment at discharge was only ASA in the majority of cases (n = 3,840, 72.2%). Any combination of DAPT was used in 25.4%. The proportion of patients discharged with ASA and ticagrelor increased from 3.6% in 2012 to 24% in 2015, while the use of ASA and clopidogrel decreased from 13% to 4.7% during the study period (Figure 16).

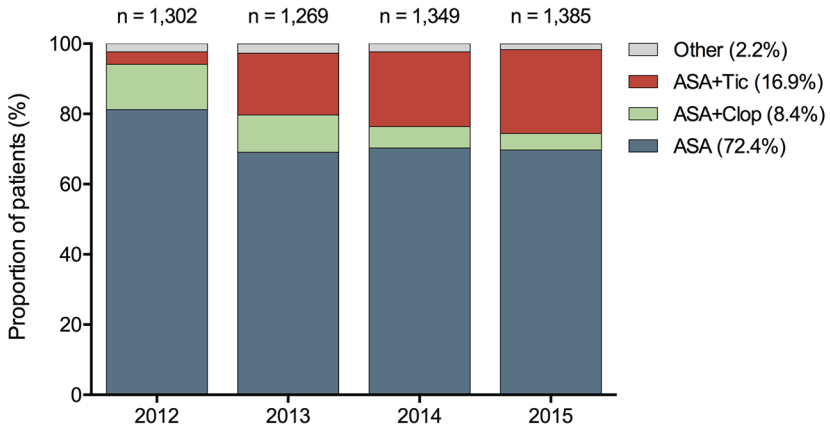

Figure 16. Proportion of ACS patients treated with different antiplatelet therapies at discharge after CABG.

4.6 Antiplatelet therapy and mortality after CABG surgery

Unadjusted mortality one year after discharge differed between the antiplatelet treatment groups: ASA only: 107 / 3840 (2.8 %); ASA and clopidogrel: 13 / 447 (2.9 %); ASA and ticagrelor: 8 / 896 (0.9 %) (p = 0.004).

DAPT with clopidogrel was not associated with increased survival compared to ASA only using either unadjusted (HR = 1.02, p = 0.95) or PS matched data (HR = 0.79, p = 0.49). ASA + ticagrelor was associated with reduced mortality in both the unadjusted (HR = 0.34, p = 0.002) and PS matched analysis (HR = 0.42 p = 0.020) (figure 17). Combining clopidogrel and ticagrelor patients in one group, data indicated a survival benefit of more intense antiplatelet therapy (unadjusted data: HR = 0.58, p = 0.02; PS matched data: HR = 0.54, p = 0.012).

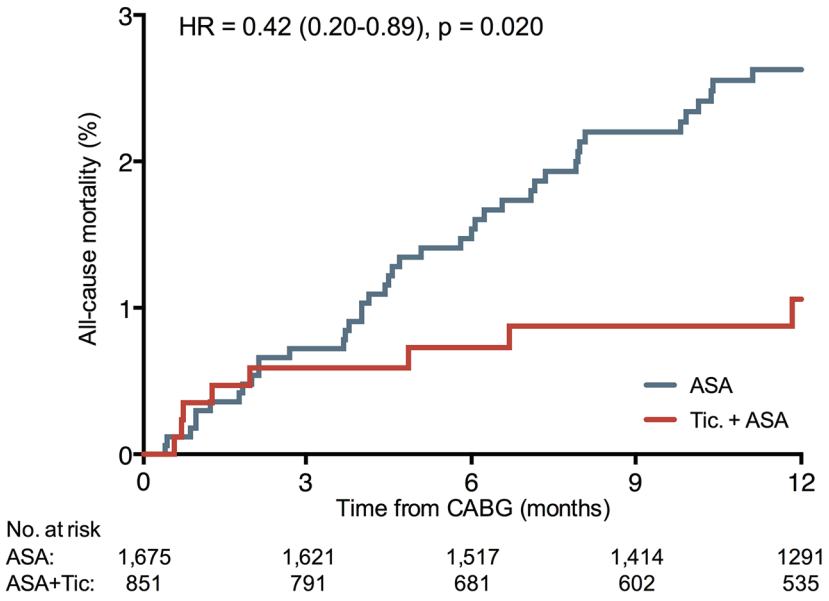

Figure 17. All-cause mortality after discharge in CABG treated ACS patients of propensity score matched groups. ASA = Acetylsalicylic acid; Tic. = ticagrelor.

Hazard ratios were calculated using multivariable Cox regression for both unmatched and PS matched groups. There was a general agreement between the univariable and multivariable regression analyses (Table 3).

	Univariable Cox regr.		Multivariable Cox regr.	
	Unmatched groups	PS matched groups	Unmatched groups	PS matched groups
DAPT vs ASA	0.58 (0.36-0.92)	0.54 (0.33-0.88)	0.57 (0.36-0.92)	0.51 (0.31-0.85)
Clop. + ASA vs ASA	1.02 (0.57-1.81)	0.79 (0.41-1.53)	0.91 (0.51-1.62)	0.76 (0.39-1.49)
Tic. + ASA vs ASA	0.34 (0.17-0.70)	0.42 (0.20-0.89)	0.35 (0.17-0.73)	0.45 (0.21-0.97)
Tic. + ASA vs clop. + ASA	0.33 (0.14-0.80)	0.39 (0.16-0.98)	0.39 (0.16-0.98)	0.41 (0.16-1.04)

Table 3. Hazard ratios for comparisons of different postoperative antiplatelet treatments calculated with univariable and multivariable Cox regression analysis for both unmatched and propensity score matched groups of patients. 95% confidence intervals are given within parenthesis. PS = propensity score; DAPT = dual antiplatelet therapy; ASA = acetylsalicylic acid; Clop. = clopidogrel; Tic. = ticagrelor

5 DISCUSSION

Guidelines recommend that a multi-disciplinary heart team involving both surgeons and cardiologists should discuss ACS patients in need of cardiac surgery[69]. Among other factors, two opposing risks should be considered in the ACS patient. First, there is the risk of additional ischaemic events. This risk is increased early after the initial event, and may be linked to thrombotic events at both culprit and non-culprit lesions[110]. Patients considered for CABG may be particularly susceptible, as they usually have a high atherosclerotic burden and more advanced coronary artery disease. Other contributory risk factors include a pro-coagulant and pro-inflammatory milieu associated with an acute myocardial infarction[111] and, if patients have recently undergone PCI treatment, the risk of early stent thrombosis[112].

Secondly, one should consider the risk of perioperative bleeding. Severe bleeding is not a trivial complication, but is associated with poor outcome –also after adjusting for confounding factors[65]. Recent treatment with antiplatelet drugs is one factor that increases the bleeding risk, although far from all of these patients are subject to this complication[73 113]. This may in part be related to a variable response of platelet inhibition, which is well-characterized in clopidogrel-treated ACS patients[114].

Previous studies of thienopyridine-treated ACS patients have shown that preoperative PFT using impedance aggregometry can predict excessive bleeding. These studies have suggested a cut-off level of ADP-dependent platelet aggregation of 22 or 30 U, to identify patients with an increased risk of bleeding[48 77].

5.1 Recovery of platelet function

The pharmacokinetics of ticagrelor have been reported in several studies based on healthy volunteers[115] or patients with stable atherosclerotic disease[71 116]. In paper I, we reported platelet recovery after discontinuation in ACS patients, and as expected, the mean platelet function gradually increased after discontinuation. Despite this seemingly predictable recovery, there was considerable variation in individual patients. A relatively narrow range of aggregation was observed at 12 h after discontinuation (0–24 U), but with increasing ranges at each subsequent time point. At 72 h, the ADP-induced

aggregation ranged from 4 to 88 U, and 6 of 24 patients (25%) had an aggregation below 22 U, indicating a possible increased risk of severe bleeding.

A slight increase in AA- and TRAP-induced aggregation after discontinuation of ticagrelor was also observed, illustrating the partial dependence on $P2Y_{12}$ signalling in these responses also[117]. The increase in AA-induced aggregation was only very minor, which can be explained by continuous treatment with aspirin throughout the study period.

The variable recovery of ADP-dependent aggregation in ACS patients was confirmed in paper II, where platelet function was measured at different discontinuation times. Grouping of these observations depending on time since last dose of ticagrelor resulted in a gradual increase in mean values (Figure 10 A), although individual measurements at comparable discontinuation times had a wide spread – resulting in some patients with a short time since discontinuation having a high platelet aggregation and vice versa (Figure 10 B).

These studies do not explain the reason for the variability, but it is probably related to differences in patients' clearance of the drug. Unlike clopidogrel, ticagrelor does not require metabolism for activation, although hepatic CYP-450 activity is needed for its clearance[115]. This activity may be reduced for a number of reasons, including chronic liver disease, interactions with other drugs, and dietary factors. If CYP-450 activity is diminished, the overall antiplatelet activity would not be reduced as is the case in clopidogrel treatment[118 119], but might very well be increased or prolonged due to impaired clearance. Indeed, one study of volunteers with mild hepatic impairment receiving a single dose of ticagrelor reported modest, but significantly higher exposure to ticagrelor and an active metabolite than in healthy control subjects[120].

5.2 Predicting the risk of bleeding

With such a variability in platelet recovery, these data suggest that PFT may improve the assessment of the operative risk when ticagrelor-treated patients are considered for surgery. This was confirmed in paper II, showing that the preoperative ADP-induced platelet aggregation capacity predicted the risk of severe bleeding complications in cardiac surgery patients treated with ticagrelor < 5 days before surgery.

The accuracy of ADP-induced platelet aggregability tested immediately before cardiac surgery to predict severe bleeding was acceptable, but not perfect (AUC under the ROC curve 0.73), underscoring the multifactorial nature of perioperative bleeding complications.

Due to the individual variation in recovery of platelet function, the use of PFT may be superior to using time alone for optimal timing in high-risk patients. The logistic regression model of the preoperative ADP test estimating the risk of severe bleeding (Figure 13) can be used to estimate the bleeding risk in an individual patient after the PFT result is obtained. This risk of bleeding, and associated morbidity and mortality, can then be weighed against the risk associated with postponing surgery.

The results from this study are supported by previous observations in thienopyridine-treated patients, where preoperative PFT also predicted bleeding[48 77]. After the publication of paper II, another study of 149 patients undergoing CABG within 48 hours of discontinuation of $P2Y_{12}$ inhibitor (clopidogrel, n = 80; prasugrel, n = 28; ticagrelor, n = 41) was reported. Preoperative PFT was performed using four different methods, including LTA and impedance aggregometry using the ADP test. Although this study did not provide a cut-off for bleeding or define the optimal PFT, the results strongly suggested a decrease in bleeding with increasing ADP-dependent platelet reactivity.

The optimal cut-off value for ADP-dependent aggregation to detect bleeding risk in our study (22 U) was the same as the cut-off value in a previous study of thienopyridine-treated patients[48]. We used Youden's index to define the optimal cut-off value. A limitation of this method is that a false negative (i.e. a falsely predicted non-bleeder) and false positive (i.e. a falsely predicted severe bleeder) are given the same statistical weight. In clinical practice, a false-negative value may confer greater risk in the management of patients, and a higher cut-off value may therefore be considered.

5.3 Platelet transfusion in ticagrelor-treated ACS patients

If ACS patients with DAPT are stable and at low risk of ischaemia, surgery should be postponed until the effect of antiplatelet therapy wears off. If the clinical situation requires acute surgery, measures

should be taken to minimize the risk of bleeding and transfusions. This includes meticulous surgical techniques, using a cell-saver device, minimizing CPB priming volumes, using antifibrinolytics, and optimizing secondary haemostasis. Ideally, an antidote for antiplatelet drugs would also be used. Although a successful antidote for ticagrelor has been reported in mice (Buchanon 2015), none is yet available for clinical use.

In both paper I and paper III, platelet supplementation with clinically relevant doses did not result in any significant improvement in ADP-induced aggregation, but large increases in AA-induced aggregation, in line with the results of previous studies[121 122]. However, the results from paper I expand the previous findings, showing no effect on ADP-induced aggregation up to four days after discontinuation of ticagrelor.

The lack of effect on ADP-induced aggregation might be explained by remaining plasma concentrations of ticagrelor or its metabolites, which may inhibit the supplemented platelets. Also, over time, storage of platelets diminishes the capacity for ADP-induced aggregation, limiting the possible improvement in this platelet signalling pathway after platelet transfusion[123].

Although these results indicate that the use of stored platelets is not a good antidote to ticagrelor, its use may still be beneficial. Most patients will have impaired AA-dependent aggregation due to treatment with ASA, but this will be greatly improved after platelet transfusion.

5.4 Antifibrinolytics in ticagrelor-treated ACS patients

Two classes of antifibrinolytic agents, the serine protease inhibitor aprotinin and the lysine analogues TA and EACA, have gained widespread acceptance and use in cardiac surgery. Similar to lysine analogues, aprotinin is a potent inhibitor of plasmin formation, but it also has additional effects. After the withdrawal and ensuing re-introduction of aprotinin, lysine analogues have continued to dominate the market, with EACA being used predominantly in the United States[124]. Although aprotinin appears to be more effective than the lysine analogues in minimizing postoperative blood loss[101], it remains unclear which patients might benefit from this treatment. Patients with

ongoing or recently stopped DAPT have an increased bleeding risk and may thus be a group that would benefit from aprotinin treatment.

The main finding of paper III was that aprotinin, but not TA, improved ADP-induced platelet aggregation in patients with ongoing treatment with aspirin and ticagrelor. The results were independent of concomitant platelet concentrate supplementation. Although the absolute improvement in ADP-dependent aggregation was limited, data from paper II show that even small improvements in platelet function can reduce the bleeding risk, especially if baseline platelet function is poor (Figure 13).

In this study, we could not identify the mechanism responsible for the improved platelet function. Aprotinin, which is a more complex molecule than TA, has several additional biological effects[95], but there are no data showing a direct interaction between aprotinin and the $P2Y_{12}$ receptor. Future mechanistic studies are needed to clarify this.

5.5 DAPT after CABG surgery

Current guidelines recommend that DAPT should be resumed as soon as possible after CABG[69 90]. These recommendations are based on limited evidence, as indicated by a C level of evidence. The results of paper IV support the idea that ACS patients benefit from DAPT after CABG. However, the benefit of DAPT was mainly due to the low mortality observed in patients treated with ticagrelor + ASA –both compared to ASA only, and compared to ASA + clopidogrel. The latter result was very much in line with the results from the CABG sub-study in the PLATO trial[41], where ticagrelor + ASA was found to be associated with a 50% reduction in all-cause one-year mortality compared to clopidogrel + ASA.

The benefit of ticagrelor treatment in ACS compared to clopidogrel was also supported by a recent study based on 45,073 patients from the SWEDEHEART registry[125]. However, this study did not include patients undergoing CABG, which was the subject of paper IV. When the results of these two SWEDEHEART studies are compared, another pattern from the PLATO study is supported: ACS patients undergoing CABG appeared to have the greatest survival benefit from ticagrelor treatment. In the SWEDEHEART study of ACS patients treated medically or with PCI, the adjusted HR for death at two years was 0.85

(95% CI 0.78–0.93), whereas the adjusted hazard ratio for death at one year was 0.36 (95% CI 0.15–0.89) in paper IV.

The adherence to the recommendation of DAPT after CABG in ACS patients was relatively low, with only 26% of ACS patients being treated with DAPT after CABG. One can speculate that the lack of strong evidence, the fear of bleeding complications, and the tradition of using ASA only after CABG contribute to surgeons' resistance to prescribe DAPT after CABG. In the Synergy between Percutaneous Coronary Intervention with Taxus and Cardiac Surgery (SYNTAX) study, where approximately one-third of the patients had ACS, only 20% of CABG patients were treated with DAPT at discharge, as compared to 97% in the PCI group[91]. It is possible that increased use of ticagrelor would improve the long-term outcome after CABG in patients with ACS.

5.6 Limitations of the studies

Haemostasis is a complex process involving the interaction of complicated systems. A single laboratory test can give important information, but cannot be expected to reflect the entire process. We used only MEA to assess platelet function, albeit with multiple agonists to activate different platelet signalling pathways. This method does not take into consideration possible platelet interactions with blood vessels, vascular endothelium, or rheological factors. Tests can be influenced by platelet count, and reliable results require a platelet count of $> 100 \times 10^9/L$[126], which was the case in all patients in papers I–III at the time of aggregometry.

However, the MEA testing is reproducible, uses whole-blood sampling, and allows study conditions to be standardized. Other PFTs might give somewhat different results, but it has been shown that there is a satisfactory correlation between MEA and LTA, which is considered the golden standard method. MEA is also a point-of-care device that is easily adopted in clinical practice.

The ex vivo models for platelet and drug supplementation used in papers I and III are simplified and do not take into consideration the effect of surgical trauma or CPB. Other studies of platelet supplementation have used autologous platelet-rich plasma, but we deliberately chose to use ABO-compatible apheresis platelet concentrate from the hospital blood bank, which reflects the clinical situation more

closely. Ideally, the results from these studies should be confirmed in clinical in vivo studies.

In paper II, the study was not performed blind; therefore, knowledge of both the time since the last dose of ticagrelor and of the preoperative impedance aggregometry results might have influenced clinical handling, including surgical management and postoperative transfusions, especially regarding platelet transfusions. The results and conclusions are applicable only to the study population (i.e. ACS patients with ticagrelor treatment within 5 days before CABG). In other populations, other factors may be equally, or more important to predict bleeding complications.

As paper IV was an observational study, the issue of selection bias was addressed by propensity score matching. However, it is only possible to match or adjust for patient characteristics that are known and included in the data. Thus, a limitation was unmeasured or unknown factors that were not accounted for (residual confounding). Another limitation was missing registry data in certain variables, which could possibly have influenced the results.

The registry data did not include information on morbidity after the initial hospitalization, including bleeding and thromboembolic complications during the 12-month study period. Furthermore, no data were available on preoperative antiplatelet therapy, the length of treatment with the $P2Y_{12}$ inhibitor, compliance with the prescription, and causes of deaths.

Ideally, the results of paper IV should be confirmed in a well-designed prospective randomized trial comparing ASA + ticagrelor with ASA alone, or with clopidogrel + ASA, after CABG. Pending the results of such a trial, this study had some merits and a number of strengths. It included a nationwide dataset with a large number of patients and the data contained a large number of relevant and relatively complete variables with none of the patients being lost to follow-up.

6 CONCLUSIONS

1. There is a large degree of inter-individual variability in recovery of ADP-induced platelet aggregation after discontinuation of ticagrelor in ACS patients awaiting CABG.
2. Administration of platelet concentrate does not improve ADP-induced aggregation after discontinuation of ticagrelor. Platelet concentrate markedly improves ADP-induced aggregation in patients with ongoing ASA treatment.
3. The use of platelet function tests can predict severe bleeding complications in ticagrelor-treated cardiac surgery patients.
4. Aprotinin improves the ADP-dependent platelet aggregation in ticagrelor treated ACS patients. Tranexamic acid was not associated with any improvement in platelet function.
5. Less than 30% of ACS patients in Sweden currently discharged with DAPT after CABG surgery.
6. Postoperative antiplatelet therapy may influence survival after CABG in patients with ACS. DAPT with ticagrelor and ASA is associated with improved one-year survival.

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