Platelet Inhibition and Bleeding Complications in Cardiac Surgery Patients

Clinical and experimental studies

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ABSTRACT

BACKGROUND AND OBJECTIVE

Dual antiplatelet therapy (DAPT) with acetylsalicylic acid and a $P2Y_{12}$ inhibitor (clopidogrel, ticagrelor, or prasugrel) reduces thrombotic events in patients with acute coronary syndrome (ACS), but it is also associated with an increased risk of bleeding complications. The aim of this project was to investigate the prevalence and effects of platelet inhibition in the context of cardiac surgery, the bleeding problems that may occur, and treatment of bleeding complications.

METHODS

Studies I and II investigated the incidence of CABG-related bleeding complications with DAPT in relation to time from discontinuation. Study I was a regional pilot study and Study II was a nationwide registry analysis. Studies III and IV were experimental ex vivo studies of platelet function in patients treated with platelet inhibitors, as measured by multiple-electrode aggregometry. Study III investigated the effects of platelet transfusion in patients with different platelet inhibitors, and Study IV examined the effects at time points after discontinuation. Study IV also investigated the recovery of platelet aggregability after discontinuation of ticagrelor. Study V examined the role of platelet inhibition in patients operated for acute aortic dissection.

RESULTS

The incidence of CABG-related major bleeding was high when DAPT was discontinued < 24 hours before surgery. Discontinuation 3 days before surgery, as opposed to 5 days, did not increase the incidence with ticagrelor, but increased the risk with clopidogrel. The overall risk of major bleeding was lower with ticagrelor than with clopidogrel. Platelet supplementation improved platelet aggregability independently of antiplatelet therapy. However, the effect on ADP-induced platelet aggregation was limited, and it was reduced further with ticagrelor compared to clopidogrel. Platelet concentrate did not improve aggregation at later time points after discontinuation of ticagrelor. Platelet aggregation recovered to levels not associated with bleeding 72 hours after ticagrelor, but with large inter-individual variation. The indication for antiplatelet therapy in patients operated for acute aortic dissection was weak or absent in most cases. Patients with ongoing platelet inhibition at the time of aortic repair had more bleeding complications, and DAPT was associated with increased early mortality.

CONCLUSIONS

DAPT with ticagrelor allows shorter discontinuation time before surgery than clopidogrel, and timing of surgery may be aided by platelet function testing. In case of bleeding, platelet transfusion can be expected to improve platelet function, but less so in ticagrelor-treated patients than in clopidogrel-treated patients. It is important to carefully consider the indication for DAPT before treatment is started in patients who may undergo surgery.

KEYWORDS: Acute coronary syndrome, bleeding complications, cardiac surgery, platelet aggregation inhibitors, platelet transfusion

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SAMMANFATTNING PÅ SVENSKA

Bakgrund och syfte

Dubbel trombocythämning med acetylsalicylsyra och $P2Y_{12}$-hämmare (clopidogrel, prasugrel eller ticagrelor) är standardmedicinering vid akut kranskärlssjukdom och minskar risken för sjukdom och död efter det första insjuknandet. Dock medför behandlingen också en ökad risk för allvarlig blödning i det fall patienten behöver opereras, eftersom läkemedlen påverkar blodets koagulationsförmåga. Internationella riktlinjer rekommenderar därför att behandlingen sätts ut innan stora kirurgiska ingrepp, om det är möjligt med hänsyn till patientens tillstånd. Avhandlingsarbetet syftar till att beskriva förekomsten av dubbel trombocythämning i samband med hjärtkirurgi, vilka effekter sådan behandling har på förekomsten av blödningskomplikationer och hur blödningsproblemen kan behandlas med transfusion av trombocyter.

Metoder

Delarbete I och II beskrev blödningskomplikationer efter kranskärlskirurgi hos patienter med olika trombocythämmare (ticagrelor och clopidogrel), i förhållande till utsättning av medicinen före ingreppet. Delarbete I var en regional pilotstudie, och delarbete II var en nationell registerstudie. Delarbete III och IV var laborativa studier av effekten av trombocyttransfusion på blodprover från patienter med trombocythämning, undersökt med trombocytfunktionstest. Delarbete III jämförde patienter med olika läkemedel och delarbete IV beskrev effekten vid flera tidpunkter efter utsättning av ett av läkemedlen. Delarbete V beskrev förekomst och effekter av trombocythämning i samband med akut aortadissektion.

Resultat

Förekomsten av blödningskomplikationer var högre om medicineringen satts ut senare inför ingreppet. Utsättning tre dagar före kirurgi istället för fem dagar ökade inte antalet blödningar med ticagrelor, men däremot med clopidogrel. Generellt orsakade ticagrelor färre blödningskomplikationer än clopidogrel. Trombocyttransfusion förbättrade trombocyternas funktion även hos patienter med dubbel trombocythämning, men i mindre grad än i kontrollgrupperna. Effekten var ytterligare försämrad bland ticagrelorpatienter jämfört med clopidogrelpatienter. Vid senare tidpunkter

efter utsättning av ticagrelor var effekten mycket begränsad. Den trombocythämmande effekten av ticagrelor avtog gradvis, och var i medeltal återställd till nivåer som inte associerats med ökad blödning efter 72 timmar, men med stor individuell variation. Bland patienter med akut aortadissektion var trombocythämmare ofta insatt på grund av misstanke om hjärtinfarkt på svaga indikationer, och behandlingen var associerad med ökad blödning och ökade blodtransfusioner. De patienter som fått dubbel trombocythämning före aortaoperationen hade också ökad dödlighet.

Slutsatser

Utsättningstiden före kirurgi kan vara kortare efter behandling med ticagrelor än med clopidogrel. Trombocytfunktionstest kan vara av värde eftersom återhämtningen av trombocytfunktionen varierar mycket mellan olika individer. I händelse av blödningskomplikationer kan man förvänta sig effekt av trombocyttransfusion, men effekten kan vara lägre om patienten fått ticagrelor än clopidogrel. Medicinering med effektiva trombocythämmande läkemedel medför ökad risk för blödningskomplikationer i samband med hjärtkirurgi, speciellt om behandlingen pågår nära inpå ingreppet. Därför är det viktigt att säkerställa att behandlingen är korrekt innan den startas, och att operationer utförs vid rätt tidpunkt efter utsättning av läkemedlet.

LIST OF PAPERS

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

I. Hansson EC, Rexius H, Dellborg M, Albertsson P, Jeppsson A.
Coronary artery bypass grafting-related bleeding complications in real-life acute coronary syndrome patients treated with clopidogrel or ticagrelor.
Eur J Cardiothorac Surg 2014;46:699-705.

II. Hansson EC, Jidéus L, Åberg B, Bjursten H, Dreifaldt M, Holmgren A, Ivert T, Nozohoor S, Barbu M, Svedjeholm R, Jeppsson A.
Coronary artery bypass grafting-related bleeding complications in patients treated with ticagrelor or clopidogrel: a nationwide study.
Eur Heart J 2016;37:189-197.

III. Hansson EC, Shams Hakimi C, Åström-Olsson K, Hesse C, Wallén H, Dellborg M, Albertsson P, Jeppsson A.
Effects of ex vivo platelet supplementation on platelet aggregability in blood samples from patients treated with acetylsalicylic acid, clopidogrel, or ticagrelor.
Br J Anaesth 2014;Mar;112(3):570-575.

IV. Hansson EC, Malm CJ, Hesse C, Hornestam B, Dellborg M, Rexius H, Jeppsson A.
Platelet function recovery and effects of ex vivo platelet transfusion after ticagrelor discontinuation in blood samples from patients waiting for coronary artery bypass grafting.
(Submitted).

V. Hansson EC, Dellborg M, Lepore V, Jeppsson A.
Prevalence, indications and appropriateness of antiplatelet therapy in patients operated for acute aortic dissection: associations with bleeding complications and mortality.
Heart 2013;99:116-121.

TABLE OF CONTENTS

ABBREVIATIONS

AA	Arachidonic acid
ADP	Adenosine diphosphate
ACS	Acute coronary syndrome
ASA	Acetylsalicylic acid (aspirin)
CPB	Cardio-pulmonary bypass
COX	Cyclooxygenase
CABG	Coronary artery bypass grafting
DAPT	Dual antiplatelet therapy
GPIIbIIIa	Glycoprotein receptor IIbIIIa
RBC	Red blood cell
MACE	Major adverse cardiovascular events
MEA	Multiple-electrode aggregometry
NSTEMI	Non-ST-elevation myocardial infarction
PCI	Percutaneous coronary intervention
STEMI	ST-elevation myocardial infarction
TRAP	Thrombin receptor activating peptide
UAP	Unstable angina pectoris

1 INTRODUCTION

1.1 Platelet inhibition in acute coronary syndrome

Since ancient times, the bark of *Salix alba* has been used for medicinal purposes, such as treatment of fevers and headaches. Extract of the bark served as the source of acetylsalicylic acid (ASA or aspirin) when it was first developed in the 19th century as an analgesic and antipyretic medication. In more recent times, the antithrombotic effect of ASA has been used in acute coronary syndrome (ACS), as first described in the Veterans Administration Cooperative Study by Lewis et al. in 1983 [1]. Since then, platelet inhibition has been the standard of care in ACS [2, 3]. ACS comprises ST-elevation myocardial infarction (STEMI), non-ST-elevation myocardial infarction (NSTEMI), and unstable angina pectoris (UAP), as all three are manifestations of the same underlying pathology, albeit with different degrees of severity and acuteness. In 2014, 27,000 patients in Sweden were diagnosed with myocardial infarction, with a mortality of 26% [4]. The mechanism behind ACS in the vast majority of patients is the gradual (in UAP) or acute (in STEMI and NSTEMI) aggravation of a coronary atherosclerotic disease with coronary thrombosis.

The role of platelet inhibition in ACS is to reduce the thrombus formation in atherosclerotic coronaries. When circulating platelets are exposed to the non-endothelial surface of a disrupted atherosclerotic plaque, they adhere via surface glycoproteins to collagen and von Willebrand factor in the subendothelium. The adherence triggers reshaping of the platelet and activation of intracellular systems, leading to degranulation of α- and δ-granules, which contain ligands that start the activation of the plasma coagulation cascade and activation in other platelets in a similar cascading manner (Figure 1). Clinically, the most important platelet agonists in this step are adenosine diphosphate (ADP), thrombin, collagen, and thromboxane [5]. The activation also induces changes in the glycoprotein (GP) IIbIIIa-receptor complex, enabling the aggregation of platelets through binding of fibrinogen. Fibrinogen is subsequently cross-linked by factor XIII to form a stable clot. As illustrated in Figure 1, these activating systems are targeted at different levels by various pharmacologic agents.

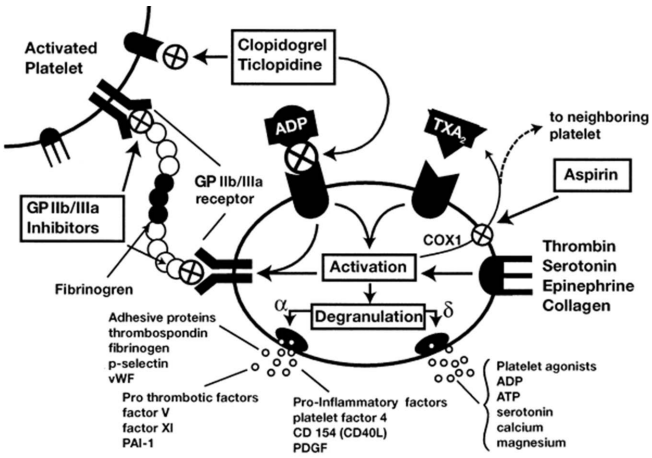

Figure 1. Platelet activation. Reprinted from Mehta SR, Salim Y. J Am Coll Cardiol 2003;41(4 Suppl S): 79S–88S with permission from Elsevier.

ASA as the first platelet inhibitor

ASA inhibits platelet activation by binding irreversibly to cyclooxygenase (COX)-1, and deforming the catalytic site of the enzyme, thereby inhibiting production of prostaglandin and thromboxane A_2, agents responsible for platelet aggregation and vasoconstriction. Complete inhibition of COX-1 occurs even at low doses of ASA, whereas the dosage most commonly used in ACS (75–100 mg daily) only partially inactivates COX-2. However, this slight inhibition gives further anti-inflammatory properties, which may also be beneficial in ACS. As the platelets lack a cellular nucleus and are therefore unable to synthesize new enzymes, the effect remains throughout the lifespan of the platelet, i.e. eight to nine days [6].

The dosage of ASA has varied over time and with geographic region, due to the fact that development of the drug as an antithrombotic agent in ACS was driven by many different randomized controlled studies, which were primarily conducted by the medical and scientific community rather than a patent-holding pharmaceutical company [6]. For example, the previously mentioned study by Lewis et al. [1] used 324 mg of ASA daily in patients with UAP, whereas the Second International Study of Infarct Survival (ISIS-2) in 1988 —which was groundbreaking in large-scale randomized trials— used 160 mg of ASA daily in STEMI patients [7]. In both studies, however, there was a

significant decrease in mortality after the initial ischaemic cardiac event, with a relative risk reduction of 20–40%, which translated into saving up to 100,000 lives annually if used clinically. And accordingly, since then ASA has been used to prevent mortality and morbidity in ACS patients.

Introduction of dual antiplatelet therapy

After ASA, a new class of platelet inhibitors arrived in the 1990s as ticlopidine and clopidogrel, the first $P2Y_{12}$ receptor antagonists, were approved for use in patients with ACS [8, 9] —starting the era of dual antiplatelet therapy (DAPT). Due to severe side effects of ticlopidine, clopidogrel rapidly became the main platelet inhibitor used in ACS patients.

Clopidogrel (Figure 2), of the thienopyridine family, is a prodrug requiring a two-step metabolism to its active form via cytochrome P450 (CYP) enzymes in the liver. The short-lived active metabolites bind irreversibly to the $P2Y_{12}$ receptor, blocking ADP-mediated platelet aggregation via GPIIbIIIa, but not ADP-mediated change in platelet shape. In addition, clopidogrel has other antithrombotic effects, e.g. reduction of circulating fibrinogen levels and inhibition of platelet-dependent endothelial tissue factor [10]. Maximum platelet inhibition by clopidogrel is achieved approximately three hours after administration of the loading dose [11], which has led to recommendations regarding preloading as soon as ACS is suspected in patients presenting with chest pain and/or ECG changes [12].

Table 1. Overview of oral $P2Y_{12}$ inhibitors

	Clopidogrel	**Prasugrel**	**Ticagrelor**
Class	Thienopyridine	Thienopyridine	Triazolopyrimidine
Metabolism to active form	Hepatic	Hepatic and intestinal	Not required
Non-responders	Up to 30%	Uncommon	No
Binding	Irreversible	Irreversible	Reversible
Onset	2–4 h	30 min	30 min
Effect duration	3–10 days	5–10 days	3–4 days

Compared to treatment with ASA alone, DAPT with ASA and clopidogrel reduced the relative risk of recurrent thrombotic events after an initial ACS by approximately 20%. The CURE trial, involving 12,562 non-ST-elevation ACS patients reported a reduction from 11.4% to 9.3% [8]. Nevertheless, this benefit came at the cost of a 38% increase in the risk of major bleeding. The CURE trial also included patients undergoing coronary artery bypass grafting (CABG), and the incidence of major bleeding was not significantly higher in the clopidogrel-treated CABG patients than in ASA-treated CABG patients [13]. However, in most patients medication was discontinued at a median of five days before surgery, which was then adopted into clinical practice and endorsed by subsequent guidelines [2].

Due to CYP polymorphisms, the individual response to clopidogrel is variable, and studies have reported between 15% and 30% non-responders [14]. Clopidogrel non-responders with coronary stents have been reported to have a higher risk of stent thrombosis [10, 15, 16] compared to responders. Attempts have been made to tailor clopidogrel treatment to patients based on platelet function testing [14], but this has not proven effective in large studies, and it has not been implemented in guidelines [17]. Instead, DAPT evolved with the addition of a new generation of $P2Y_{12}$ receptor antagonists.

New generation of platelet inhibitors

Due to the less-than-ideal pharmacological properties of clopidogrel, development of platelet inhibitors continued, with prasugrel appearing in 2009 [18] and ticagrelor appearing in 2010 [19]. Like clopidogrel, prasugrel is a thienopyridine and a prodrug. However, unlike clopidogrel, metabolism of prasugrel is less variable and occurs in both hepatic and intestinal CYP enzymes, leading to a more rapid onset of effect—approximately 30 minutes after administration [11]. Prasugrel binds irreversibly to the $P2Y_{12}$ receptor and the duration of effect thereby corresponds to the half-life of platelets, approximately five to ten days. It gives a higher degree of platelet inhibition than clopidogrel, possibly due to the lower inter-individual pharmacokinetic variability, rather than a more potent platelet inhibition per se [10]. The TRITON-TIMI 38-study of 13,608 ACS patients planned for interventional treatment showed a significant reduction in major adverse cardiovascular events (MACE), but at the cost of increased major and fatal bleeding [18].

This was especially true in the CABG population, where prasugrel treatment yielded an almost five-fold increase in major bleeding compared to clopidogrel treatment. In spite of more bleeding, there was still improved survival with prasugrel compared to clopidogrel in CABG patients [20].

Ticagrelor (Figure 3) is instead a triazolopyrimidine, a slightly different class of ADP receptor antagonist. It is direct-acting, and therefore avoids the problem of non-responsiveness due to heterogeneity in metabolism. It binds reversibly to the $P2Y_{12}$ receptor, changes the configuration of the receptor, and causes a non-competitive ADP inhibition [10, 11]. The onset of effect is more rapid than with clopidogrel, and it is a more potent inhibitor of platelet aggregation than the thienopyridines. Clearance of the inhibitory effect is faster than with clopidogrel, as shown in patients with stable coronary artery disease [21]. Maximum plasma concentration and platelet inhibition is achieved after 1–3 hours, whereas plasma half-life is 6–8 hours, resulting in the need for administration twice a day. The reversible binding leads to the possibility of an antidote, and although development is under way, there is no direct antidote currently available clinically [22].

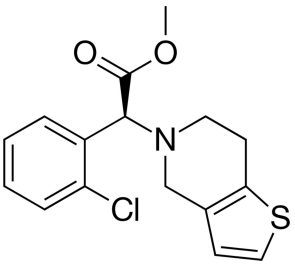

Figure 2. The molecular structure of clopidogrel. https://commons.wikimedia.org/w/index.php?curid=40964449 Public domain.

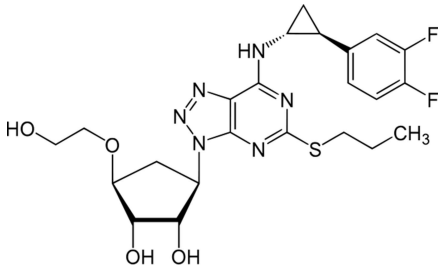

Figure 3. The molecular structure of ticagrelor. https://commons.wikimedia.org/w/index.php?curid=16901595 Public domain.

The PLATO trial [19], involving 18,624 ACS patients, showed a greater benefit of DAPT with ticagrelor compared to DAPT with clopidogrel. There was a relative reduction in MACE of 16% and an absolute reduction in cardiovascular mortality of 1%, from 5.1% to 4.0% in one year. However, the more potent platelet inhibition yielded more spontaneous major bleeding, a relative increase of 18%. In the PLATO-CABG substudy, treatment with ticagrelor improved survival with a relative reduction in both cardiovascular

and all-cause mortality of almost 50% [23]. There was no overall difference in CABG-related major bleeding between the platelet inhibitors in this study.

When all the currently available data are considered, DAPT remains a cornerstone in the treatment of ACS with or without ST-segment elevation, irrespective of interventional strategy [2, 3, 24]. The use of prasugrel has been given lower priority than the use of ticagrelor in guidelines [2, 3], due to the less fortunate bleeding profile.

1.2 Cardiac surgery

The history of cardiac surgery began during the 19th century with attempts to treat stab wounds to the heart, most often without successful clinical outcome. The first reported successful attempt at suturing the heart was made in 1896 by Dr Ludwig Rehn in Frankfurt, Germany, who repaired a 1.5-cm wound of the right ventricle. At this time, there had also been developments in anaesthesia and aseptic technique, which aided the pioneering surgeons [25].

Surgical treatment of patients with congenital defects of the heart and great vessels proved even more successful, spearheaded by—among many others—Dr Clarence Crafoord at the Karolinska Institute, who performed the first successful repair of aortic coarctation [26], and Dr Alfred Blalock of Johns Hopkins, who, along with Dr Helen Taussig and Mr Vivien Thomas, developed the surgical cure for "blue babies" [25]. Cardiac surgery then advanced with the development of cardio-pulmonary bypass (CPB) in the 1950s, a technical development brought about by fruitful collaboration between clinicians (such as Drs Viking Olof Björk and John Gibbon) and engineers. The use of CPB was also facilitated by the discovery of heparin [27], enabling the use of extracorporeal circuits without clot formation. This allowed open heart surgery, and led forward to surgical repair of acquired valve disease. Surgery for acute aortic dissection was first reported by Dr Morris and associates in 1963 [28].

Surgical treatment of coronary disease started with various attempts at increasing blood flow to the myocardium, by stimulating collateral circulation from the pericardium through grafting of the pectoral muscle [29] or induction of chemical pericarditis [25]. Dr Arthur Vineberg developed the technique of

grafting the internal mammary artery to the myocardium, with clinically viable results [30]. Direct grafting of the internal mammary artery to the coronary arteries originated as something of a salvage procedure when Dr William Longmire attempted to perform a thrombendarterectomy on a stenotic coronary artery in 1958, but only "later decided it was a good operation" [25, 31]. Subsequently, CABG was developed as a treatment for coronary disease and myocardial infarction by Dr René Favaloro, among others [32]. In the 1970s, CABG as we know it today started to emerge, with use of cardioplegia, and complete revascularization of three vessel-disease by grafting with internal mammary artery and saphenous vein grafts [25].

Over the last 20 years, endovascular treatment by percutaneous coronary intervention (PCI) with balloon angioplasty and stenting has taken over a large proportion of the revascularization procedures in ACS patients. Around 22,000 PCI procedures are done in Sweden every year, with a 30-day mortality rate of just over 2% for NSTEMI patients and around 7% for STEMI patients [4]. Nevertheless, surgical treatment still remains an important clinical weapon in the treatment arsenal against ACS, especially in patients with complex coronary disease [33]. In 2015, approximately 5,800 cardiac operations were performed in Sweden, 3,300 of them being CABG procedures with a 30-day mortality rate of 1.9%.

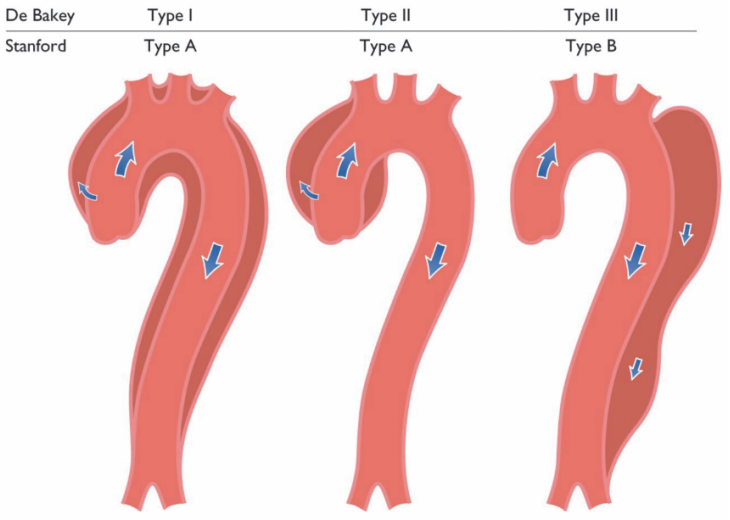

Figure 4. Classification of acute aortic dissection. Reprinted from Erbel R et al., Eur Heart J 2014;35:2873-2926 with permission from Oxford University Press.

At Sahlgrenska University Hospital today, CABG is mainly performed with normothermia and haemodilution, using non-pulsatile CPB technique with a hollow-fibre membrane oxygenator. Before cannulation, heparin is given and supplemented as required to maintain an activated clotting time of more than 480 seconds, and after weaning off CPB, protamine is given to reverse the effect of heparin. For cardioprotection, cold blood cardioplegia is used. To prevent excess bleeding, all patients receive tranexamic acid before surgery and after skin closure. Routinely, the aim of the procedure is complete revascularization of stenotic vessels as determined by coronary angiography. In most cases, the left internal mammary artery and a saphenous vein graft are used, but arterial revascularization with bilateral internal mammary arteries and/or radial artery are also used regularly.

Acute aortic dissection is a rare but very serious condition affecting the aortic wall, with rupture of the intima and development of a false lumen (Figure 4). Mortality if left untreated is high, approximately 2% per hour [34]. Surgical repair is the only curative treatment for type-A dissection, i.e. involvement of the ascending aorta and/or aortic arch. The aim of surgical repair is to remove the affected portion of the ascending aorta in order to prevent rupture and proximal advancement of the dissection. Surgery is often performed in hypothermia and circulatory arrest with selective cerebral perfusion [35].

Bleeding in cardiac surgery

Bleeding is common in cardiac surgery, and patients normally bleed approximately 500 mL after the procedure, due to the surgical trauma and impaired haemostasis attributable to haemodilution, exposure to extracorporeal circulation, platelet dysfunction, and increased fibrinolysis [36]. Nevertheless, excess bleeding is an important clinical issue, as bleeding complications are strongly associated with poor outcome in ACS patients [37, 38], and even more so in cardiac surgery [39, 40]. Major bleeding in the setting of adult cardiac surgery is associated with an eightfold increase in mortality, after adjustment for other factors (Figure 5). Bleeding in itself, reoperation for bleeding, and transfusions appear to have a role in the increased morbidity [41-43].

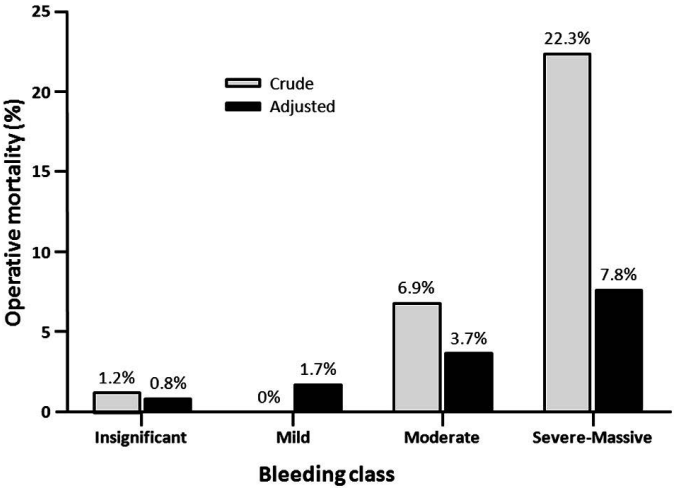

Figure 5. Mortality with severe bleeding after adult cardiac surgery (n = 1,144). Reprinted from Dyke C et al. J Thorac Cardiovasc Surg 2014;147:1458-1463.e1451 with permission from Elsevier.

Reoperation for bleeding occurs in approximately 5% of procedures [4], and is associated with increased morbidity and mortality. However, the decision to bring the patient back to surgery is multifactorial. The frequency and timing therefore vary a great deal between centres and individual surgeons [41, 42, 44]. Transfusion of blood products, especially red cell transfusion, has also been linked to increased short- and long-term mortality in retrospective observational studies [43], even if the patient is transfused with as little as one or two units of blood [45]. In contrast, a recent large randomized study of liberal or restrictive transfusion after cardiac surgery failed to demonstrate any difference in the primary endpoint (morbidity after three months), whereas mortality was increased with a more restrictive transfusion regimen [46].

The risk of bleeding complications in cardiac surgery is increased in patients with DAPT [8, 18, 47], and current guidelines recommend discontinuation of $P2Y_{12}$ inhibitors five to seven days before elective or subacute major surgical procedures [3, 24]. ASA treatment is usually maintained until surgery, and a recent randomized trial showed no excess bleeding with preoperative ASA use in CABG surgery [48]. Recommendations regarding timing of discontinuation are based on pharmacological data and observational studies of the different platelet inhibitors. Even though discontinuation of platelet inhibition is

favourable in order to minimize bleeding risk, it may also increase the risk of thrombosis [49], and it is therefore important to optimize timing of surgery after discontinuation. Additionally, in acute surgery where discontinuation is clinically impossible —such as acute CABG or acute surgery for aortic dissection— or undesirable —e.g. recurrent ischaemia in spite of optimal medical treatment, and/or threatening coronary anatomy— patients will have to undergo surgery regardless of sustained or recently discontinued high-grade platelet inhibition.

1.3 Platelet transfusion

Platelet transfusion is used to improve haemostasis in patients with both hereditary and acquired platelet dysfunction, and in case of ongoing bleeding. The earliest cases of effective platelet transfusion came about as transfusion of large amounts of whole blood were noted to alleviate thrombocytopenia and stop haemorrhage [50]. In the 1960s and 1970s, platelet transfusion became more readily available, as technical developments facilitated storage of separated blood products. Currently in Sweden, approximately 50,000 units of platelets are administered by transfusion every year [51]. Transfusion of allogenic blood products presents a risk of severe side effects, even though strict safety precautions are in place to minimize the risk of transmitting pathogens. Transfusions can also cause immunological reactions and transfusion-related acute lung injury (TRALI) [52].

Preparation of platelet concentrate for donation can be done in two different ways. This is done either by centrifuging donated whole blood, and thereby obtaining buffy-coat platelets, which are pooled—usually from four donors—to obtain one unit of platelet concentrate; or by apheresis, which is automated separation of platelets from one donor, thus minimizing collection of erythrocytes and leukocytes. The advantage of single-donor apheresis platelets is the lower number of donors per unit of platelet concentrate, while the apheresis concentrate may contain a higher number of erythrocytes, which may, in rare cases, induce haemolysis or Rh-immunization [52].

Transfusion algorithms are recommended to help clinicians in prescribing appropriate transfusions, but adherence to guidelines often varies [53]. At the Department of Cardiothoracic Surgery at Sahlgrenska University Hospital,

transfusion protocols recommend transfusion of red blood cells (RBCs) during CPB at haematocrit below 20%, and postoperatively at haemoglobin levels below 7 g/dL—or below 10 g/dL with symptoms of anaemia or ongoing bleeding. Transfusion of plasma is recommended when there is bleeding in excess of 200 mL/h and documented dysfunction of coagulation factors and/or platelets from platelet function tests. Platelet transfusion is recommended in patients with bleeding and documented or suspected platelet dysfunction, e.g. in those with ongoing or recently stopped antiplatelet therapy.

There are limited options available besides platelet transfusion if major bleeding occurs in patients on antiplatelet therapy. Desmopressin, a synthetic vasopressin analogue that increases factor VIII and von Willebrand factor [54], and aprotinin, an antifibrinolytic, may be used in high-risk patients and in case of severe perioperative bleeding, but the potentially limited efficacy of desmopressin [55, 56] and side effects of aprotinin have restricted the use [57]. Tranexamic acid, a lysine analogue, is instead the recommended antifibrinolytic agent in cardiac surgery [58]. Oral platelet inhibitors have long half-lives, and currently there are no direct antidotes. Little is known about the efficacy of platelet transfusion in patients who are on platelet inhibition with new-generation DAPT. Studies have shown that platelet inhibition caused by ASA may be amended by ex vivo platelet supplementation [59]. Furthermore, clopidogrel could be reversed—at least partially—by means of platelet concentrate in blood samples from healthy volunteers [60], whereas DAPT-effect could be partially restored in ASA- and clopidogrel-treated healthy subjects in vivo after supplementation with autologous platelets [61]. Only recently, in 2015, O'Connor et al. reported the efficacy of in vivo platelet transfusion in patients on DAPT [62].

1.4 Platelet function testing

Because platelets have such an important role in haemostasis, and are at risk of dysfunction in cardiac surgery patients, the prospect of determining platelet function in case of major bleeding is appealing. However, coagulation and clot formation is an intricate process, and laboratory tests of the functions involved are often complicated and time consuming. The gold standard for

platelet function tests is Born aggregometry or light transmission aggregometry [63] using platelet-rich plasma that is exposed to a platelet agonist, causing the platelets to aggregate and absorb less light, which can be detected by a photosensitive detector. This method is available in clinical laboratories, with the expected delay in response time for logistical reasons, whereas severe bleeding in a perioperative setting often calls for prompt decisions. Hence, several different point-of-care methods for testing of platelet function have been developed, e.g. impedance aggregometry, light absorption aggregometry, and a flow cytometric vasodilator-stimulated phosphoprotein assay, as well as viscoelastic devices assessing clot formation [64]. There is no consensus regarding which devices to use in cardiac surgery [64], but one recent study has suggested that platelet function tests may be better than clot formation tests at predicting bleeding in cardiac surgery patients with ongoing platelet inhibition [65]. The viscoelastic tests are also unable to detect pharmacological platelet inhibition [64]. Multiple-electrode impedance aggregometry (MEA; Multiplate®) is one of the widely used platelet function test devices, and studies to conclusively confirm its ability to predict bleeding complications in cardiac surgery are under way [64, 66-70]. Platelet function tests have also been used to facilitate timing of surgery after discontinuation of clopidogrel [71].

Ranucci and colleagues have studied the use of MEA to assess bleeding risk after CABG in clopidogrel- and prasugrel-treated patients [67,72], and they suggested a cut-off of 31 U or 22 U in ADP-initiated platelet aggregation as a predictor of major bleeding. However, ticagrelor-treated patients have not been studied until recently, as reported by Malm and co-workers [68]. They found that ADP-induced aggregation under 22 U is a predictor of major bleeding also in ticagrelor-treated CABG patients.

1.5 Study objectives

At the start of this thesis project, the incidence of bleeding after CABG in patients with ticagrelor treatment had not yet been reported outside the registration trials. Thus, we wanted to study the risk of major bleeding after CABG in relation to the type of platelet inhibitor (clopidogrel or ticagrelor), and also whether shorter discontinuation times before surgery would increase

the risk of major bleeding with any of the platelet inhibitors in a "real-life" setting. We therefore designed **Study I** as a regional observational study of ACS patients with DAPT before acute and urgent CABG. **Study I** served as a pilot for **Study II**, which was a nationwide registry study over two years of all patients operated with acute or urgent CABG and treated preoperatively with DAPT.

Since there was no evidence of the effect of platelet transfusion in patients treated with DAPT, **Studies III and IV** were carried out to assess the efficacy of platelet transfusion in ACS patients on DAPT. In both cases, the study design was an experimental ex vivo set-up simulating platelet transfusion, where platelet aggregation in whole blood was measured before and after supplementation with increasing doses of allogeneic platelet concentrate. **Study III** included patients with recent exposure to different platelet inhibitors and healthy controls, whereas **Study IV** tested the effect of platelet supplementation at consecutive time points up to five days after discontinuation of ticagrelor.

As recommendations regarding optimal timing of cardiac surgery after discontinuation of platelet inhibitors are based primarily on pharmacological data from pre-registration trials, **Study IV** was also designed to determine the recovery of platelet function in ACS patients, where ticagrelor was discontinued before urgent CABG.

Initiation of DAPT has been recommended as soon as possible after suspected ACS, because of the slow onset of the first-generation $P2Y_{12}$ inhibitors. This would lead to a risk of inappropriate treatment if there is misdiagnosis. Other severe diagnoses with presentation similar to that of ACS, such as acute aortic dissection, may therefore be mistreated with platelet inhibitors and thereby be subject to a higher risk of bleeding when surgery subsequently is necessitated. So, in **Study V** we aimed to determine the prevalence, indications, and appropriateness of antiplatelet therapy in patients who were later operated for acute aortic dissection, and its associations with bleeding complications, transfusion requirements, and short-term mortality.

2 GENERAL AIM

The general aim of this work was to investigate the effect of platelet inhibition on bleeding complications and transfusion requirements in cardiac surgery patients, to examine what role platelet transfusions can play in cases of bleeding in the cardiac surgery setting, and to assess the bleeding-related risks of potentially inappropriate antiplatelet treatment in patients with acute aortic dissection.

2.1 Study aims

1. To compare the incidence of CABG-related bleeding complications in patients treated with ticagrelor and clopidogrel, first in a regional pilot study (**Study I**), and then in a nationwide setting (**Study II**).
2. To determine the effect of discontinuation of ticagrelor or clopidogrel on the incidence of CABG-related bleeding complications (**Studies I and II**).
3. To describe the effect of platelet transfusion in patients with platelet inhibition, early after intake (**Study III**), and at later time points after discontinuation (**Study IV**).
4. To compare the effects of platelet transfusion in patients with different platelet inhibitors (**Study III**).
5. To characterize the recovery of platelet function after discontinuation of ticagrelor (**Study IV**).
6. To examine the indications and appropriateness of platelet inhibition in acute aortic dissection type A (**Study V**).
7. To determine the effects of platelet inhibition on bleeding in patients operated for acute aortic dissection type A (**Study V**).

3 PATIENTS AND METHODS

3.1 Patients

All studies were conducted in accordance with the Declaration of Helsinki, and were approved by the Regional Research Ethics Committee in Gothenburg. For Studies I, II, and V, the committee waived the need for individual consent from the patients before inclusion, and in Studies III and IV, all patients were included after obtaining written informed consent. Patient characteristics are summarized in Table 2.

Table 2. Patient characteristics. Number with percentage or mean ± SD

	Study I	Study II	Study III	Study IV	Study V
n	405 *	2244	50	25	133
Female gender	76 (19%)	474 (21%)	0	2 (8%)	46 (34%)
Age, years	67 ± 10	68 ± 9.4	66 ± 12	68 ± 9	60 ± 11
BMI, kg/m²	27.3 ± 4.5	27.3 ± 4.1	26.7 ± 3.9	26.8 ± 3.5	26.2 ± 4.5
Euroscore I	5.9 ± 3.7	5.6 ± 3.2	-	-	-
Diabetes mellitus	108 (27%)	599 (27%)	6 (12%)	3 (12%)	-
Haemoglobin, g/L	137 ± 14	137 ± 16	140 ± 11	139 ± 14	134 ± 15
Platelet count, × 10^9/L	262 ± 69	248 ± 73	239 ± 65	236 ± 63	227 ± 76
Any platelet inhibition	405 (100%)	2244 (100%)	40 (80%)	25 (100%)	43 (32%)
DAPT with clopidogrel	232 (57%)	978 (44%)	15 (30%)	-	24 (18%)
DAPT with ticagrelor	173 (43%)	1266 (56%)	15 (30%)	25 (100%)	-
Operation time, min	193 ± 26	191 ± 62	-	-	412 ± 148
CPB time, min	79 ± 31	79 ± 34	-	-	199 ± 59
Cross-clamp time, min	48 ± 20	46 ± 21	-	-	87 ± 35
Acute surgery	75 (19%)	258 (11%)	-	-	133 (100%)

* Also included in Study II.

3.2 Methods

Study I

All CABG patients with ACS who were treated with DAPT and operated at Sahlgrenska University Hospital between January 2012 and July 2013 were included in an observational study (n = 405). Data were collected from medical records and the hospital's surgical database. The patients were treated with ASA and ticagrelor (n = 173) or ASA and clopidogrel (n = 232). Whenever clinically possible, the $P2Y_{12}$ inhibitor was discontinued 5 days before surgery. Major bleeding complications according to modified Blood Conservation Using Antifibrinolytics in a Randomized Trial (BART) criteria [58] were compared overall, and when ticagrelor or clopidogrel was discontinued ≥ 5 days (n = 280), 2–4 days (n = 40), or 0–1 day before surgery (n = 85). Study I served as a pilot for Study II, so the patients from Study I are also included in Study II.

Study II

All ACS patients on DAPT who underwent acute or urgent CABG at any of the eight cardiothoracic units in Sweden from January 2012 to December 2013 were included in a retrospective analysis. The patients were treated preoperatively with ASA and either ticagrelor (n = 1,266) or clopidogrel (n = 978) within the last 14 days before surgery (Figure 6). Prasugrel treatment was

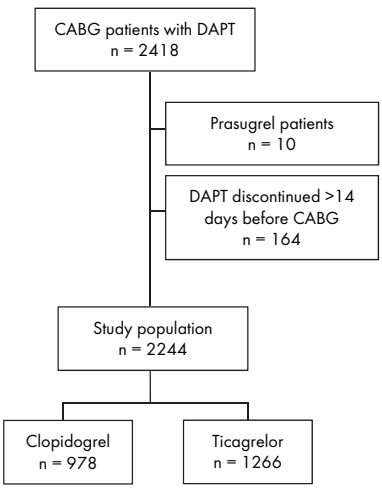

Figure 6. Flow chart for Study II.

used in only ten patients, and they were excluded from further analysis. Data on preoperative medication, bleeding, and transfusions were obtained from the SWEDEHEART registry [73], hospital records, and the surgical databases of the participating hospitals, and they were compiled in a nationwide registry. We compared the incidence of major bleeding complications and transfusions in ticagrelor- and clopidogrel-treated patients overall, and between and within the platelet inhibitors in relation to discontinuation time.

Studies III and IV

The design of Studies III and IV was ex vivo analysis of platelet aggregability in whole blood samples from coronary artery disease patients and, in Study III, from healthy controls. Platelet aggregability was investigated with MEA (Multiplate®; Roche Diagnostics, Basel, Switzerland, [74]; described below) using ADP, arachidonic acid (AA), and thrombin receptor activating peptide-6 (TRAP) as activators. The ADP test with prostaglandin detects $P2Y_{12}$-dependent aggregation with a high degree of sensitivity. The AA test assesses COX-dependent (i.e. ASA-sensitive) platelet aggregation. The TRAP test detects protease-activated receptor-1-dependent platelet aggregation and is commonly used to evaluate the effect of GPIIbIIIa antagonists. The manufacturer's normal range for ADP high-sensitivity test with hirudin-anticoagulated samples is 43–100 U, for the AA test it is 71–115 U, and for the TRAP test it is 84–128 U.

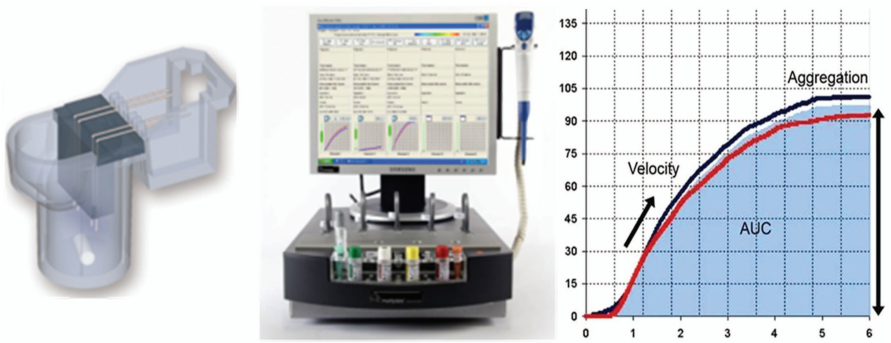

Figure 7. The Multiplate® device (left, test cell with two paired electrodes; middle, the device with automatic pipette attached; right, aggregation curve).

MEA uses whole blood samples, activated by the agonist in the test cell of the aggregometer (Figure 7), initiating aggregation of platelets on two paired electrodes in the test cell. This results in a change in impedance expressed as

a graph where the area under the curve is a quantification of platelet aggregability, which is reported in arbitrary aggregation units (U). Single analyses with one test cell (containing two paired electrodes) were performed. The maximum tolerated difference between the two electrode pairs is 20%.

Blood samples were collected from three groups of patients on antiplatelet therapy in Study III: ASA (n = 10), ASA + clopidogrel (n = 15), and ASA + ticagrelor (n = 15); and one group of healthy controls (n = 10). Aggregability was measured in baseline samples collected two hours after ingestion of platelet inhibitors, and after addition of three increasing doses of ABO-compatible fresh apheresis platelet concentrate to the baseline samples. The increase in platelet count was calculated to correspond to the increase in platelet count achieved by in vivo transfusion of approximately 2–5 units of platelets in a 70-kg patient.

In Study IV, ACS patients on DAPT with ticagrelor (n = 15) who were planned for urgent—but not acute—CABG, where DAPT was discontinued before surgery, were included in a prospective observational study. Platelet aggregability in blood samples was analyzed with MEA before and after ex vivo supplementation with two doses of platelet concentrate, corresponding to approximately 2–4 units of apheresis platelets given to a 70-kg patient. The same agonists as in Study III were used, but the analysis was repeated at five consecutive time points: 12, 24, 48, 72 and 96 hours after the last dose of ticagrelor.

The other part of Study IV included 25 ACS patients on DAPT with ticagrelor who were accepted for CABG, where DAPT was discontinued before surgery. We evaluated the recovery of platelet aggregation with MEA at five consecutive time points 12–96 hours after discontinuation of ticagrelor. The same agonists as previously described, i.e. ADP, AA, and TRAP, were used.

Study V

One hundred and thirty-three consecutive patients who underwent surgery for acute dissection of the ascending aorta (Stanford type A) at Sahlgrenska University Hospital from January 2007 to June 2011 were included in a retrospective single-centre observational study. During the time period studied, DAPT was limited to treatment with clopidogrel in addition to ASA.

Indication for platelet inhibition was collected from medical records. Appropriate DAPT according to guidelines [12] was defined retrospectively by two independent observers; differences of opinion were resolved by consensus. Perioperative bleeding, transfusions, and mortality were compared between patients with and patients without ongoing platelet inhibition.

Definitions

Major bleeding was classified according to four published definitions (Table 3): Bleeding Academic Research Consortium (BARC) type 4, CABG-related [75]; modified BART criteria [58], PLATO life-threatening bleeding, and PLATO major bleeding [19]. All four definitions were used in Studies I and II, and the BART major bleeding definition was used in Study V.

Table 3. Definitions of major bleeding

BARC type 4 (CABG-related bleeding) [75]	**BART major bleeding [58]**	**PLATO life-threatening bleeding [19]**	**PLATO major bleeding [19]**
Intracranial bleeding	Fatal bleeding	Fatal bleeding	
Re-exploration for bleeding	Re-exploration for bleeding	Re-exploration for bleeding	Re-exploration for bleeding
Transfusion of ≥5 U red blood cells	Transfusion of ≥10 U red blood cells	Transfusion of ≥4 U red blood cells	Transfusion of ≥2 U red blood cells
Chest tube output ≥2 L/24 h	Chest tube output ≥1.5 L/12 h	-	-
-	-	Drop in haemoglobin ≥50 g/L	Drop in haemoglobin ≥30 g/L

3.3 Statistics

For all studies, data are 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 per cent, as indicated. Statistical significance was assumed with two-sided p-values of < 0.05.

Study I

Continuous variables were compared using Mann–Whitney U-test for both normally distributed values (due to inequality in group size) and for comparing distribution across groups of data that were not normally distributed. Normality of data was tested with the Kolmogorov–Smirnov test. Categorical variables were compared with Fisher's exact test. For statistical analysis, SPSS Statistics 20 was used (IBM Corp., Armonk, NY, USA).

Study II

The two groups were compared by Fisher's exact test for dichotomous variables, the Mantel–Haenszel Chi-square test for ordered categorical variables, and the Mann–Whitney U-test for continuous variables. Logistic regression modelling was used to identify factors related to major bleeding and to compare the incidence of bleeding between discontinuation groups. Factors that were significantly different between platelet inhibitors and associated with major bleeding with a p-value of < 0.10 were included in a multivariable logistic regression model for adjustment. Based on the previous regional study, the sample size (at least 500 patients in each group) was chosen to achieve 80% power in finding a significant difference in incidence of major bleeding between clopidogrel and ticagrelor after stratification by time from discontinuation of medication to surgery. No adjustment for multiplicity was performed. SAS software version 9.4 was used (SAS Institute, Cary, NC, USA).

Study III

Changes from baseline within a group were analyzed with paired t-test. Group comparisons of aggregability at baseline were made with one-way ANOVA (for more than two groups) or with Student's t-test (for two-group comparisons). Differences in response to the different doses of platelet transfusion between groups were analyzed by ANOVA for repeated measurements. For statistical analysis, we used STATISTICA 10 software (StatSoft, Tulsa, OK, USA).

Study IV

Change in platelet aggregation over time was tested with a general linear model for repeated measurements. A mixed-effects model for repeated measurements was used to analyze the efficacy of addition of platelet

concentrate and interaction between time point and dose of platelets. No formal adjustment for multiplicity was assumed to be necessary. Normal distribution of the data was tested with the Shapiro–Wilk test. Statistical analysis was done with IBM SPSS Statistics 23 software, except for the mixed model, where SAS software version 9.4 was used.

Study V

Continuous variables were compared using Student's t-test for normally distributed values and the Mann–Whitney U-test for values that were not normally distributed. Categorical variables were compared with Chi-square test. Survival was estimated by Kaplan–Meier survival analysis and compared by log-rank test. Factors univariately associated with 30-day mortality and bleeding of more than 1,000 mL were identified with a logistic regression model.

4 RESULTS

4.1 Incidence of major bleeding with dual antiplatelet therapy

Major bleeding

In Study I, the regional pilot study, there was no overall difference in modified BART major bleeding between patients treated with clopidogrel and those treated with ticagrelor, 13.8% vs. 14.5% (p = 0.89). However, in Study II there were significantly less CABG-related major bleeding complications in ticagrelor-treated patients according to three of the four definitions (Figure 8): BARC CABG, 12.9% vs. 17.6% (p=0.0024); BART major bleeding, 8.8% vs. 11.6% (p = 0.041), and PLATO life-threatening major bleeding, 46.8% vs. 54.0% (p = 0.001) for ticagrelor- and clopidogrel-treated patients, respectively. There was no significant difference in PLATO major bleeding: 89.9% vs. 92.1% (p = 0.076).

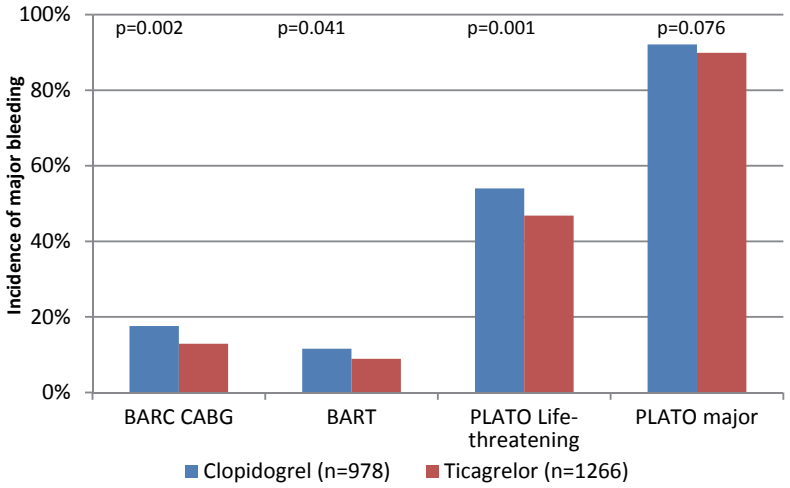

Figure 8. Incidence of major bleeding complications according to BARC-CABG, BART, PLATO life-threatening, and PLATO major bleeding (p-values from Fisher's exact test between ticagrelor and clopidogrel).

In Study II, the use of ticagrelor was associated with a reduced risk of BARC major bleeding, both before adjustment (odds ratio (OR) 0.69 (95% confidence interval (CI) 0.55–0.87), p = 0.002) and after adjustment for time since discontinuation and the other factors that significantly influenced risk of

bleeding in the univariable analysis (adjusted OR 0.72 (95% CI 0.56–0.92), p = 0.012). Mortality was significantly higher in patients with BARC major bleeding (9.9% vs. 0.7%, unadjusted OR 14.78 (95% CI 7.82–27.9), p < 0.0001).

Bleeding volume and transfusions

In Study I, there tended to be more reoperations due to bleeding and more transfusions of RBCs, plasma, and platelets in the group treated with ticagrelor. The rate of reoperation due to bleeding was almost doubled in the ticagrelor group compared to that in the clopidogrel group (11% vs. 23%), but the difference did not reach statistical significance (p = 0.15)

In Study II, overall, ticagrelor-treated patients bled significantly less over the first 12 hours after surgery (median 470 mL (IQR 350–665 mL) vs. 500 mL (IQR 370–730 mL), p = 0.0017), and they received fewer transfusions of RBCs (median 0 U (IQR 0–3) vs. 1 U (IQR 0–3), p = 0.0012). There was no significant difference in reoperation due to bleeding in the two groups. However, when medication was discontinued less than 24 hours before surgery, ticagrelor-treated patients bled more and received more transfusions than clopidogrel-treated patients (Table 4).

Table 4. Bleeding and transfusion after CABG according to time from discontinuation of platelet inhibitor (Study II)

	Discont.	Clopidogrel	Ticagrelor	p-value
Postop. bleeding in first 12 h (mL)	**0–24 h**	488 (340–721)	670 (498–1103)	< 0.001
	24–48 h	600 (415–890)	585 (400–753)	0.514
	48–72 h	570 (440–815)	510 (370–735)	0.207
	72–96 h	560 (400–790)	450 (343–738)	0.118
	96–120 h	520 (405–800)	450 (350–698)	0.036
	Over 120 h	480 (358–653)	450 (349–610)	0.021
Any transfusion (U)	**0–24 h**	4 (2–11)	8.5 (4–17)	0.001
	24–48 h	3 (0–8)	4 (2–10.5)	0.111
	48–72 h	3 (0–6)	3 (0–7)	0.779
	72–96 h	2 (0–6)	2 (0–3)	0.013
	96–120 h	2 (0–6)	0 (0–3)	0.025
	Over 120 h	0 (0–3)	0 (0–2)	0.044

Median with IQR. p-values from Mann–Whitney U-test.

Impact of time since discontinuation

In Study I, 70% of patients had their $P2Y_{12}$ inhibitor discontinued 5 days before surgery as recommended in guidelines, 10% had it discontinued 2–4 days before surgery, and approximately 20% were maintained on DAPT 0–1 day before the procedure. Mean discontinuation time was longer in clopidogrel-treated patients than in ticagrelor-treated patients (9.3 ± 8.4 vs. 7.5 ± 6.1 days, p = 0.029).

When the groups were stratified by time from discontinuation of platelet inhibitor to surgery, there were no statistically significant differences in incidence of major bleeding. However, in patients with discontinuation 2–4 days, there was a large numerical difference in major bleeding (6.3% for ticagrelor vs. 25% for clopidogrel, p = 0.21). When treatment was discontinued 0–1 days before surgery, there was a trend of a higher incidence in ticagrelor-treated patients than in clopidogrel-treated patients (41% vs. 22%, p = 0.063). For clopidogrel-treated patients, there was a significantly higher incidence of major bleeding when treatment was discontinued 2–4 days

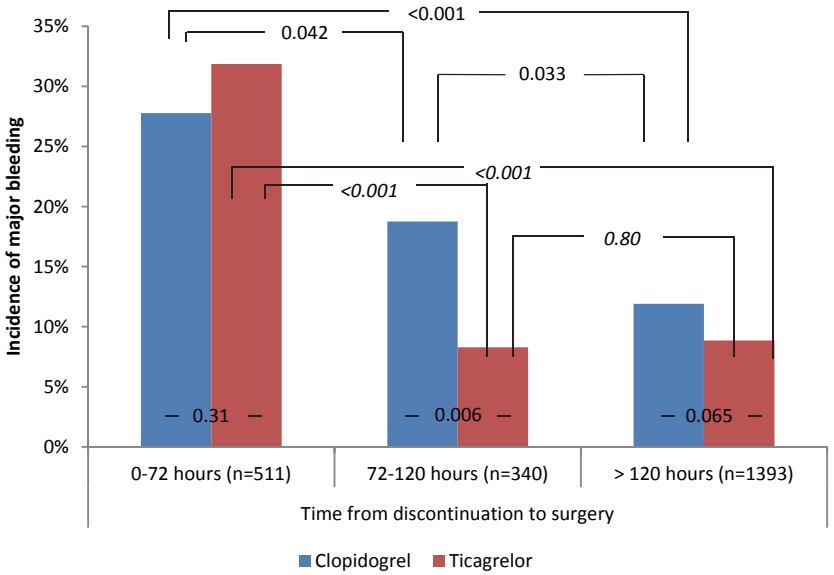

Figure 9. Incidence of BARC major bleeding within and between platelet inhibitor groups, stratified by time from discontinuation to surgery (p-values from a logistic regression model, lower values between inhibitor groups, upper values within clopidogrel group, comparison within ticagrelor group in italics).

or 0–1 day before surgery rather than five or more days, whereas only ticagrelor- treated patients with treatment discontinued 0–1 day before surgery had a higher incidence than patients with discontinuation five or more days.

In Study II, 44% of the clopidogrel-treated patients had the $P2Y_{12}$ inhibitor discontinued less than 5 days before surgery, as compared to 33% in the ticagrelor group ($p < 0.0001$), and the mean discontinuation time was 5.2 ± 3.6 days before surgery for the clopidogrel-treated patients and 5.9 ± 3.5 days before surgery for the ticagrelor-treated patients ($p < 0.0001$).

The difference in incidence of major bleeding between ticagrelor and clopidogrel was mainly driven by a significant reduction in major bleeding complications in the ticagrelor group when clopidogrel or ticagrelor was discontinued 72–120 hours before surgery (OR 0.39 (95% CI 0.20–0.76), $p = 0.006$) (Figure 9). When either drug was discontinued according to current guidelines (> 120 hours before surgery), there was no significant difference in the incidence of major bleeding complications between ticagrelor- and clopidogrel-treated patients (OR 0.72 (95% CI 0.51–1.02); 9% vs. 12%, $p = 0.065$) (Figure 9).

Within the ticagrelor group, there was no significant difference in major bleeding complications between patients with discontinuation 72–120 hours or > 120 hours before surgery (OR 0.93 (95% CI 0.53–1.64), $p = 0.80$), whereas discontinuation 0–72 hours before surgery was associated with a significantly higher rate of major bleeding compared to both 72–120 hours (OR 5.17 (95% 2.89–9.27), $p < 0.0001$) and > 120 hours (OR 4.81 (95% CI 3.34–6.95), $p < 0.0001$). In contrast, clopidogrel-treated patients had a higher

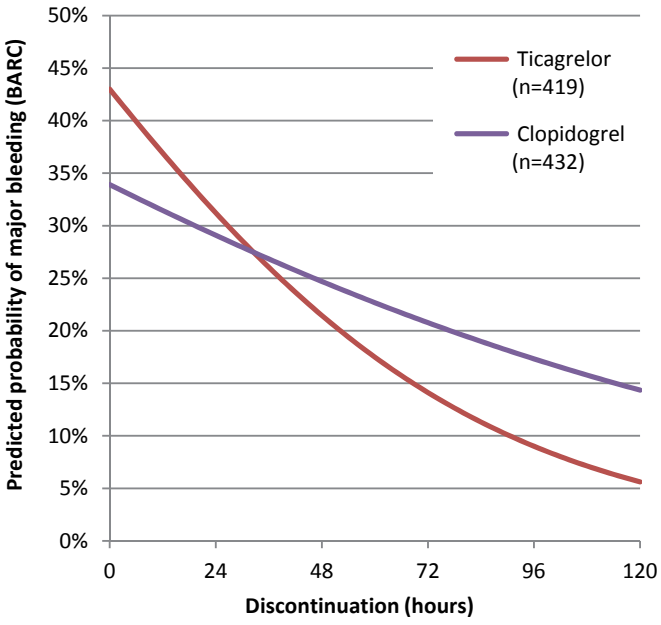

Figure 10. Predicted risk of major bleeding (BARC) in CABG patients with discontinuation of clopidogrel or ticagrelor < 5 days before surgery (logistic regression model).

incidence of major bleeding when treatment was discontinued 72–120 hours before surgery rather than > 120 hours before surgery (OR 1.71 (95% CI 1.04–2.79), p = 0.033). Likewise, in the clopidogrel group, discontinuation 0–72 hours before surgery was associated with a higher incidence of major bleeding than discontinuation 72–120 hours before surgery (OR 1.67 (95% CI 1.02–2.73), p = 0.042) or > 120 hours before surgery (OR 2.85 (95% CI 1.98–4.10), p < 0.0001). The predicted risk of major bleeding is plotted as a function of time between discontinuation of treatment and surgery in Figure 10.

4.2 Inhibition of platelet aggregation with different platelet inhibitors

In Study III, there was a significant difference in ADP-induced aggregation between the four groups at baseline (ANOVA $p < 0.001$), as illustrated in Figure 11. Patients treated with DAPT had significantly lower aggregation than ASA-treated patients and healthy controls, but there was no significant difference between clopidogrel- and ticagrelor-treated patients ($p = 0.90$). The same pattern was found in the TRAP test, with significantly lower aggregation in the two DAPT groups compared to ASA-treated patients ($p = 0.004$ for clopidogrel and $p = 0.006$ for ticagrelor) and healthy controls ($p = 0.012$ for clopidogrel and $p = 0.017$ for ticagrelor), with no significant difference between the clopidogrel-treated patients and the ticagrelor-treated patients ($p = 0.67$), and no significant difference between the controls and the ASA-treated patients ($p = 0.89$).

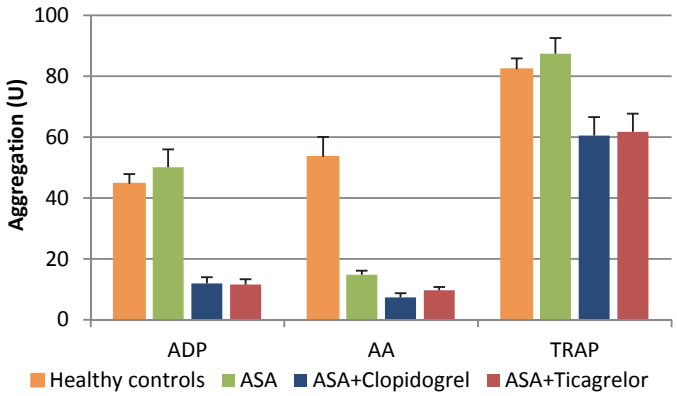

Figure 11. Platelet aggregation at baseline in patients treated with different platelet inhibitors and in healthy controls (mean with SEM).

AA-induced aggregation was significantly reduced in all three patient groups compared to healthy controls (ANOVA, $p < 0.001$). As expected, the AA test also showed significant inhibition in patients treated with ASA alone. Furthermore, AA-induced aggregation was significantly lower in both DAPT groups than in the ASA group ($p = 0.001$ for clopidogrel and $p = 0.006$ for ticagrelor). There was no significant difference between clopidogrel and ticagrelor in this test ($p = 0.19$).

4.3 Recovery of platelet function after discontinuation of ticagrelor

In Study IV, ADP-induced platelet aggregation gradually increased after discontinuation of ticagrelor ($p < 0.001$) (Figure 12a), from 10 ± 7.5 U at 12 hours, and reached a mean level of 55 ± 31 U 96 hours after discontinuation of ticagrelor. There was a large degree of inter-individual variability in recovery of platelet function at all sampling times: range 0–24 U at 12 h; 3–34 U at 24 hours; 4–75 U at 48 hours; 4–88 U at 72 hours; and 11–114 U at 96 hours (Figure 12b). Also, mean AA-induced platelet aggregation increased significantly with time, but at a low level, from 10 ± 8 U at 12 hours to 17 ± 9 U at 96 hours ($p = 0.003$) (Figure 12a). Mean TRAP-induced platelet aggregation increased from 86 ± 17 U at 12 hours to 112 ± 31 U at 96 hours ($p < 0.001$) (Figure 12a).

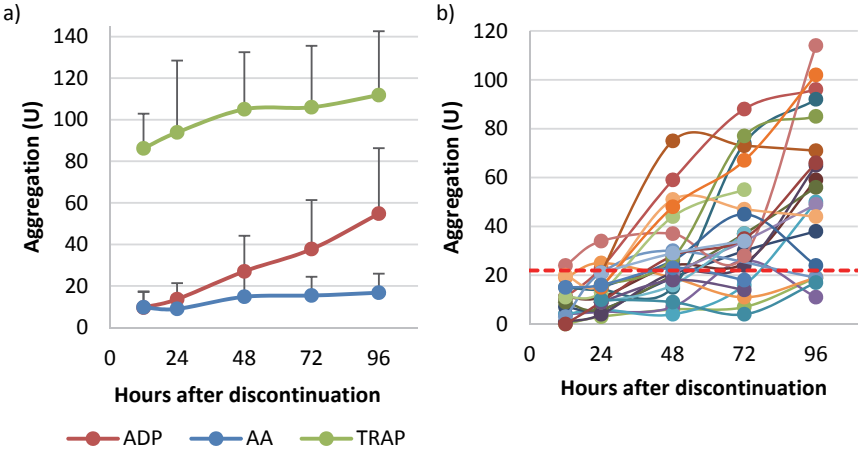

Figure 12. a) Platelet aggregation at consecutive time points after discontinuation of ticagrelor (mean with upper whisker of standard deviation). b) Inter-individual variation in recovery of ADP-initiated platelet function after discontinuation of ticagrelor. Points from the same patients are connected by Bezier splines for illustrative purposes. The dashed line indicates U = 22, a previously suggested cut-off value for risk of major bleeding.

4.4 Effect of platelet transfusion on platelet aggregability

In Study III, the effect of ex vivo platelet supplementation in three doses was tested at two hours after ingestion of platelet inhibitors. Platelet supplementation improved ADP-induced aggregation significantly in the two

DAPT groups, but not in the ASA group or in healthy controls (Figure 13). When comparing the effect of platelet supplementation between clopidogrel and ticagrelor, it was significantly inferior in the ticagrelor-treated patients at the highest dose of platelets ($p = 0.021$).

AA-induced aggregation was improved after supplementation in all four groups, with the highest response in the ASA-only group and the lowest response in the ticagrelor group (Figure 13). In a direct comparison between clopidogrel and ticagrelor, the response to platelet supplementation was significantly lower in ticagrelor-treated patients at all doses of platelets ($p = 0.025$, $p = 0.040$, and $p = 0.004$).

Platelet supplementation increased TRAP-induced aggregation in all four groups, with the largest increase in the clopidogrel and ticagrelor groups (Figure 13). There was no significant difference in response between the clopidogrel group and the ticagrelor group ($p = 0.67$).

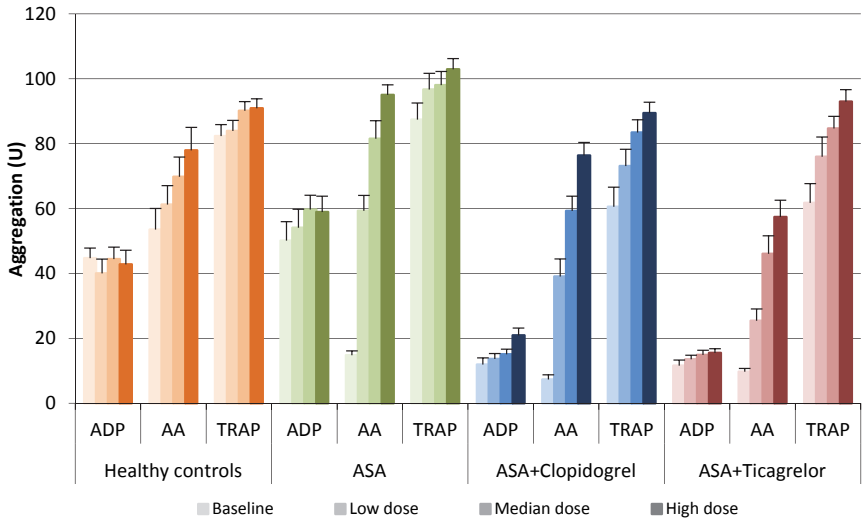

Figure 13. Effect of ex vivo platelet supplementation on samples from healthy controls and patients on ASA or DAPT with clopidogrel and ticagrelor (mean with SEM).

In Study IV, ex vivo supplementation with two doses of platelet concentrate was tested at five consecutive time points after discontinuation of ticagrelor. Supplementation with either the higher dose or the lower dose did not increase

ADP-induced platelet aggregation at any of the time points (Figure 14a). There was no significant difference between the lower dose and the higher dose of platelet concentrate ($p = 0.98$), but there was a minor interaction between time and dose for both the lower dose and the higher dose, with less effect at later time points.

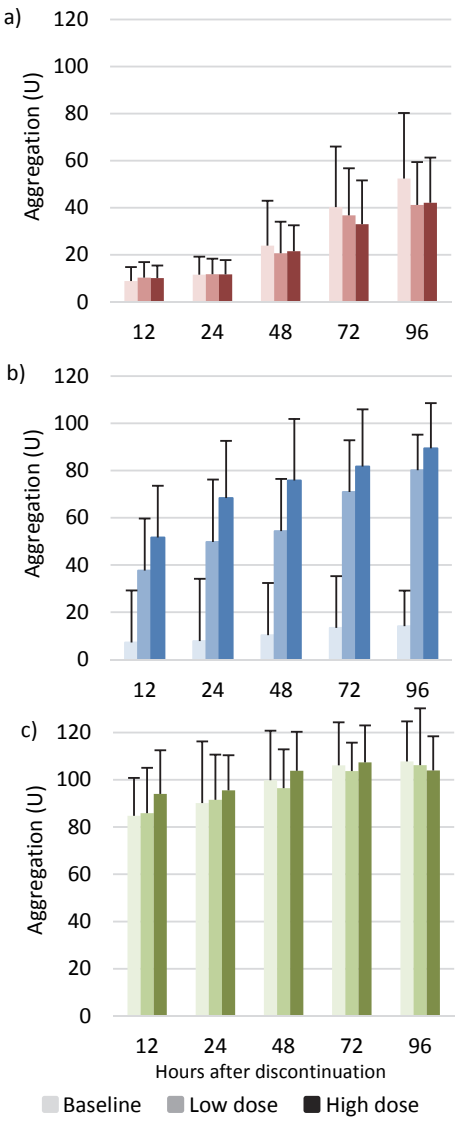

Figure 14. Platelet aggregation at consecutive time points after discontinuation of ticagrelor, at baseline and after addition of a low or high dose of platelet concentrate. Mean with upper whisker of standard deviation. Platelet aggregation induced by (a) adenosine diphosphate, (b) arachidonic acid, or (c) thrombin receptor activating peptide.

In contrast, AA-induced platelet aggregation was significantly increased when platelet concentrate was added (Figure 14b). It increased to levels close to the normal range (71–115 U) for the lower dose of platelet concentrate, and to levels within the normal range for the higher dose at all times, irrespective of the level before supplementation. The dose-response effect—with an increase of 21 U with the higher dose compared to the lower dose—was statistically significant in the mixed model ($p = 0.0013$).

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 14c).

4.5 Platelet inhibition in patients operated for acute aortic dissection

Prevalence, indications, and appropriateness of platelet inhibition

Forty-three of 133 patients (32%) were treated with one or two antiplatelet agents at the time of surgery, 19 (14%) with ASA alone and 24 (18%) with DAPT. In the 24 patients treated with DAPT, the indication for clopidogrel treatment was STEMI in one case, release of cardiac enzymes in five patients, and unspecific ECG changes or ST depression in eight patients. In seven cases, treatment was started due to chest pain alone. Two patients were given DAPT in the pre-hospital setting without a clearly stated indication, and one patient had DAPT prior to presentation of acute aortic dissection. Compared to indications for DAPT according to guidelines [12], treatment was appropriate in seven of the 24 patients (29%) who were treated with clopidogrel and it was considered less appropriate in 17 cases (71%).

Bleeding and transfusions

Bleeding volumes during the first 12 hours after surgery were greater in patients who received platelet inhibition, median 800 mL (IQR 390–1,070 mL) vs. 500 mL (IQR 420–1,605 mL) in the group without inhibition ($p = 0.037$). There was no significant difference in postoperative bleeding between patients treated with ASA or DAPT. In univariate testing, time on CPB was a significant predictor of bleeding in excess of 1,000 mL, whereas intraoperative

use of aprotinin was associated with a reduced risk (OR 0.26, 95% CI 0.07–0.92).

There was a trend of having more transfusions in patients on ASA or DAPT: median 14 units of RBCs (IQR 7.5–24) as opposed to 9 units (IQR 3–17) in patients with no antiplatelet therapy (p = 0.066), 12 units of plasma (IQR 6–25) as opposed to 9 units (IQR 3–19) (p = 0.17), and 5 units of platelets (IQR 3–8) as opposed to 3 units (IQR 2–7) (p = 0.08). There was no significant difference in the number of transfusions between patients who were treated with ASA and patients who were treated with DAPT.

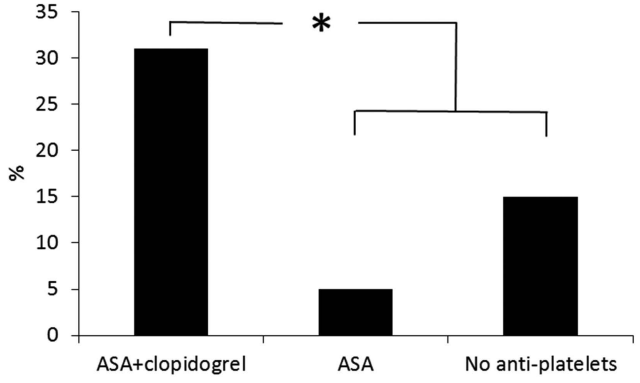

Figure 15. Comparison of 30-day mortality after surgery for acute aortic dissection type A in patients on DAPT with clopidogrel, ASA alone, or no platelet inhibition (p = 0.038).

Thirty-day mortality

Mortality during the first 30 days after surgery was not significantly different in patients with antiplatelet therapy and those without: 15% vs. 19% (p = 0.56). In contrast, in the subgroup treated with DAPT, mortality during the first 30 days after surgery was significantly higher than in patients treated with ASA alone or in those with no platelet inhibition: 30% vs. 5% and 15%, respectively (p = 0.038) (Figure 15). DAPT was associated with a threefold increase in 30-day mortality (unadjusted OR 2.94, 95% CI 1.03–8.4, p = 0.044). Other factors associated with 30-day mortality were transfusion with RBCs (OR 1.04 per unit, 95% CI 1.016–1.067), postoperative bleeding (OR 1.045 per 100 mL, 95% CI 1.010–1.085), and cardiac tamponade at presentation (OR 3.00, 95% CI 1.12–8.0).

5 DISCUSSION

One of the main observations in this work is that patients in the cardiac surgery setting who are subject to ongoing potent platelet inhibition at the time of surgery bleed more, and receive more transfusions than patients without ongoing antiplatelet treatment. This holds true whether patients are under appropriate treatment—i.e. the acute coronary syndrome patients with an unstable disease in need of urgent surgery—or the aortic dissection patients who receive medication because their clinical presentation is difficult to distinguish from the more common ACS. Earlier studies [37-43] have shown that increased bleeding is an important risk factor for increased morbidity and mortality in ACS and cardiac surgery patients. It is not possible to completely avoid bleeding in cardiac surgery, but the risk appears to be tied to the amount of bleeding [37, 39]. Bleeding in the setting of cardiac surgery is multifactorial. Procedural considerations such as the acuteness of the operation, the presence of CPB, the technical skills of the surgeon, the underlying disease and its inflammatory response, and concurrent medication are all factors that may contribute.

Likewise, the detrimental effect of bleeding may be mediated by a number of factors. The trauma in itself, the need for transfusions, and the underlying cause of bleeding may be both mediators and confounders in the causative relationship between major bleeding and increased mortality. However, as shown by Dyke et al. [39] among others, the harmful effects remain even after adjustment for other factors, indicating that the issue of bleeding is not trivial in itself. Increased mortality in patients with major bleeding was observed in Study II, where unadjusted 30-day mortality was almost 15 times greater in patients with major bleeding than in patients with no bleeding complications (9.9% vs. 0.7%, OR 14.8). The severity of major bleeding was also further confirmed by the higher mortality seen in aortic dissection patients treated with DAPT at the time of surgery (Study V). This confirms the previous studies about the negative effects of bleeding complications and emphasises the importance of—if possible—avoiding excessive bleeding in cardiac surgery. This will require continued improvement in surgical technique, and also developments in preoperative treatment and medication. Timely

discontinuation of platelet inhibitors before less acute surgery would help to achieve this.

Timing of discontinuation of platelet inhibitors

It is well understood that discontinuation of platelet inhibitors is associated with an increase in thrombotic complications such as stent thrombosis, early and late after discontinuation of platelet inhibitors [76-78]. Furthermore, it has been suggested that withdrawal of platelet inhibition is an independent risk factor for cardiac events in ACS patients in general [79], but few studies have reported ischaemic events in patients waiting for CABG [80]. The timing of discontinuation of platelet inhibitors is influenced by a number of factors, including institutional guidelines, the patient's condition, logistical considerations, and the decision of the individual surgeon. The variability was apparent in Studies I and II, where 30–45% of the patients were operated after a shorter discontinuation than the five days recommended in guidelines. However, the current European guidelines do make allowances for case-by-case decisions regarding discontinuation when patients are at risk of both bleeding and thrombosis [24], to allow some degree of restoration of platelet function. This is supported by the results of Study II, where there was no difference in major bleeding complications when ticagrelor was discontinued three days before surgery rather than five days. It may also be expanded to suggest that it is safe to recommend that ACS patients treated with ticagrelor can be operated as early as three days after discontinuation. This conclusion was also drawn in another recent study of CABG patients with DAPT within five days before surgery [81]. A reduction in the waiting time from five to three days would reduce the risk of thrombotic events while waiting for CABG, and save hospital resources, as it would shorten hospital length of stay.

Study IV lent further support to shorter discontinuation of ticagrelor, as it showed that on average 72 hours of discontinuation allowed adequate recovery of ADP-induced platelet function in ticagrelor-treated patients. At 72 hours, mean ADP-induced aggregation with MEA was 38 U, which is well above the previously suggested cut-off levels for increased bleeding risk (22 U [67, 68] or 31 U [72]). As shown in Study II (Table 4), the bleeding volumes and incidence of transfusions were correspondingly lower in ticagrelor patients who were operated when the $P2Y_{12}$ inhibitor had been discontinued

more than 72 hours before surgery, compared to clopidogrel patients at the same time point.

The difference in pharmacological properties of ticagrelor and clopidogrel may explain the overall lower incidence of major bleeding with ticagrelor in Study II. Ticagrelor and its active metabolites are cleared from plasma with a half-life of 6–13 hours [11], and the decrease in platelet inhibition follows the same pattern due to the reversible binding. For clopidogrel, the half-life of the drug in plasma is much shorter, with active metabolites only present in plasma during the first hours after ingestion [82]. However, due to the irreversible binding of clopidogrel to the ADP receptor, the recovery of platelet function will instead be based on generation of new platelets rather than plasma clearance of the drug.

The difference in incidence of major bleeding in Study II was driven mainly by a lower incidence in ticagrelor patients where platelet inhibition was discontinued between 72 and 120 hours. It could not be fully explained by the slightly longer discontinuation time in the ticagrelor group, since the difference was also maintained after adjustment for this factor. The antiplatelet effect of ticagrelor therefore returns to baseline faster than that of clopidogrel, which is why the remaining effect of ticagrelor at this time point can be expected to be lower than the remaining effect of clopidogrel. It should be noted that the result in this regard is hypothesis-generating rather than conclusive, given the retrospective observational design of the study. However, it supports the findings of previous studies [21, 47], where ticagrelor had a more rapid offset than clopidogrel. In the study by Becker et al., platelet inhibition by ticagrelor at day three after discontinuation was comparable to that of clopidogrel at day five [47].

Role of platelet function tests in timing of surgery

Despite the known difference in offset time of the antiplatelet effect of the different $P2Y_{12}$ inhibitors, current guidelines recommend, as previously mentioned, that both ticagrelor and clopidogrel should be discontinued five days before cardiac surgery [2, 24]. The results of Study II instead support the use of differentiated discontinuation times for ticagrelor and clopidogrel, i.e. three days for ticagrelor and five days for clopidogrel. Another option in timing of surgery after discontinuation of platelet inhibitors is to be guided by

platelet function tests, an approach endorsed in the most recent updates of the European guidelines, but with a low level of evidence [2].

In Study IV, there appeared to be a good correspondence between the discontinuation time of three days for ticagrelor suggested by Study II and the mean time needed to recover platelet function to levels over the previously suggested cut-off above 22 U or 31 U of ADP-initiated platelet aggregation [67, 68, 72]. Combining the results from Study II and IV, the patients with recently discontinued ticagrelor are likely to have a higher degree of residual platelet inhibition. This is corroborated by Study II, where patients with DAPT discontinued 72–120 hours before surgery were less likely to suffer from major bleeding than patients with discontinuation of less than 72 hours. As suggested by Ranucci and Malm [67, 68, 72], platelet function testing may be used in this group to identify patients with a higher risk of bleeding. In patients for whom surgery cannot be postponed further despite high residual platelet inhibition, platelet function testing may instead be used to identify the patients in whom counter-measures against bleeding should be initiated early.

Despite a relatively narrow range of aggregation at 12 hours after discontinuation (0–24 U), the range increased at subsequent time points. At 72 hours, the range was 4–88 U, and six out of 25 patients still had ADP-initiated aggregation under 22 U. This large inter-individual variability in recovery of platelet function supports the use of platelet function tests in timing of surgery. The approach was tested in a study by Mahla and associates [71] using thromboelastography in clopidogrel-treated patients, where it was found to be feasible and safe—and that waiting time was reduced by approximately 50%. A recent study by Plicner et al. similarly found that high platelet aggregation, above 50% as measured by LTA, was associated with less bleeding and a lower rate of reoperation after CABG in patients with clopidogrel treatment within five days before surgery [69]. A randomized study comparing timing by a set number of days with timing by platelet function test is warranted. Such a study would preferably include patients with ticagrelor, where the benefit in terms of waiting time may be even greater, given the more rapid recovery of platelet function expected. The benefits of this approach must be weighed against the logistic problems that would arise. Platelet function testing to determine timing of surgery would require

increased flexibility from the healthcare system, as procedures may be postponed at the last minute. Hence, it would also be important to study recovery of platelet function in more detail in a larger patient material, to ascertain whether testing could be performed on the day before surgery with sufficient predictability. These questions were not studied in the current work.

Increased bleeding with ongoing platelet inhibition

In addition, Studies I and II illustrate another important clinical problem. Patients with ACS suffer from an acute disease that develops over time, and it is not always possible to predict the clinical course that each patient may take. Thus, it is impossible to avoid operating ACS patients with ongoing or recently discontinued DAPT altogether. Instead, it is important to more closely characterize the bleeding risks at different time points—on medication and after discontinuation. The results from Studies I and II suggest that patients requiring urgent surgery, especially those treated with ticagrelor, may benefit from discontinuation of medication at least 72 hours before surgery, if the overall clinical condition permits. In Study IV, the majority of ticagrelor-treated patients had sufficiently recovered platelet function at this point, in concordance with the ONSET-OFFSET study [21]. The recent study by Malm et al. also confirmed the correlation between residual platelet inhibition by ticagrelor as measured by MEA and the risk of major bleeding after CABG [68].

In patients with ongoing treatment with platelet inhibitors, i.e. when the platelet inhibitor was discontinued within 24 hours before surgery (Study II), there was a higher incidence of transfusions and greater postoperative bleeding volume in the ticagrelor group than in the clopidogrel group. This finding differs from that of the PLATO-CABG substudy, where timing of discontinuation was unrelated to incidence of major bleeding. However, the PLATO definition of major bleeding included a drop in haemoglobin [23], which may present in cardiac surgery patients due to haemodilution rather than bleeding.

The higher incidence of bleeding after recent discontinuation of ticagrelor may be understood as a reflection of the pharmacological properties of the platelet inhibitors, as ticagrelor is known to generate a stronger platelet inhibition than clopidogrel [11, 21], in addition to the absence of non-

responders. In patients with a high risk of acute CABG or with an uncertain diagnosis, this should be considered before administration of a ticagrelor loading dose. Since the onset of effect of ticagrelor is quicker than with clopidogrel, it may be hypothesized that it would be sufficient to administer the inhibitor when the revascularization strategy has been decided. A recent study of preloading with ticagrelor in STEMI patients failed to show a definite positive effect as well [83]. Avoiding a pre-hospital loading dose may also reduce the number of patients with aortic dissection, or other conditions presenting with acute chest pain, being incorrectly treated with potent platelet inhibitors.

In Study V, we showed that approximately one-third of patients with acute aortic dissection at some point are mistaken for ACS patients and treated with platelet inhibitors. In some cases, treatment was initiated in the ambulance before arrival at the hospital, and the indication for treatment was not always documented in medical records. ACS is approximately 100 times more common than acute aortic dissection [4, 84], but our results highlight the importance of a correct diagnosis before initiating potent treatment, even more so since treatment with antiplatelet drugs increased the already substantial risk of bleeding in these patients. Also, there was a twofold increase in mortality in aortic dissection patients who received DAPT before correct diagnosis and subsequent surgery, compared to patients who were treated with ASA only or did not receive any antiplatelet treatment. However, it is important to note that the study did not have the statistical power for mortality analysis, and the results were not adjusted for other factors that might influence mortality. It would therefore be of merit to analyze mortality in association with DAPT in aortic dissection in a larger material.

Efficacy of platelet transfusion with DAPT

When cardiac surgery patients bleed, platelet transfusion is often used to improve haemostasis. Although platelet transfusion increased ADP-induced aggregation significantly in patients on DAPT with clopidogrel or ticagrelor in Study III, aggregation did not reach more than 50% of the baseline levels in healthy subjects—even with the highest dose of platelets. The amounts of platelet concentrate used in Studies III and IV correspond to 2–5 units of platelet concentrate transfused to a patient weighing 70 kg. These doses are

within the clinically relevant spectrum of transfusion to a patient suffering from bleeding complications after cardiac surgery. The levels of aggregation obtained with the highest dose of platelets in clopidogrel- and ticagrelor-treated patients (21 and 16 U, respectively) were below the previously suggested cut-off level for increased bleeding risk in cardiac surgery patients. Furthermore, patients on DAPT with clopidogrel or ticagrelor had approximately 25% lower baseline TRAP-induced aggregation than healthy volunteers and ASA-treated patients. This may have been due to $P2Y_{12}$ inhibitors preventing the release of ADP from dense granules in the platelets, thereby also inhibiting TRAP, which partly depends on this signalling for activation [85]. A similar mechanism in the thromboxane pathway may explain the significantly lower AA-induced aggregation in patients treated with ASA only, despite the fact that they had received the same dose of ASA (75 mg daily) [86].

Study III indicated that platelet supplementation may have less effect in patients treated with the newer platelet inhibitor ticagrelor than in those treated with clopidogrel. In the APTITUDE study [62], where platelet transfusion was given to cardiac surgery patients on DAPT with ongoing perioperative bleeding, it had less effect in patients on DAPT with prasugrel or ticagrelor than in those on DAPT with clopidogrel. However, the DAPT group in this study was heterogeneous, as it compared patients treated with clopidogrel with a mixture of patients treated with prasugrel and ticagrelor, in spite of their different pharmacological properties. Few other studies have investigated the effect of platelet transfusions given to patients on DAPT with ticagrelor. Our findings are similar to those of Bonhomme et al. [87], where platelet supplementation was found to increase platelet reactivity more in prasugrel-treated patients than in ticagrelor-treated patients, and also to those in an in vitro study of ticagrelor-treated samples from healthy volunteers [88].

One reason for the lower effect of platelet supplementation may be the reversible binding of ticagrelor to the $P2Y_{12}$ receptor [11], indicating that remaining systemic ticagrelor—or its active metabolites—can be redistributed to the transfused platelets, whereas clopidogrel or prasugrel are irreversibly bound to the native platelets. This is further supported by the results of a study where inhibition of donor platelets was tested after exposure to plasma from

patients treated with clopidogrel, prasugrel, and ticagrelor, and ticagrelor plasma alone was capable of affecting platelet aggregation [89]. Furthermore, in Study IV, the effect of platelet transfusion was also low at later time points after discontinuation, which might be explained by continued redistribution as long as ticagrelor remains in the circulation. The problem of ticagrelor redistribution may be solved using a direct antidote, which is under development [22], but it is not yet clinically available.

However, some clinical effect of platelet transfusion can still be expected also in patients treated with ticagrelor, since AA-induced aggregation was restored to normal or supernormal levels in both Study III and Study IV, in accordance with other studies [87, 88]. The restoration was achieved even though patients with DAPT were more inhibited than patients on monotherapy with ASA at baseline. TRAP-induced aggregation was also enhanced by platelet supplementation to levels above baseline, both for patients on ASA alone and for those on DAPT in Study III—but not in Study IV. The lower effect in Study IV than in Study III may be explained by the age of the platelet concentrates, and lesions acquired through storage. Stored platelets have been suggested to be more vulnerable to desensitization of ADP-induced aggregation [90]. Only fresh apheresis platelets were used in Study III, whereas in Study IV, only stored platelet concentrates where available—with a mean age of 1.5 days.

Limitations of the studies

Studies I, II, and V had all the inherent limitations of observational studies, including selection bias and unregistered confounders. This included—but was not limited to—history of bleeding, liver disease, heart failure, and renal failure. In order to conclusively prove the findings of Study II, a randomized clinical trial with different discontinuation times would be required. Unfortunately, such a study is unlikely ever to be conducted. In spite of this, Study II in particular also had strengths; it represented a complete nationwide cohort of ACS patients on DAPT with ticagrelor or clopidogrel who were operated with acute or urgent CABG in Sweden over a two-year period, giving a relatively large cohort of similar patients. The data in Studies I and II were collected over 2012 and 2013. During this period, ticagrelor replaced clopidogrel as first choice $P2Y_{12}$ antagonist for ACS patients in the Swedish guidelines, but the

introduction of ticagrelor did not occur simultaneously in all parts of Sweden [4]. The gradual introduction of the new inhibitor over the whole country gave an opportunity for comparison, even though the groups were not randomized. The reported baseline characteristics of the two patient groups were correspondingly similar, even though there may have been differences in unregistered factors that we were unaware of.

The ex vivo set-up of Study III and IV naturally had limitations. Coagulation and platelet activation are multifaceted physiological systems, and one laboratory assay cannot be expected to cover the entire process. We chose to focus on MEA alone, and it is possible that other methods of determining platelet function might have yielded different results. MEA has certain benefits in that it uses whole blood, and it is a quick and relatively easy point-of-care method that has acceptable correlation with the gold standard, light transmission aggregometry [91, 92]. However, it does not take account of the influence of circulation, the properties of blood vessels, or interaction of platelets with endothelium or extravascular tissue. Nevertheless, MEA has been shown to have a satisfactory correlation with not only the gold standard, as previously mentioned, but also with important clinical endpoints such as bleeding and reoperations [67-69]. The ability of MEA to discern clinically relevant platelet inhibition in the cardiac surgery setting is advantageous.

Supplementation with platelet concentrate ex vivo in Studies III and IV is also merely a model of in vivo platelet transfusion. In other studies, various ex vivo models of platelet supplementation have been tried, often using autologous platelet-rich plasma as a model of platelet concentrate [59, 60, 87]. In our set-up, we instead used allogenic apheresis platelets from the hospital blood bank, which reflects the clinical setting more closely than autologous platelets. It would, however, also be most interesting to study the effects of platelet transfusion in vivo, comparing efficacy between patients treated with different $P2Y_{12}$ inhibitors.

The limited study sample and the lack of a control group were other limitations of Study IV. Currently, almost all the ACS patients at our centre are treated with ticagrelor, so no observational control group exists. However, we still believe that our data add insight for the clinical setting in which ticagrelor is used. Recovery of platelet function after discontinuation of

clopidogrel and prasugrel in ACS patients has been described previously [66], but ticagrelor has different pharmacodynamic and pharmacokinetic properties. Previous studies investigating recovery of platelet function after discontinuation of ticagrelor have been conducted in patients with stable coronary disease rather than in ACS patients [21].

The descriptive and retrospective nature of Study V limits the interpretation to some extent; it is not possible to conclusively determine whether the poorer outcome in DAPT patients is due to the medication or whether it is instead indicative of the more severely ill patient. On the other hand, it would not be ethical to knowingly subject patients with a lethal and most acute condition to what is, in fact, a mistreatment. Thus, observational studies will remain the main source of knowledge in conditions like this, but the conclusions would be strengthened if they were repeated in future larger studies with sufficient statistical power to allow performance of adjusted analyses.

6 SUMMARY

1. Ongoing or recently discontinued DAPT at the time of surgery was associated with a higher rate of bleeding complications after CABG (**Studies I and II**). The overall incidence of major CABG-related bleeding complications was lower with ticagrelor than with clopidogrel.
2. The difference in CABG-related bleeding between ticagrelor and clopidogrel was mainly driven by a lower incidence when clopidogrel/ticagrelor was discontinued 72–120 hours before surgery (**Study II**). Discontinuation of ticagrelor three days before CABG, as opposed to five days, did not increase the incidence of major bleeding complications (**Study II**).
3. Platelet transfusion improved platelet aggregation in all patients treated with platelet inhibitors (**Studies III and IV**). Time since discontinuation did not influence the effect of platelet transfusion on ADP-dependent platelet aggregation (**Study IV**).
4. AA-induced platelet aggregation was completely restored by platelet supplementation in all patients treated with platelet inhibitors, while platelet concentrate had a limited effect on ADP-induced aggregation in DAPT patients (**Study III**).
5. Mean ADP-induced aggregation recovered to levels not associated with increased bleeding 72 hours after discontinuation of ticagrelor, but with a large inter-individual variability (**Study IV**).
6. The indication for antiplatelet therapy in patients who were later operated for acute aortic dissection was weak or absent in the majority of cases (**Study V**).
7. Patients with ongoing platelet inhibition had more bleeding complications. DAPT was associated with increased early mortality in patients who were operated for acute aortic dissection compared to patients with no antiplatelet therapy or ASA alone (**Study V**).

7 CONCLUSIONS AND FUTURE PERSPECTIVES

More potent platelet inhibition is associated with a higher rate of bleeding in cardiac surgery patients, especially if the treatment is continued until the time of surgery. It is therefore important to carefully consider the indications for treatment and timing of surgery, weighing the risk of bleeding against the risk of thrombosis. Due to the large degree of variability in recovery of platelet function after discontinuation, platelet function testing may prove to be a valuable tool in timing of surgery, especially in patients with a high risk of bleeding and thrombosis. Bleeding complications in patients on DAPT may also prove to be more difficult to treat, especially in patients who are treated with ticagrelor, since platelet concentrate may be less effective. In case of bleeding, platelet transfusion could be recommended, but a lower effect may be expected with ticagrelor than with clopidogrel.

As bleeding complications will most likely remain an important issue in cardiac surgery in spite of future technical developments, the pursuit of understanding and prevention of bleeding will continue. As an increasing number of patients are treated with potent platelet inhibitors and anticoagulation [4], and with longer duration of treatment [93], more patients will be in need of major surgery —cardiac and non-cardiac— despite ongoing anticoagulation and platelet inhibition, with the associated higher risk of bleeding. In such cases, and also with spontaneous and traumatic bleeding, platelet function tests may be used, especially when patients are unconscious or otherwise unable to give account of their medication.

The proposed shorter discontinuation of ticagrelor before surgery should be validated in other settings, as well as the use of platelet function tests in timing of surgery. Individualizing treatment and timing of surgery will require a greater amount of flexibility from hospitals and care providers, but will most likely improve outcome for patients and thereby prove to be cost-effective.

The results of our studies of platelet transfusion warrant further investigation, preferably in vivo, of the effect of platelet transfusion in patients on DAPT who suffer from bleeding complications after major surgery. The use of allogenic blood products is, however, associated with appreciable costs and

the risk of transmissible diseases, as well as the issue of limited supply. Therefore, finding and developing other pro-haemostatic agents that are able to counteract major bleeding without increasing the risk of thrombotic events is of the essence. However, there are impending pharmacological developments that may alleviate some of these issues, such as bridging of DAPT with short-action intravenous platelet inhibitors [94] and the development of direct antidotes [22].

Even so, it is important to note that treatment with DAPT is beneficial for the ACS patient with lower morbidity and mortality also in the cardiac surgery setting [18, 19, 23]. Thus, DAPT should not be regarded as an enemy of the cardiac surgeon, but rather as a potent tool to be used with precision, at the right time, and on the right patient—much like any surgical instrument wielded by the skilled professional in day-to-day surgical practice.

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