Improving outcomes in patients with coronary heart disease using national registers and platelet function testing

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Improving outcomes in patients with coronary heart disease using national registers and platelet function testing

Abstract **Introduction:** Ischemic heart disease is the leading cause of death worldwide. The aims of this thesis were to evaluate different contemporary approaches in coronary care with regard to mortality and myocardial infarction. **Methods:** The thesis consists of 6 papers with 4 papers evaluating different exposures and their outcomes (clopidogrel pre-treatment, 5 different anti-platelet protocols, ticagrelor pre-treatment and treatment delays to PCI). Two papers involved evaluation of interventions: platelet function testing in patients with clopidogrel and clinical evaluation of bivalirudin versus heparin in patients with acute coronary syndromes, respectively. Patients with ischemic heart disease were identified using national and local registers. All patients in the six included papers were treated with PCI. **Results:** Pre-treatment with clopidogrel prior to primary PCI was associated with improved cardiovascular outcomes compared to peri-procedural clopidogrel. The composite end point of one-year mortality and MI was improved as well as mortality alone. Pre-treatement with ticagrelor compared to peri-procedural ticagrelor did not affect 30-day mortality. In one of three statistical models, 30-day myocardial infarction was reduced with ticagrelor pre-treatment. Platelet function testing failed to identify a clinically applicable cut-off value to predict stent thrombosis or new onset MI in patients with dual anti-platelet therapy. Using platelet function testing, a loading dose of either prasugrel or ticagrelor on top of a previous loading dose of clopidogrel, did not cause a pharmacodynamic overshoot of platelet inhibition. Treatment delays from first medical contact to PCI exceeding one hour were associated with higher mortality in patients with STEMI undergoing primary PCI. Finally, we showed that a register based randomized clinical trial designed to evaluate bivalirudin versus heparin in patients with ACS and PCI is feasible, with a higher degree of enrollment of "real-life" patients than in in conventional randomized clinical trials. **Conclusions**: This thesis has potential implications for acute care of patients with myocardial infarction.

Key words: STEMI, pre-treatment, ticagrelor, clopidogrel, prasugrel, system delay, bivalirudin, heparin

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”If we knew what it was we were doing, it would not be called research, would it?”
Albert Einstein (1879-1955)

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To my parents

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List of papers

I. Koul S, Smith JG, Scherstén F, James S, Lagerqvist B, Erlinge D. Effect of upstream clopidogrel treatment in patients with ST-segment elevation myocardial infarction undergoing primary percutaneous coronary intervention. Eur Heart J 2011; 32(23): 2989-97.

II. Varenhorst C, Koul S, Erlinge D, Lagerqvist B, Siegbahn A, Wallentin L, James S. Relationship between clopidogrel-induced platelet P2Y12 inhibition and stent thrombosis or myocardial infarction after percutaneous coronary intervention-a case-control study. Am Heart J 2011; 162(2): 363-71.

III. Koul S, Andell P, Martinsson A, Smith JG, Scherstén F, Harnek J, Götberg M, Norström E, Björnsson S, Erlinge D. A pharmacodynamic comparison of 5 anti-platelet protocols in patients with ST-elevation myocardial infarction undergoing primary PCI. BMC Cardiovasc Disord 2014; 14: 189.

IV Koul S, Smith JG, Götberg M, Venetsianos D, Alfredsson J, Omerovic E, Lagerqvist B, James S, Erlinge D. Ticagrelor pre-treatment compared to treatment during PCI in patients with ST-elevation myocardial infarction undergoing primary PCI. Manuscript.

V Koul S, Andell P, Martinsson A, Smith JG, van der Pals J, Scherstén F, Jernberg T, Lagerqvist B, Erlinge D. Delay from first medical contact to primary PCI and all-cause mortality: A nationwide study of patients with ST-elevation myocardial infarction. Journal of the American Heart Association 2014;3(2):e000486.

VI Erlinge D, Koul S, Eriksson P, Scherstén F, Omerovic E, Linder R, Östlund OP, Wallentin L, Fröbert O, James S. Bivalirudin versus Heparin in non-ST and ST-segment elevation myocardial infarction - a registry-based randomized clinical trial (RRCT) in the SWEDEHEART registry (the VALIDATE-SWEDEHEART-trial). Submitted.

Paper I was awarded the Young Investigator Award – Thrombosis at the European Society of Cardiology Conference 2010 (Stockholm).

Paper V was awarded Article of the Month in April 2014 (Lund Medical Faculty).

In addition to the articles above, the author has published 21 other articles in international peer-reviewed journals.

Abbreviations

ACS	Acute coronary syndrome
CABG	Coronary Artery Bypass Grafting
CI	Confidence Interval
DSMC	Data Safety and Monitoring Committee
ECG	Electrocardiogram
GPIIb/IIIa	Glycoprotein IIb/IIIa
HR	Hazard Ratio
IQR	Interquartile Range
LMWH	Low Molecular Weight Heparin
MI	Myocardial Infarction
NSTE-ACS	Non-ST-elevation Acute Coronary Syndromes
NSTEMI	Non-ST-elevation Myocardial Infarction
STEMI	ST-elevation Myocardial Infarction
PCI	Percutaneous Coronary Intervention
PRU	Platelet Reactivity Units
RIKS-HIA	Register of Information and Knowledge about Swedish Heart Intensive-Care Admissions
SCAAR	Swedish Coronary Angiography and Angioplasty Register
SD	Standard Deviation
SWEDEHEART	Swedish Web-system for Enhancement and Development of Evidence-based care in Heart disease Evaluated According to Recommended Therapies
VALIDATE	Bivalirudin versus Heparin in non-ST and ST-segment elevation myocardial infarction in patients on

	modern antiplatelet therapy in the SWEDEHEART register
VASP	VAsodilator-Stimulated Phosphoprotein

Introduction

History and epidemiology

Ischemic heart disease is the leading cause of death in men and women globally.[1] Coronary artery thrombosis was recognized as a cause of death in the 19th century, and acute myocardial infarction (MI), the main presentation of ischemic heart disease, resulting from coronary thrombosis was recognized as a clinical diagnosis in the early 20th century.[2] In-hospital mortality for MI was approximately 30-40% as late as in the 1960s.[3]

Initial improvements in mortality were achieved with the introduction of dedicated cardiac intensive care units with specialized staff, including nurses and with access to early defibrillation and proper monitoring, improved mortality in acute myocardial infarction.[4] The main root cause, the thrombotic coronary artery, was addressed first with the introduction of aspirin and thrombolysis for ST-elevation myocardial infarction (STEMI) in the 1980s, resulting in a substantial decline in mortality and prevention of new thrombotic episodes.[5, 6] Newer pharmacological drugs[7-9] such as P2Y12 receptor antagonists, anticoagulants, statins, betablockers and ACE inhibitors as well as wide implementation of primary PCI has further improved outcomes in these patients in the 1990s and 21st century.[10-13]

Risk factors for coronary artery disease include besides age: hyperlipidemia, smoking, diet, sedentary lifestyle, hypertension, diabetes, hyperlipidemia, obesity and psychosocial factors.[14, 15] Reducing these risk factors is of outmost importance, both regarding primary as well as secondary prevention.[15] With modern therapy, in-hospital mortality is well below 10% and in many instances below 5% today in patients with an acute coronary syndrome (ACS).[16-18] However, still 10% of patients in Sweden experience death within one year after occurrence of a myocardial infarction (figure 1).[17] With the high prevalence of coronary artery disease as such with increasing age, this death rate accounts for a substantial degree of total mortality in the population.[1] Escalating costs of health care is an increasing economic burden that requires newer treatments to show a good cost/benefit profile in order to be implemented. Optimizing patient care for the individual myocardial infarction patient with maximum medical benefit and minimum risk at an affordable cost is a constantly evolving challenge.[19-21]

Definitions

The definition of a myocardial infarction is stated in guidelines (3rd Universal definition).[22] For the diagnosis of a myocardial infarction, a characteristic rise or fall in cardiac troponins is required in combination with either symptoms, characteristic ECG changes, imaging suggestive of an MI, angiographic findings or autopsy indicative of MI. Special rules of definition apply to MI in the setting of death before cardiac markers are taken and procedure related MI in conjunction with PCI, stent thrombosis or coronary artery bypass grafting (CABG).[22]

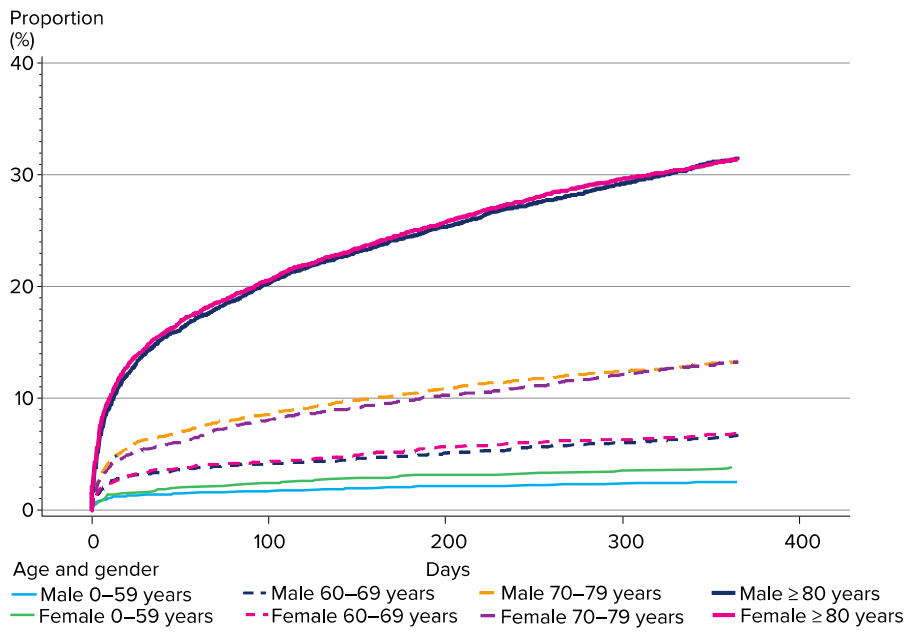

Figure 1.
One-year mortality in Sweden following a myocardial infarction 2014. Reprinted with permission from SWEDEHEART.

Myocardial infarctions are also of various types. The "classical" myocardial infarction involving rupture of a vulnerable plaque and subsequent thrombosis with either complete or partial occlusion of a coronary artery is designated as a type I myocardial infarction.[23, 24] These patients have an unstable coronary artery disease and need swift pharmacological and invasive strategies.[25] However there are multiple clinical situations in which patients present with elevated myocardial biomarkers but not due to plaque rupture. Clinical situations causing relative hypoxia that result in infarctions are labelled as type II myocardial infarctions and include increased stress on the heart (tachycardia, septicemia, pulmonary embolism, anemia, etc).[22-24, 26] The prevalence of

type I versus type II myocardial infarction is highly variable in different studies.[24] However there is an elevated risk of mortality with both types of MI, with comparable event rates.[27]

Pathophysiology

The vulnerable plaque

The core pathogenetic feature of a myocardial infarction is the rupture or ulceration of a vulnerable atherosclerotic plaque.[28, 29] Some vascular atherosclerotic features can be seen in early age, however progression into a manifest atherosclerotic plaque takes often decades and many mechanisms behind this development are still poorly understood.[30] However early onset of atherosclerosis is thought to be characterized by lipid accumulation and oxidation, especially in the intima of the arterial wall.[31] This is followed by release of cytokines and migration of inflammatory cells including monocytes that differentiate into macrophages in the intima of the vessels.[32, 33] These macrophages in turn start accumulating lipids, especially lipids modified by oxidative stress and turn into large scavenger cells, so-called foam cells.[34] At this early point in development, the plaque is present as a so-called fatty streak, which has been reported to be present already in the late teens and early 20s in many humans.[30, 35] Foam cells in turn perpetuate the atherosclerotic process by secreting additional inflammatory cytokines as well as releasing highly oxidative substances that continue oxidative stress. Furthermore foam cells replicate as well as migrate from the circulation and thus increase in numbers.[34, 36] In response to all above, smooth muscle cells migrate into the intima and start replicating as well as producing fibrous tissue into the intimal wall adding to plaque progression (figure 2).

As the disease progresses, a fibrous capsule encapsulates the growing atherosclerotic plaque that will contain (in varying proportions) an inner core of lipids, macrophages, smooth muscle cells and fibroblasts intermixed with dead necrotic cells.[37-39] To support the plaque, neovascularization occurs, often with immature blood vessels that could hemorrhage, further adding to plaque progression. Calcifications of plaque take place in later stages of development (figure 2).[38]

Growth of the early atherosclerotic plaque occurs initially in an outward direction, positive remodeling, without obstruction of luminal flow and will thus not be visible by a conventional coronary angiography. However as the plaque progresses intraluminal growth generally follows.[40, 41] Although the concentration of lipids are relatively uniform in the blood, not all segments of the arterial tree progress into manifest atherosclerosis. The scientific basis behind why certain areas tend to progress more aggressively into plaque formation whereas other areas might just exhibit

intimal thickening is not fully understood. Factors such as shear stresses and turbulent flow, for example at bifurcations that show a higher degree of plaque formation, suggest some degree of mechanical involvement in this process.[42, 43]

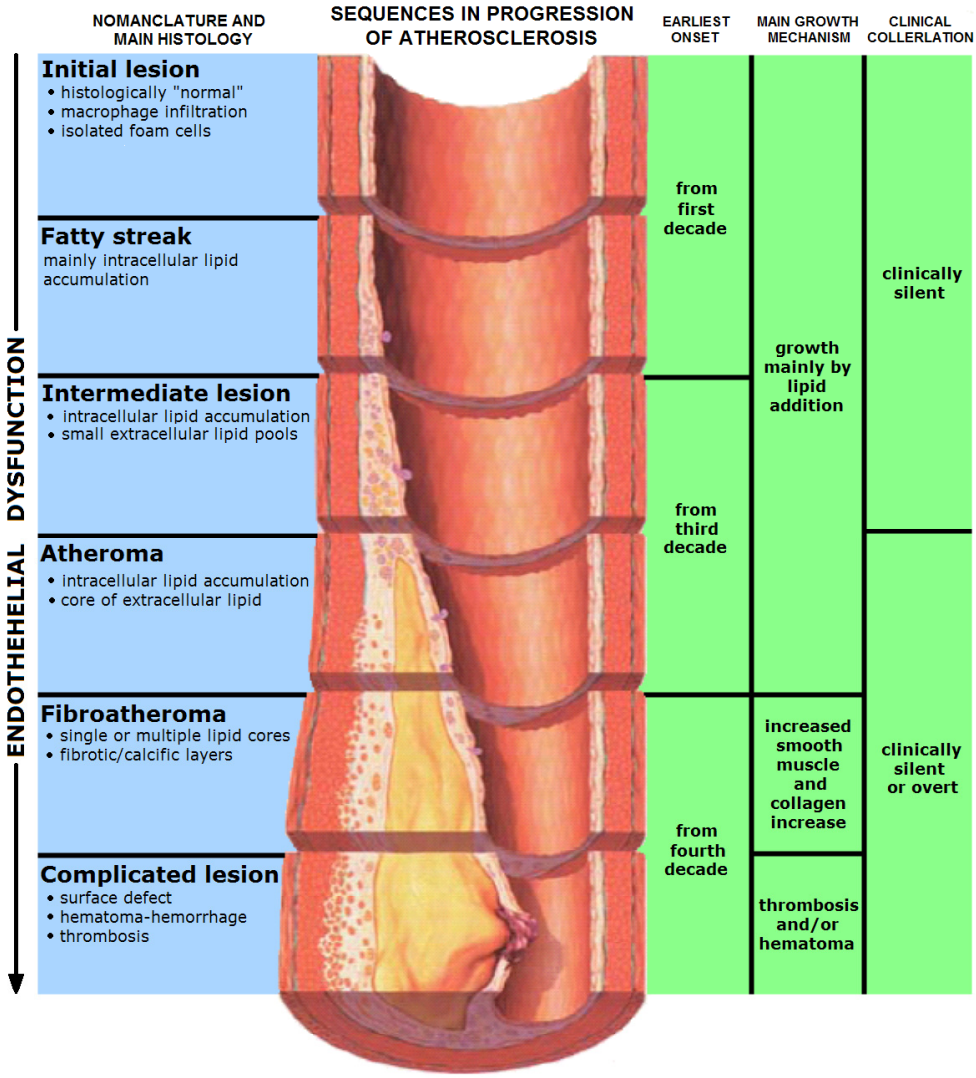

Figure 2.
Atherosclerosis progression over time.

Factors influencing plaque rupture

A high proportion of myocardial infarctions occur in plaques that have not manifested clinically prior to the infarction, i.e. have not caused sufficient degree of luminal obstruction to cause angina, which complicates screening and interventional primary preventive measures.[38, 44, 45] Plaque disruption is the main singular cause of myocardial infarction. The process wherein the fibrous cap of a plaque ruptures and exposes the blood for underlying pro-thrombotic products and subsequent thrombus formation is complex.[39, 44] Decreased collagen synthesis or increased degradation of extracellular matrix all contribute to this process. A lower degree of smooth muscle cells (with concomitant decreased matrix production) as well as a high degree of lipids, inflammatory cells and a thin fibrous cap are common features seen in ruptured plaques.[46] Thrombosis can also occur due to "superficial erosion", where endothelial denudation in atherosclerotic plaques trigger thrombosis by exposure of sub-endothelial tissue to blood. Besides plaque specific characteristics, there are circulatory characteristics like blood viscosity, increased fibrinogen levels, circulating platelet levels and function, etc that all contribute to whether a specific plaque rupture leads to a clinical event or ends up as a silent thrombus with subsequent plaque healing.[38] Activated systemic inflammation is a clear risk factor for MI with substantially increased risk for MI following a respiratory infection.[47, 48] Patients with atherosclerotic disease have increased levels of CRP and interleukins (like IL-6) suggesting a connection between systemic inflammation and atherothrombosis.[48, 49]

Thrombus formation

Following a plaque rupture or erosion, subendothelial products like tissue factor come into contact with and activate platelets. Platelets are non-nucleic cells, however they contain receptors and granules that exert a host of different reactions in the body.[50] The main granules in platelets are alfa-granules and dense granules. Alfa-granules are rich in larger polypeptides like von Willebrand factor (vWF), platelet derived growth factor (PDGF), P-selectin, CD40-ligand, glycoprotein IIb/IIIa (GPIIb/IIIa), etc. The dense granules contain small substances like ADP, ATP and serotonin that like proteins from the alfa-granules regulate platelet function as well as exert other pleiotropic effects.[50, 51] Platelet activation has been classically described as a multistep reaction involving an initiation phase, extension phase and stabilization phase.

The initiation phase is triggered by platelet exposure to extracellular matrix substances like collagen, vWF, fibronectin, etc. Binding of vWF to platelets is mediated primarily by the GpIb/IX/V complex. Collagen also binds to platelets via the GpIb/IX/V complex as well as through other collagen binding receptors on the platelet surface (like GpIa/IIa and GpVI). The GpIb/IX/V complex also binds to other proteins like thrombin and other proteins in the coagulation cascade and is critical for initial platelet response.[50-52] These vWF/collagen bound platelets form a

monolayer of activated platelets that secrete their granules and activate other platelets, which triggers the extension phase. Important mediators in this process include thromboxane A2 (inhibited by aspirin), ADP (inhibited by P2Y12-inhibitors) and thrombin (inhibited by bivalirudin, dabigatran, heparin and low-molecular weight heparin).[53] The final downstream step in platelet activation is the expression of GPIIb/IIIa-receptors on the platelet surface that cross-link with fibrinogen and vWF to bind other activated platelets.[52, 53] The platelet clot formation subsequently undergoes the stabilization phase, wherein the platelets form a close network. Several receptors have been implicated in this process, including the previously mentioned GPIIb/IIIa-receptors as well as CD40 and its ligand (CD40L).[51] The final step in thrombus formation is the activation of the coagulation cascade with the deposition of fibrin to stabilize the thrombus. This process is started by exposure of tissue factor to the coagulation system, thrombin generation and final conversion of insoluble fibrinogen into fibrin.[54]

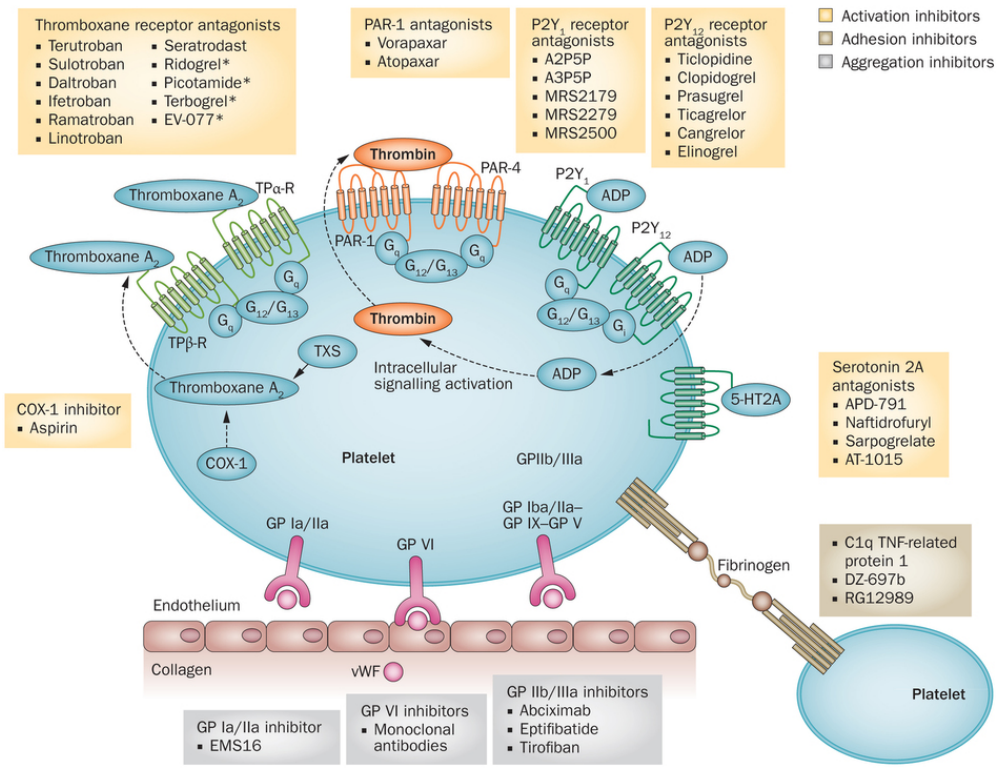

Figure 3.
Major receptors and ligands in platelet activation and current and future potential drug targets.
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Infarct progression

Necrosis of the heart starts to commence after about 20-30 minutes complete ischemia as a wave-front phenomenon.[55] The time duration for complete infarction to take place varies from patient to patient and depends on the existence of collaterals, intermittent spontaneous revascularization, pre-conditioning, temperature and other factors.[22, 56, 57] More recent data using single photon emission computed tomography (SPECT) and cardiac magnetic resonance imaging (cMRI) suggest a longer time period for infarct evolution with 288 minutes to reach 50% of infarct size with correction for area at risk, compared to previous data.[58]

Symptom onset and time to intervention

After thrombus formation, several factors determine whether this results in a clinical event with symptoms. If the thrombus does not limit blood flow or cause embolization, the thrombus will most likely go unnoticed. In fact it has been suggested that asymptomatic thrombus formation and subsequent healing is one feature of atherosclerotic plaque progression. However if the thrombus formation leads to sufficient impaired blood flow or embolization, clinical symptoms often rapidly arise. [38]

The symptoms of myocardial infarction although typically described as a "pressure" or "squeezing" with radiation to arms, back or neck can vary greatly from patient to patient.[59, 60] Thus the exact timing of symptom onset can be difficult both for the patient as well as for the health care chain to ascertain. If the symptom onset is difficult to establish due to various biases, using symptom-to-PCI as a treatment delay metric will be inherently flawed.[61, 62] Time from first medical contact to PCI (FMC-to-PCI or system delay) might be a more appropriate treatment delay metric with a less degree of bias.[61-63] Having fewer but higher volume centers could improve mortality due to increased operator and intensive care experience as well as better access to advanced cardiac care.[62, 64, 65] However having fewer centers with increased geographical distances could theoretically lead to longer treatment delays and thereby increased mortality.[66, 67] The organization of STEMI networks requires proper treatment delay metrics and corresponding outcome data in order to optimize patient flow and improve mortality.[62]

Anti-thrombotic treatment prior to and during PCI

The extent and effect of pre-treatment with various anti-thrombotic medications prior to arrival in the catheterization laboratory (cath lab) of ACS patients is highly debated. Although pre-treatment with anti-thrombotic drugs might appear to be of clear benefit in patients with STEMI, the problem is intricate. Pre-treatment might prevent further clot formation and mediate in the body's own fibrinolysis with subsequent spontaneous reperfusion as well as decreased peri-procedural complications. However patients might present as a suspected STEMI on the ECG but in fact have normal coronary vessels. Their symptoms might thus be due to other diagnoses like perimyocarditis, pulmonary embolism, aortic dissection/rupture, cardiac tamponade, etc. These patients will thus have no beneficial effect of pre-treatment with anti-thrombotics and might even take harm from it, especially if acute/sub-acute surgery is required. The issue of pre-treatment is therefore complex and requires careful consideration.[68, 69]

Aspirin

Aspirin treatment constitutes a cornerstone in the treatment of ACS. However no modern randomized study exists that has evaluated aspirin versus placebo in ACS patients. In fact nearly all randomized aspirin versus placebo studies have been conducted prior to the introduction of PCI.[5, 70-72] High (≥300 mg) versus low (75-100 mg) maintenance doses of aspirin have been compared in a modern randomized trial, including PCI patients, suggestive of no additional beneficial effects of high maintenance dose aspirin.[73] Despite the scarcity of modern placebo controlled randomized trials with aspirin, it is still recommended for all ACS patients (STEMI, non-ST-elevation myocardial infarction (NSTEMI) and unstable angina), with a loading dose followed by a low (75-100 mg) maintenance dose.[25, 74]

Clopidogrel

Clopidogrel has been, prior to the introduction of prasugrel and ticagrelor, the P2Y12-inhibitor of choice in the treatment of ACS.[25, 74] Treatment with clopidogrel is widely practiced, especially in countries where more modern P2Y12-inhibitors are not widely used.[75] Clopidogrel has shown beneficial effects in both patients with unstable angina/NSTEMI (NSTE-ACS) as well as STEMI patients undergoing fibrinolysis or medical management.[76-78] The effect of pre-treatment with clopidogrel in STEMI patients undergoing primary PCI has been studied in various register studies, however the majority of them have been small and underpowered for hard clinical events.[79-83]

One single small randomized study showed favorable trends for clopidogrel pre-treatment in patients with ST-elevation MI undergoing primary PCI (discussed in further detail under "discussion").[84]

Prasugrel and ticagrelor

Modern P2Y12-inhibitors like prasugrel and ticagrelor have been shown to be more effective than clopidogrel in the treatment of acute coronary syndromes (STEMI and NSTEMI). Both these drugs are considerably more potent as well as have a faster onset than clopidogrel, which might be an advantage in a pre-hospital setting.[7, 8] However this could also lead to more complications if given to the wrong patient. Prasugrel pre-treatment for STEMI patients undergoing primary PCI has not been selectively studied in a prospective randomized fashion. However register studies indicate a potential benefit of prasugrel pre-treatment in the setting of STEMI and primary PCI.[85, 86] The *A Comparison of prasugrel at the time of percutaneous Coronary intervention or as pretreatment at the time of diagnosis in patients with non–ST-segment elevation myocardial infarction* (ACCOAST) trial randomized NSTEMI patients to prasugrel prior to cath lab arrival or prasugrel given in the cath lab. The results were neutral concerning ischemic end-points, however with increased bleeding rate in the prasugrel pre-treatment group. Prasugrel pre-treatment is thus not recommended for NSTEMI patients prior to coronary catheterization.[74, 87]

Ticagrelor treatment has, like prasugrel, been shown to be superior to clopidogrel in patients with ACS. In the pivotal *Platelet inhibition and patient Outcomes* (PLATO) study comparing ticagrelor to clopidogrel, ticagrelor was allowed as pre-treatment, irrespective of STEMI or NSTE-ACS.[8] Ticagrelor can thus, in contrast to prasugrel, be administered prior to coronary catheterization in NSTEMI patients in current European guidelines.[74] Ticagrelor pre-treatment in STEMI patients undergoing primary PCI was evaluated in the *Ambulance or in-catheterization laboratory administration of ticagrelor for primary percutaneous coronary intervention for ST-segment elevation myocardial infarction* (ATLANTIC) study. The results were mixed with a neutral effect on the primary end point of ST-resolution and infarct vessel patency at initial coronary angiography, but with beneficial effects on stent thrombosis, however with a trend towards increased mortality (discussed in further detail under "discussion").[88]

Glycoprotein IIb/IIIa-inhibitors

GPIIb/IIIa-inhibitors have previously been widely used, both as pre-treatment as well as adjunctive treatment, for ACS patients during PCI. This was based upon early studies before the advent of routine use of dual anti-platelet inhibition.[89, 90] Contemporary studies on GPIIb/IIIa-inhibitors with dual anti-platelet therapy have

shown largely negative results on ACS patients undergoing PCI with modern anti-platelet drugs. The use of GPIIb/IIIa-inhibitors have thus been downgraded in current guidelines for both NSTEMI and STEMI.[9, 25, 74, 91-94] However some study data suggest that GPIIb/IIIa-inhibitors might be beneficial in STEMI patients that are early presenters.[95, 96] Furthermore expert consensus suggest that patients with large thrombus burden or thrombotic complications during PCI can be given GPIIb/IIIa-inhibitors, although this practice of selective use only has not been prospectively evaluated in a randomized trial.[25, 74]

Heparin and bivalirudin

Heparin treatment in patients with NSTE-ACS has been shown to be inferior to low molecular weight heparin (LMWH) in 3 previous randomized studies. However these studies were conducted before the use of dual anti-platelet therapy and routine use of coronary angiography for NSTE-ACS.[97-99] In the *Superior Yield of the New Strategy of Enoxaparin, Revascularization and Glycoprotein IIb/IIIa-Inhibitors* (SYNERGY) trial, comparable ischemic event rates were shown for heparin compared to LMWH in patients with NSTE-ACS. However an increased bleeding rate was noted in the LMWH group. The majority of the patients in SYNERGY underwent PCI and received dual anti-platelet therapy.[100] The data have thus been inconclusive regarding heparin versus LMWH in patients with NSTE-ACS. However the factor Xa-inhibitor, fondaparinux, demonstrated reduced bleeding and death compared to LMWH in this patient group. Fondaparinux therefore became the anti-coagulant of choice in NSTE-ACS in Sweden and is also endorsed in current guidelines.[74, 101] However, fondaparinux was also associated with an increased rate of catheter thrombosis during PCI compared to LMWH. This was remedied by adding a bolus dose of heparin during PCI which is also recommended by guidelines.[74, 102] The combination of early fondaparinux, in combination with heparin during PCI, thus became common practice in Sweden for patients with NSTE-ACS and has been associated with improved outcomes compared to a LMWH based strategy.[103] Whether bivalirudin, instead of heparin, might improve clinical outcomes in this patient group is unknown and is elaborated further under “results” and “discussion”.

Bivalirudin has in patients with NSTE-ACS been shown to be superior to heparin in combination with GPIIb/IIIa-inhibitors.[93, 94] Furthermore, in the *Harmonizing Outcomes with RevascularIZatiON and Stents in Acute Myocardial Infarction* (HORIZONS) trial, bivalirudin was superior to heparin in combination with GPIIb/IIIa-inhibitors in STEMI patients undergoing primary PCI. This included a reduction in all-cause mortality, driven primarily by a reduced bleeding rate.[93] Bivalirudin versus heparin monotherapy (without GPIIb/IIIa-inhibitors) in ACS patients undergoing PCI has been addressed in some recent trials. The results have been conflicting and are discussed in more detail under “discussion”.[75, 104, 105]

Platelet function testing

Patients on clopidogrel exhibit a considerable variation in platelet response. The main reason for clopidogrel resistance lies in the hepatic conversion of clopidogrel (an inactive pro-drug) to its active metabolite, where different liver enzyme genotypes lead to varying degrees of conversion and generation of active metabolite.[106] An often cited prevalence rate is 1 out of 5 patients being clopidogrel resistant.[107] However clopidogrel resistance prevalence rates vary between 5-44% depending on usage of different platelet function tests, with different concentrations of agonists and at different time periods during treatment.[108, 109] Measurement of platelet reactivity after exposure of platelet inhibitors has attracted attention as a feasible way of individualizing platelet inhibitor medication for patients with coronary heart disease undergoing PCI. Although not yet properly assessed in a large randomized trial, a "therapeutic window" might exist for platelet inhibition with sufficient degree of inhibition to prevent new ischemic episodes, at the cost of minimum amounts of bleedings.[110-113] Several methods to measure platelet reactivity have been developed. Two methods widely used and that have been studied in paper II and III are VerifyNow and vasodilator-stimulated phosphoprotein phosphorylation assay (VASP assay). Both methods have been demonstrated to accurately measure the biological effect of activated clopidogrel metabolite when compared to other tests and have been suggested as suitable tests for the P2Y12-pathway.[114-116]

VASP assay

Several studies using the VASP assay have suggested a correlation between decreased VASP PRI values and worse clinical outcomes in stented patients. A cut-off value of <50% in VASP PRI has been identified as a good marker of worse clinical outcomes.[117-119] In a small randomized study (n=162) that has been widely cited, the loading dose of clopidogrel pre-PCI was successively increased in clopidogrel resistant patients scheduled for PCI (in some patients up to 2,4 grams) till a VASP PRI of <50% was reached pre-PCI. This group was compared to a control group that also consisted of clopidogrel resistant patients, however receiving usual treatment only (single loading dose of 600 mg clopidogrel). Patients in the VASP-controlled group showed significantly better cardiovascular outcomes without an increase in bleeding risk, despite repeated loading doses of 600 mg clopidogrel.[119]

VerifyNow

The VerifyNow system is a point-of-care system to measure platelet inhibition. The system is in comparison to other platelet measurement systems easy to use and the

point-of-care nature allows quick results that can directly influence clinical decision making.[114] Various different cut-offs have been proposed for the VerifyNow P2Y12 system with >208, >230 or >235 platelet reactivity units (PRU) suggested as cut-off for clopidogrel resistance. Currently employed cut-off at Skåne University Hospital is >230 PRU.[120, 121]

Several studies using the VerifyNow P2Y12 system have reported a correlation between high platelet reactivity on treatment and worse cardiovascular outcomes.[111, 120, 122] Prospective and randomized interventional trials have been performed using the VerifyNow P2Y12 system to guide therapy, and are discussed in further detail under "discussion".[123, 124]

Randomized clinical trials versus observational data

Medical clinical registers serve several important functions. Firstly they are an excellent tool for quality of care assessment of clinical practice. Although causality is always difficult to establish, studies have shown that usage of this kind of quality control has led to improvement of patient outcomes over the years.[125, 126]

For cardiovascular outcomes research, comparing various exposures and clinical events, registries cannot balance confounding factors as well as prospective randomized clinical trials (RCTs) and are thus considered a lower level of evidence. However registers offer other advantages. Patients included in RCTs tend to be younger and healthier compared to "real-life" patients. However data from this, often healthier cohort, are extrapolated to encompass the entire patient group. In comparison, dedicated cardiovascular trials for high-risk group patients (especially patients 80+ in age) are few. Registers offer data on "real-life" patients that traditionally are not included in RCTs. Furthermore registers can include considerably more patients than RCTs and thus add considerable statistical power to clinical studies. In addition, very rare events that might be missed in classical RCTs due to lower patient sample size, can be discovered in larger registries.[127-130]

Regsisters can also be used to screen for patients to be included in studies. For example finding subjects to be included in case-control studies can be done by screening medical registries. This is especially useful to find rare outcomes, such as stent thrombosis. Paper II used a register as a screening tool for finding patients with stent thrombosis, myocardial infarction or matching controls that all subsequently underwent platelet function testing. This, of course, requires patient informed consent like in a regular RCT.

Registers are now increasingly being used to prospectively randomize patients in clinical trials with register acquisition of baseline demographics and clinical end points follow-up. This register based randomized clinical trial (RRCT) concept might

represent an efficient way of combining the randomized nature of classical RCTs with the large sample size, low cost and "all-comer" nature of registers.[127, 130]

Aims

The general aims of this thesis were to explore different strategies to improve clinical outcomes in patients with coronary heart disease, mainly ST-elevation myocardial infarction. In all papers, registers were used in different ways to study patients.

I) To investigate whether pre-treatment with clopidogrel in STEMI patients undergoing primary PCI from the SWEDEHEART register was associated with improved outcomes as compared to clopidogrel administered first in the catheterization laboratory.

II) To investigate whether platelet function testing could identify a cut-off value for high risk of stent thrombosis or myocardial re-infarction. Patients with stent thrombosis were identified from the SWEDEHEART register.

III) To characterize the pharmacodynamic profile of 5 different anti-platelet strategies by platelet function testing during ST-elevation MI using a locally created register for this purpose (Lund platelet register) that later was linked to information from SWEDEHEART.

IV) To investigate whether pre-treatment with ticagrelor in patients with ST-elevation MI, undergoing primary PCI from the SWEDEHEART register, was associated with improved outcomes as compared to ticagrelor first in the catheterization laboratory.

V) To investigate the association of various measures of time delay to PCI, especially first medical contact-to-PCI, with mortality in patients with ST-elevation MI using the SWEDEHEART register.

VI) To design a prospective, clinically controlled, randomized trial comparing bivalirudin and heparin monotherapy during PCI in patients with acute coronary syndromes using the SWEDEHEART register.

Methods

Complete details of material and methods are found in each individual paper. This chapter aims to present a summary of the methods used.

Patient populations

Registries and clinical end point acquisition

In Sweden, all MI patients that have received treatment at a cardiac intensive care unit are registered in the national Register of Information and Knowledge about Swedish Heart Intensive-Care Admissions (RIKS-HIA) database. This database includes a wealth of data pertaining to the hospital stay. Information includes data regarding bleeding complications, cardiac arrest, need for pacemaker, ejection fraction, previous medication, medication at discharge, type of myocardial infarction, results from blood tests, etc. Details regarding the PCI procedure are registered in the Swedish Coronary Angiography and Angioplasty Register (SCAAR). The SCAAR register includes procedural data from all 29 centres that perform coronary angiography and PCI in Sweden. Parameters include treated coronary segment, type of procedure, size and type of stent used, pressure during balloon inflation, pre-treatment and treatment in cath lab with anti-thrombotic medications, use of percutaneous devices, etc. Outpatients under the age of 75 years that are followed policlinically are registered in the Swedish National Register of Secondary Prevention (SEPHIA) register. These four registers all constitute parts of the larger Swedish Web-system for Enhancement and Development of Evidence-based care in Heart disease Evaluated According to Recommended Therapies (SWEDEHEART) register.[131] Other registries that contribute to SWEDEHEART are the cardiothoracic surgery register and the TAVI register. The national cardiogenetics register is currently in early stages and will also become a part of the SWEDEHEART register.

Using the individually unique Swedish personal identification numbers, data from the SWEDEHEART register can be merged with other nation-wide registries, for additional information on background demographics not covered by SWEDEHEART, including death, cause of death as well clinical end points leading to hospitalization (stroke, heart failure, MI, kidney failure, etc).[131] Two particularly

important nation-wide registers are the national hospital discharge register containing information on all discharge codes from Swedish hospitals and the cause of death register containing information on time and underlying cause of death.

In paper I, II, IV, V and VI patients were identified (and in the case of paper VI are currently being identified) using the SWEDEHEART register. In papers I, IV and V, the SWEDEHEART register was used for direct statistical clinical research, including end point acquisition, using unidentified databases. In paper II, patients were screened using SWDEHEART for performing a case-control study with platelet function testing as primary end point.

In paper VI, SWEDEHEART is used for including and randomizing patients in the ongoing national *Bivalirudin versus Heparin in non-ST and ST- segment elevation myocardial infarction in patients on modern antiplatelet therapy in the SWEDEHEART register* (VALIDATE-SWEDEHEART) study. The primary composite end point (death, MI and major bleeding events at 180 days) as well as its individual components will be actively screened for with central adjudication. Type of myocardial infarction (type I-V according to the universal definition)[22] will also be actively screened for with central adjudication. Remaining secondary end points (outlined in paper VI) will be acquired using national registers (SWEDEHEART, Swedish hospital discharge register and the Swedish population register). Following randomization, an e-CRF with specific questions pertinent to the VALIDATE study will follow in SCAAR which is to be filled in by the angiographer after the procedure is completed. Baseline demographics will be acquired using SWEDEHEART.

In paper III patients with STEMI and undergoing primary PCI were identified prospectively at the cath lab and included in a local register, Lund platelet register (LPR). Symptom onset, timing of P2Y12-inhibitor initiation, time of blood sampling, etc were all included in LPR. Background demographics and clinical outcome data were obtained by matching the register with SWEDEHEART.

Study samples

In papers I, III, IV and V exclusively STEMI patients undergoing primary PCI were studied (table 1). In paper II patients with ACS or stable angina as well as matched controls were identified using SCAAR. This was followed by an invitation by letter to participate in the study. In paper VI, 3000 patients with STEMI and 3000 patients with NSTEMI will be included. All patients should have an intention to treat with PCI (table 1).

Table 1.
Summary of patient populations and the primary study purpose in all 6 papers.

Paper	Study sample	Sample size	Study purpose
I	STEMI patients undergoing primary PCI 2003-2008	13847	Evaluation of clopidogrel pre-treatment – register analysis
II	Patients with ACS or stable angina that have experienced stent throbosis or MI under dual anti-platelet therapy and moatched controles (2005-2007)	156	Identifying platelet function testing cut-offs to predict stent thrombosis and MI – case/control analysis
III	STEMI patients undergoing primary PCI 2009-2012	223	Montoring effects of different anti-platelet protocols using platelet function testing - register
IV	STEMI patients undergpoing primary PCI 2010-2014	5438	Evaluation of ticagrelor pre-treatment – register analysis
V	STEMI patients undergoing primary PCI 2003-2008	13 790 (main analysis)	Evaluation of treatment delay to PCI and mortality – register analysis
VI	STEMI and NSTEMI patients undergoing PCI 2014-	6000	Evaluation of bivalirudin versus heparin during PCI – design paper of a prospective and randomized clinical trial

Medical interventions

Paper II

In paper II, patients that no longer were on clopidogrel were given 600 mg clopidogrel with subsequent blood sampling, including platelet function testing (VASP and VerifyNow) within 16 to 24 hours. For patients already on clopidogrel no renewed clopidogrel loading dose was needed and blood sampling was done when possible. The study was approved by a local ethics committee.

Paper VI

In paper VI, the design of the VALIDATE-SWEDEHEART trial is outlined. Inclusion and exclusion criteria are listed in table 2. The study is currently ongoing and will randomize 3000 STEMI and 3000 NSTEMI patients to either bivalirudin (Angiox®, The Medicines Company, USA) or heparin monotherapy (Leo Pharma, Sweden). In the bivalirudin arm, a maximum of 5000 U of heparin in STEMI and 3000 U in NSTEMI is allowed pre-procedurally. The inclusion and exclusion criteria are presented in table 2. The bivalirudin loading dose is 0.75 mg/kilogram, followed by an infusion of 1.75 mg/kilogram/hour. After completion of PCI it is

recommended, but not mandatory, to continue bivalirudin as an infusion (1.75 mg/kilogram/hour) in all STEMI patients and in NSTEMI patients that received P2Y12-inhibitors less than 4 hours before PCI. Treatment with unfractionated heparin will be administered using a dose of 70-100U/kg. Additional bolus doses of either bivalirudin (bivalirudin arm) or heparin (heparin arm) can be given in case ACT does not reach at least 250 seconds during procedure. The study was approved by a local ethics committee.

Table 2.
Inclusion and exclusion criteria for the VALIDATE trial

Inclusion criteria:

- Patients with a diagnosis of NSTEMI as judged by the physician in accordance with current guideline definitions (positive troponin) or

patients with a diagnosis of STEMI as defined by chest pain suggestive for myocardial ischemia for at least 30 minutes before hospital admission, time from onset of symptoms of less than 24 hours, and an ECG with new ST-segment elevation in two or more contiguous leads of ≥0.2 mV in leads V2-V3 and/or ≥0.1 mV in other leads or a probable new-onset left bundle branch block.

- PCI of culprit lesion is intended (therapeutic PCI, not primarily diagnostic PCI).
- Ability to provide informed consent
- Age 18 years or older
- Treated with bolus dose of ticagrelor, prasugrel or cangrelor before start of PCI

Exclusion criteria:

- Previous randomization in the VALIDATE-SWEDEHEART trial
- Known terminal disease with life expectancy less than one year.
- Patients with known ongoing bleeding
- Patients with uncontrolled hypertension in the opinion of the investigator
- Patients with known subacute bacterial endocarditis
- Patients with known severe renal (GFR < 30 ml/min) and /or liver dysfunctions
- Patients with known thrombocytopenia or thrombocyte function defects
- Any other contraindication for the study medications
- Heparin >5000U before arriving to PCI lab or >3000U given in the beginning of the procedure.
- GpIIb/IIIa-inhibitors have been given or are pre-planned to be given during the procedure.

Platelet function testing

VASP assay

The vasodilator-stimulated phosphoprotein phosphorylation assay (VASP) is a flow cytometric analysis, specific for the P2Y12-pathway (Biocytex Platelet VASP kit, Marseille, France). Patients on aspirin or GPIIb/IIIa-inhibitors can thus be analyzed with no interference. The vasodilator-stimulated phosphoprotein in platelets exist in a phosphorylated (VASP-P) or dephosphorylated form (figure 4). Phosphorylation of this protein keeps the platelet inactive and is stimulated by prostaglandins (like prostaglandin E1 [PGE1]) or by inhibition of the P2Y12-receptor (by giving clopidogrel, prasugrel or ticagrelor). VASP dephosphorylation is stimulated by ADP and thus induces platelet aggregation. The balance between VASP-P and VASP is vital for the activation of GPIIb/IIIa-receptors on the platelet surface (figure 4). In short, whole blood is sampled in citrate tubes and mixed with PGE1 or with ADP + PGE1. The platelets are then incubated with VASP-P specific antibodies and in a secondary step these antibodies in turn are tagged with a fluorescein-labeled antibody. The cells are then analyzed using fluorescence-activated cell sorting (FACS) where the presence of VASP-P will be detected by increased fluorescence. A VASP platelet reactivity index (VASP-PRI) is determined by comparing VASP-P median fluorescence intensity (MFI) between samples incubated with PGE1 and ADP + PGE1 according to a specific formula.[115-117]

$$PRI\ \% = [(MFIPGE1 - MFI(PGE1+ADP))/MFIPGE1] \times 100\%$$

VerifyNow

The VerifyNow system (Accumetrics, San Diego, California) is like the VASP analysis a whole blood analysis based on citrate-treated blood. Samples are inserted into a cartridge containing different chambers with fibrinogen coated beads as well as specific platelet agonists. Three different VerifyNow cartridges exist depending on what platelet system is tested; the IIb/IIIa assay, aspirin assay and P2Y12 assay. The P2Y12 assay was used in paper II and thus only the P2Y12 assay will be further discussed. Blood from patients are separated into two chambers (figure 5). One chamber contains Thrombin Receptor Activating Peptide (TRAP) that is a strong platelet activator with biological effects to a large degree independent of other pathways like the activation of the P2Y12-receptor or aspirin-mediated TxA2 production. Thus interaction of whole blood with TRAP in the VerifyNow cartridge will give an estimate of basal platelet activity in a patient, even if the patient has

already been treated with P2Y12-inhibitors or aspirin. The second chamber contains ADP and PGE1 and interaction with whole blood is used for measurement of P2Y12-inhibition. Blood from patients with clopidogrel resistance will thus aggregate with the fibrinogen coated beads present in the chamber. The resultant effect is an increase in light transmittance which is measured by the VerifyNow apparatus and results given as PRU units (figure 5). Results can also be given as percentage platelet inhibition (based upon TRAP-stimulation for baseline platelet reactivity). The VerifyNow system is a point-of-care system to measure platelet inhibition. The system is in comparison to other platelet measurement systems easy to use and the point-of-care nature allows quick results that can directly influence clinical decision making. Various different cut-offs have been proposed for the VerifyNow P2Y12 system with >208, >230 or >235 PRU units suggested as cut-off for clopidogrel resistance. The currently used cut-off at Skåne University Hospital is >230 PRU.[120, 121, 123]

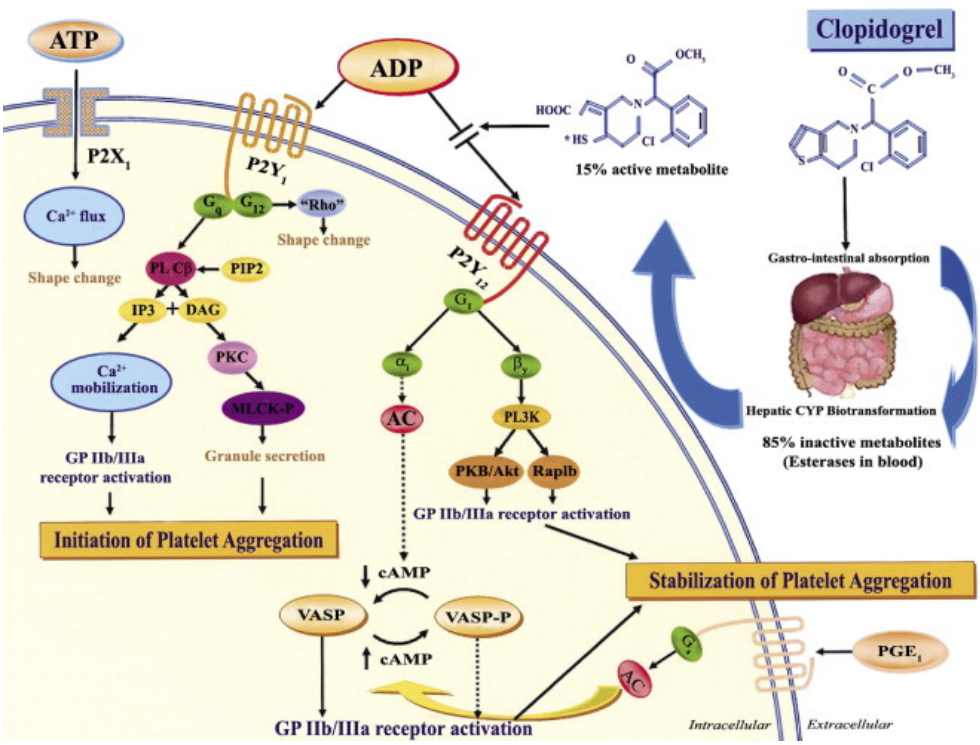

Figure 4.
Overview of platelet activation with ratio of VASP-P and VASP determining GPIIb/IIIa-receptor activation. Reprinted with permission from JACC.

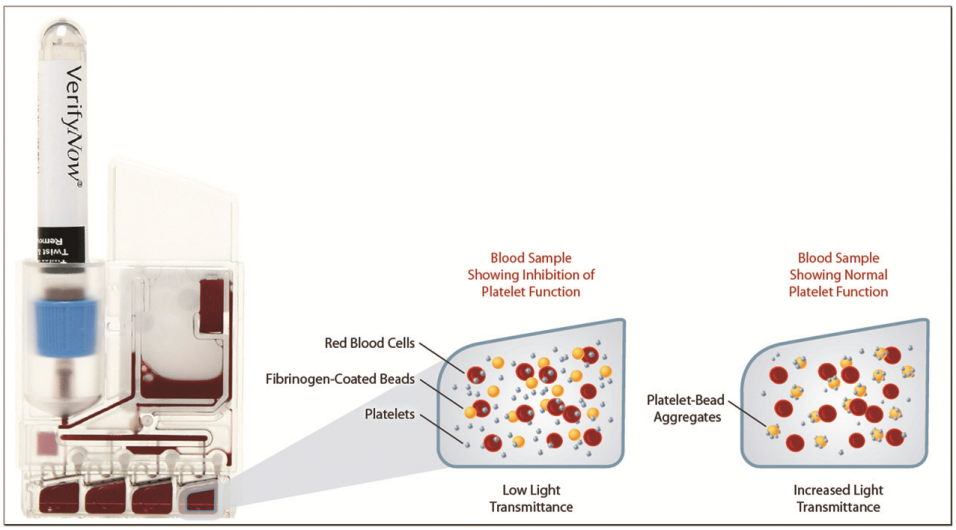

Figure 5.
Overview of the VerifyNow system. Reprinted with permission from Platelets.

Statistical methods

Baseline characteristics between groups were compared using Student's T-test or ANOVA for continuous parametric data and Mann-Whitney's U-test for continuous non-parametric data. Pearson's chi-squared test was used to compare categorical data. Crude event rates were estimated using the Kaplan-Meier estimator, with the log-rank test for significance testing. For adjustments of potential confounders, Cox proporational hazards regression analysis was used incorporating different sets of covariates. Multivariable adjustments in Cox regression models were performed by direct adjustment (paper IV and V) or using propensity scores (paper I and IV). In paper IV, a matched analysis based on the propensity score was also performed. In paper III, time separation curves were created using linear regression models as a function of linear or log-transformed time, as deemed most suitable from cluster plots. Power calculations to estimate required sample sizes were performed in paper II and VI. In paper II, power calculations were estimated on the width of the 95% CI of PRU for the 10th percentile in patients with stent thrombosis. In paper VI sample size calculation was performed to provide a statistical power of 80% for a HR of 0.75 between bivalirudin and heparin in each subset of patients, (STEMI and NSTEMI) including for patients not completing the trial or lost to follow up. All analyses were performed using SPSS (versions 18-22, SPSS Inc, Chicago) or SAS (version 9.4, SAS Institute, Cary, NC, USA).

Results

Paper I

A total of 13847 patients were included in study I with 9813 patients (71%) having received clopidogrel pre-treatment (prior to arrival at the catheter lab) and 4034 patients (29%) with no upstream clopidogrel pre-treatment. The patients were relatively well balanced in several baseline parameters; however, differences were also noted, in particular concerning the use of upstream heparin as well as LMWH.

Propensity-adjusted incidence curves for the primary end point (composite end point of death or MI) are shown in figure 6. The combined primary end point occurred in 1325 patients in the upstream clopidogrel group (14.3%) and in 712 patients (17.9%) in the non-upstream group at 1 year (Table 3). Using propensity score methods to balance out covariates, there were significant risk reductions both short-term (30 days, HR 0.83, 95% CI 0.71–0.97) as well as more long-term (one year, HR 0.82, 95% CI 0.73–0.93) (table 3).

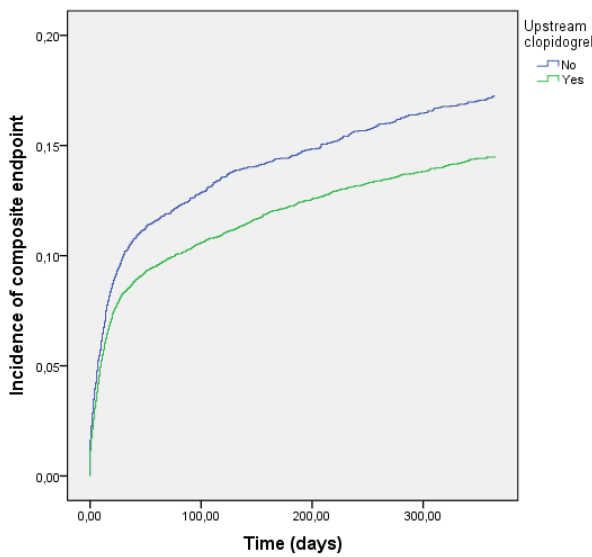

Figure 6.
Composite end point (mortality or myocardial infarction) as a function of time (Cox-plot).

Looking at mortality alone, clopidogrel pre-treatment was associated with a reduction in mortality, both unadjusted as well as adjusted. A total of 376 (9.4%) deaths occurred in patients with no clopidogrel pre-treatment and 665 deaths (7.2%) occurred in patients with clopidogrel pre-treatment at one year (Table 3). The results were significant even after using propensity score methods to balance out covariates with an adjusted mortality reduction both at 30 days (HR 0.70, 95% CI 0.57–0.85) as well as at one year (HR 0.76, 95% CI 0.64–0.90) (Table 3). The results remained significant after additional multivariate analysis on top of propensity scoring.

Table 3.
Summary of clinical end points in paper I.

	Events at 30 days			
	Death/MI	**Death**	**MI**	**Stent thrombosis**
Non-upstream group (n= 4034)	420 (10.4%)	252 (6.3%)	176 (4.6%)	18 (0.47%)
Upstream group (n= 9813)	797 (8.2%)	419 (4.3%)	385 (4.1%)	52 (0.55%)
Unadjusted hazard ratio (95% CI)	0.78 (0.69-0.87)	0.68 (0.58-0.80)	0.89 (0.75-1.07)	1.86 (1.09-3.18)
Propensity adjusted hazard ratio (95% CI)	0.83 (0.71-0.97)	0.70 (0.57-0.85)	1.00 (0.79-1.26)	1.55 (0.80-3.00)
	Events at one year			
	Death/MI	**Death**	**MI**	**Stent thrombosis**
Non-upstream group (n= 4034)	712 (17.9%)	376 (9.4%)	369 (9.9%)	29 (0.79%)
Upstream group (n= 9813)	1325 (14.3%)	665 (7.2%)	719 (8.2%)	88 (1.05%)
Unadjusted hazard ratio (95% CI)	0.78 (0.71-0.86)	0.74 (0.66-0.84)	0.82 (0.72-0.93)	1.32 (0.87-2.01)
Propensity adjusted hazard ratio (95% CI)	0.82 (0.73-0.93)	0.76 (0.64-0.90)	0.90 (0.77-1.06)	0.94 (0.56-1.59)

Concerning myocardial infarction, a total of 369 (9.9%) and 719 (8.2%) MIs occurred in the no pre-treatment versus pre-treatment groups at one year. The difference was not statistically significant after propensity score adjustment both at 30 days and at one year, (table 3). No difference in the rate of stent thrombosis between the groups was shown.

The vast majority of patients (>90%) were discharged with clopidogrel. In a separate model, patients not discharged on dual anti-platelet therapy were excluded with a continued statistically significant decrease in the composite end point as well as total mortality at one year. No difference was shown for MI, as in the previous analyses.

All adjusted results presented above results were similar after additional multivariate analysis on top of propensity scoring.

Accounting for cardiogenic shock in the propensity score model, there was a continued significant reduction in the composite end point and a strong trend towards reduced mortality at one year with clopidogrel pre-treatment. With additional multivariate analysis on top of propensity scoring, both the composite end point and mortality alone at one year showed a significant reduction with clopidogrel pre-treatment.

No differences were noted between major in-hospital bleedings between the two arms. Clopidogrel pre-treatment was given to approximately 1/3 of STEMI patients undergoing primary PCI in 2003. The same number for 2008 was almost 90%. Despite these differences, clopidogrel pre-treatment was associated with a lower one-year mortality across all time periods (figure 7).

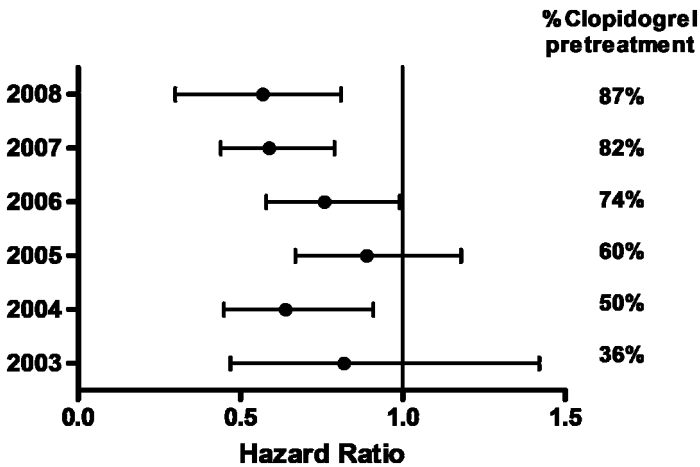

Figure 7.
Forrest plot showing one year mortality as a function of year of procedure with hazard ratio <1.0 indicating benefit with clopidogrel pre-treatment. Percentage clopidogrel pre-treatment on a year basis is visualized in the right column.

Paper II

A total of 988 patients were identified for inclusion using the SCAAR register. Out of these, 169 patients agreed to further screening and 156 patients satisfied inclusion and exclusion criteria. A total of four patient groups were included in this study: a) Patients with previous definite stent thrombosis (n = 48) and b) patients with previous MI, excluding stent thrombosis (n = 30). Both of these groups had to have had an event within 6 months of previous coronary stenting and while on dual

antiplatelet therapy. The remaining two groups consisted of matching controls without any coronary event after stenting; c) n = 50 stent thrombosis controls and d) n = 28 MI controls (table 4). The time period for inclusion was February 2009 and March 2010. Initially a total of 100 patients with a stent thrombosis and 100 patients with an MI on dual antiplatelet therapy were to be included in the study, however due to slow inclusion rate, the study was stopped at current patient numbers.

Table 4.
Inclusion and exclusion criteria for the paper II. STh = stent thrombosis.

Patients in the trial needed to fulfill the following inclusion criteria
- Providing signed informed consent
- Male or female patients older than 18 y
- Previous coronary stenting for coronary artery disease
- Previous (after coronary stenting) or current dual antiplatelet treatment with aspirin 75 mg once daily and clopidogrel 75 mg once daily. All patients needed to be on treatment with ASA 75 mg once daily at least 7 days before enrollment

Plus one of the following alternatives

(*a*) STh within 6 months of coronary stenting while on dual antiplatelet treatment

or

(*b*) Experienced MI within 6 month after coronary stenting while on dual antiplatelet treatment

or

(*c*) No experience of STh or MI for at least 6 months and until inclusion (matched control)

The initial purpose was to include only patients with an ACS (including unstable angina). However due to slow inclusion rate, patients with stable angina were also included, although they constituted a minority of patients. Patients with stent thrombosis or MI did have more previous myocardial infarction as well as heart failure than their matched controls. Stent thrombosis patients were more overweight, had more PCI performed against restenosis lesions and had more stents implanted than their controls. MI patients tended to have more hyperlipidemia and proton pump inhibitor use than their matched controls. Out of all patients in the stent thrombosis group, 40 had early stent thrombosis (0-30 days) and 8 patients had late stent thrombosis (> 30 days). The median time from stent procedure to stent thrombosis was 5 days (interquartile range [IQR], 3.0-11.5 days), and a total of 29 patients (60.4%) presented with ST-elevation MI at the time of stent thrombosis. The median time in the MI group from stent procedure to new-onset MI was 64 days (IQR, 17.0-118 days).

As outlined under "Material and methods" all patients above that were on chronic clopidogrel therapy, had blood sampling including platelet function testing by VerifyNow and VASP done. Patients that were not on chronic clopidogrel therapy, were exposed to a loading dose clopidogrel followed by the same blood sampling 16-26 hours later.

Results from the platelet function tests showed that the PRU using the VerifyNow P2Y12 system in patients with stent thrombosis (group a) was significantly higher than in their matched controls (246.8 ± 75.9 vs 200.0 ± 82.7, p = 0.001), suggesting that patients with stent thrombosis had more platelet reactivity on treatment (figure 8). Device-reported percent inhibition for patients with and without stent thrombosis was 23 ± 20.9 and 37.5 ± 23.1, p = 0.0007, again showing that patients with stent thrombosis had lower degree of platelet inhibition (= higher degree of platelet reactivity) than matched controls. Platelet function testing using the VASP assay did not show any difference in VASP PRI (%) between patients with stent thrombosis and controls (61.4% ± 18.6 vs 58.4% ± 20.2, p = .47).

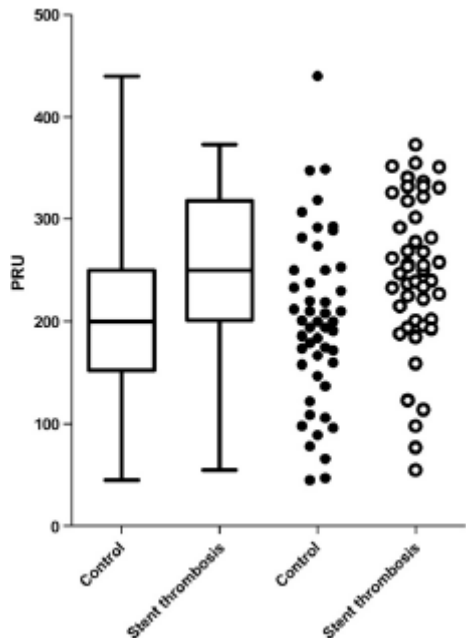

Figure 8.
Platelet reactivity measured as VerifyNow PRU units in stent thrombosis patients and matching controls (median, IQR)

Receiver-operated curves (ROC curves) showed an area under the curve of 0.688 to distinguish between stent thrombosis and matched controls. A cut-off value of ≥222 PRU for impaired clopidogrel response gave a sensitivity of 70% and specificity of 67% for stent thrombosis (figure 9).

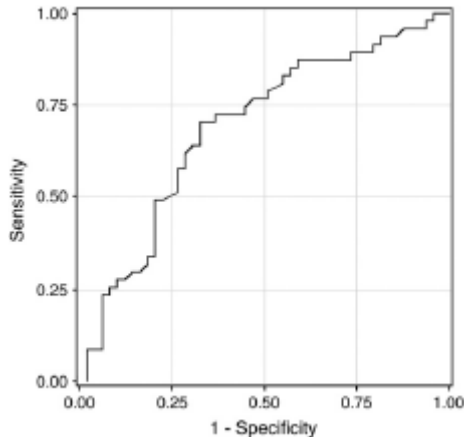

Figure 9.
ROC curve for VerifyNow for identifying stent thrombosis. Area under the curve corresponded to 0.69 ($p<0.001$).

In patients with MI on dual antiplatelet therapy (group b), both platelet function testing using VerifyNow as well as the VASP assay was not predictive of new onset MI (excluding stent thrombosis). In fact, the MI group had numerically lower PRU values as well as VASP PRI% (indicating greater platelet inhibition in the MI group)

Patients not on chronic clopidogrel therapy at inclusion were reexposed to clopidogrel with subsequent blood sampling 16 to 26 hours later. In a pre-study to the current study, 23 patients previously included in another clopidogrel pharmacodynamic study, were reexposed 2-3 years later to clopidogrel in order to test the long-term stability of clopidogrel response. The Pearson correlation coefficient for VASP PRI% was 0.74 for initial platelet response and platelet response 2-3 years later and the corresponding correlation coefficient for VerifyNow was 0.80.

Paper III

In paper III, patients with an ST-elevation myocardial infarction undergoing primary PCI were included in Lund Platelet register and blood samples (VASP assay) were taken pre-PCI, post-PCI and the day after PCI. The patient population consisted of 5 different treatment cohorts (figure 10): 1) clopidogrel pre-treatment only (*upstream clopidogrel group)* 2) clopidogrel pre-treatment, followed by

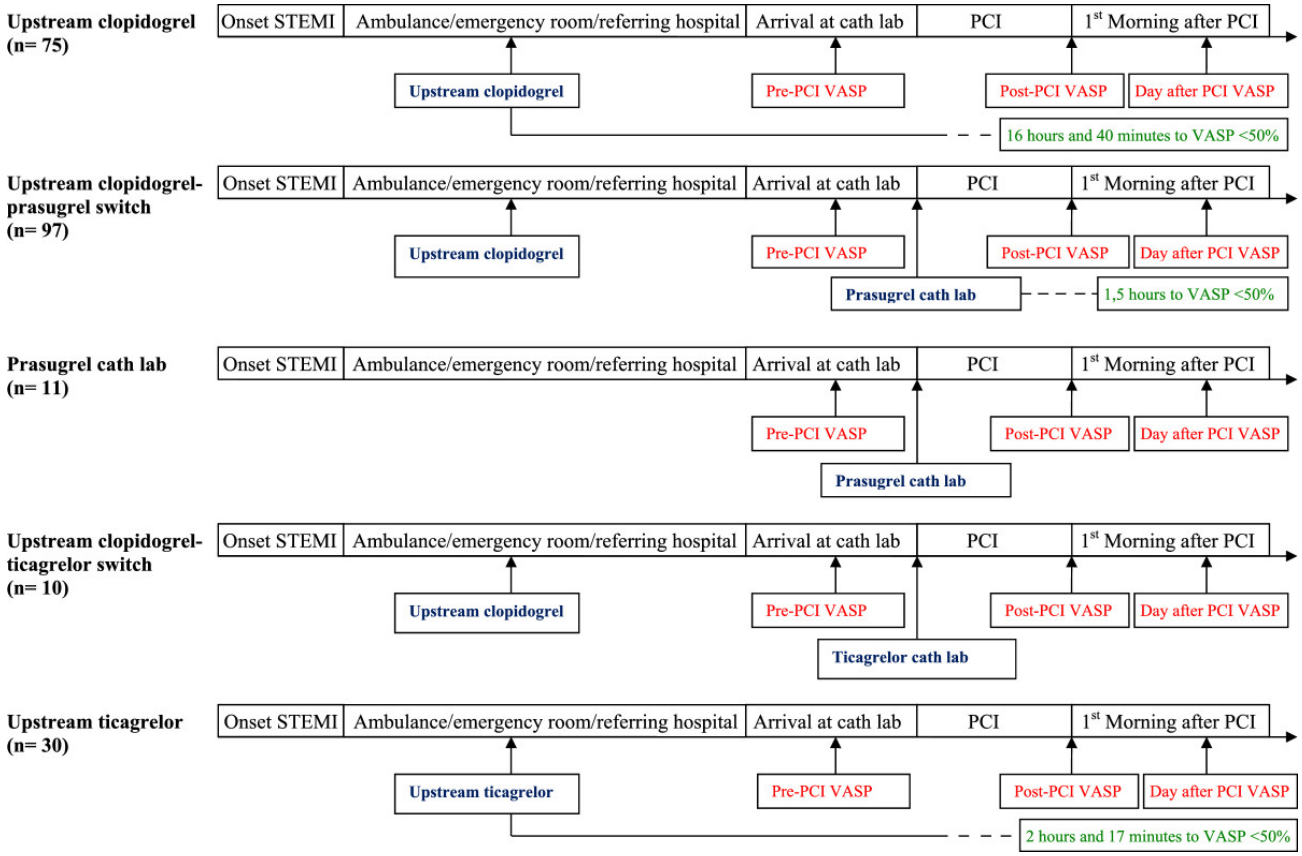

Figure 10.
Flowchart describing the 5 patient cohorts in paper III and blood sampling time points.

prasugrel loading dose in the cath lab (*upstream clopidogrel-prasugrel switch)* 3) prasugrel in the cath lab onl (*prasugrel cath lab)* 4) clopidogrel pre-treatment followed by ticagrelor loading dose in the cath lab (*upstream clopidogrel-ticagrelor switch)* and 5) pre-treatment with ticagrelor only (*upstream ticagrelor)*.

Results of the VASP PRI values in the different patient cohorts are presented in table 5. In the upstream clopidogrel group, there was a statistically significant reduction in VASP PRI the day after PCI compared to pre-PCI and post-PCI. However only 32% of patients managed to reach the pre-specified end point of VASP PRI <50% the day after PCI. Time separation curves showed significant heterogeneity in the group with a weak linear association between increasing time and clopidogrel response with an r^2 of 0.17. Using the linear correlation, an estimated average time of 16.7 hours was noted between clopidogrel administration until a VASP PRI <50% was achieved.

Table 5.
VASP PRI% in the 5 treatment groups stratified by sampling time point.

	Upstream clopidogrel	Upstream clopidogrel-prasugrel switch	Prasugrel cath lab	Upstream clopidogrel-ticagrelor switch	Upstream ticagrelor
Pre-PCI VASP	**74% (SD 19)**	**79% (SD 13)**	**80% (SD 15)**	**79% (SD 16)**	**64% (SD 29)**
Post-PCI VASP	**74% (SD 20)**	**74% (SD 21)**	**69% (SD 34)**	**77% (SD 20)**	**53% (SD 30)**
Day after PCI VASP	**56% (SD 27)**	**17% (SD 21)**	**19% (SD 18)**	**15% (SD 8)**	**29% (SD 25)**
Percentage of patients with VASP-PRI <50% day after PCI	**32%**	**90%**	**91%**	**100%**	**83%**

In the upstream clopidogrel-prasugrel switch group, there was a reduction in VASP PRI% already post-PCI compared to pre-PCI. A very high degree of platelet inhibition was achieved the day after PCI (VASP PRI% 17) with 90% of patients managing to reach a value of <50% the day after PCI. The average estimated time between prasugrel administration to VASP PRI <50% was 1.5 hours (figure 11). Similar results were obtained for the prasugrel cath lab group, however the sample size was considerably smaller for this group and no time separation curves were attempted.

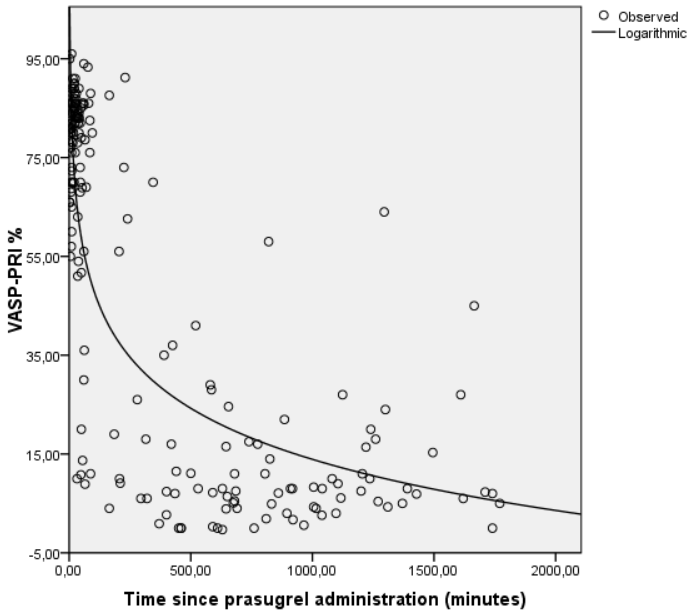

Figure 11.
Time separation curves for upstream clopidogrel-prasugrel switch group.

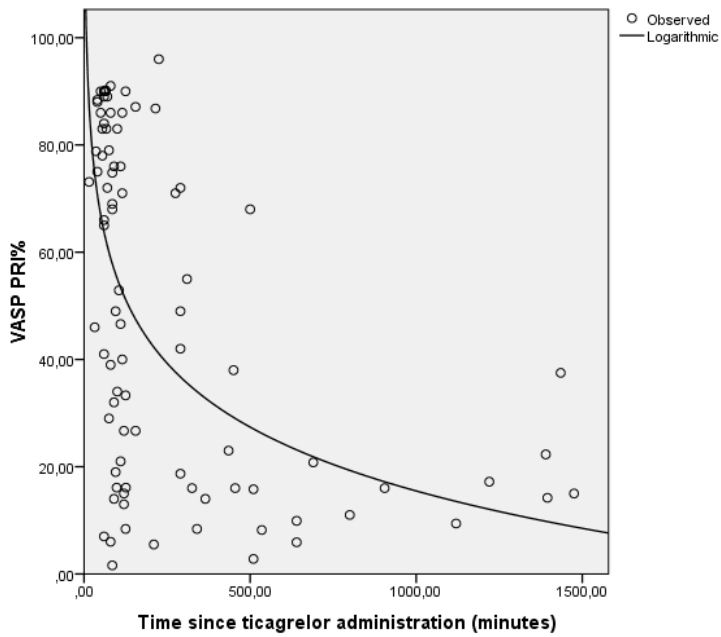

Figure 12.
Time separation curves for the upstream ticagrelor group

In the upstream ticagrelor group, there was numerically a higher degree of platelet inhibition (i.e. lower VASP PRI value) at the start of PCI (not reaching significance) as well as at the end of PCI ($p<0.05$), compared to all the other four patient groups. Half of upstream ticagrelor treated patients reached VASP PRI <50% already at completion of PCI. Just as in prasugrel treated patient groups, a high degree of platelet inhibition was noted the day after PCI with all patients being responders to treatment the day after PCI (VASP PRI <50%). The average estimated time between ticagrelor administration and good responder status was 2.2 hours (figure 12). In the upstream clopidogrel-ticagrelor switch group, results were similar to the upstream ticagrelor group, however the small sample size prevented any time separation curves.

Paper IV

In paper IV, ticagrelor pre-treatment in patients with STEMI and primary PCI was evaluated. A total of 7433 patients were included in the study, with 5438 patients receiving ticagrelor pre-treatment and 1995 patients receiving ticagrelor in the cath lab without pre-treatment. The patients were relatively similar in background characteristics, however ticagrelor pre-treatment patients were on an average older and had PCI performed during the years 2013 and 2014 to a higher extent compared to patients without pre-treatment. Pre-treated patients also received more often heparin and aspirin pre-treatment compared to patients with no ticagrelor pre-treatment. Patients with no ticagrelor pre-treatment instead had more clopidogrel pre-treatment (followed by ticagrelor in the cath lab) as well as heparin and aspirin treatment in the cath lab.

Crude mortality rates did not differ between the patient groups (4.5% in pre-treatment group versus 4.7% in no pre-treatment group) (figure 13 for Kaplan-Meier curves). After adjustment for potential confounding factors, there was no statistically significant difference in mortality between ticagrelor pre-treatment and no pre-treatment. Although numerically the results trended in favor of pre-treatment, with the strongest association shown using direct multivariable analysis (HR 0.71, 95% CI: 0.48-1.06, table 6), none of the models could statistically significantly prove a direct beneficial effect on mortality.

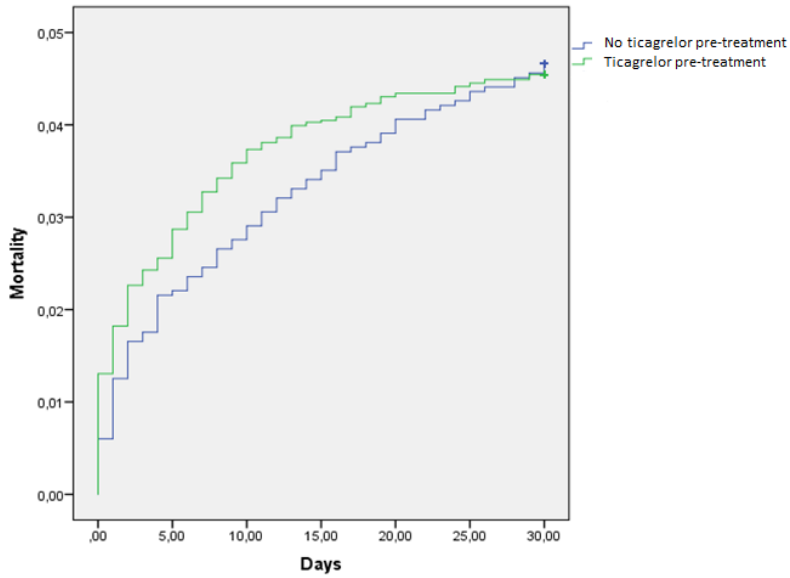

Figure 13.
Kaplan-Meier curve showing mortality as function of time stratified by ticagrelor pre-treatment or not.

Crude event rates for myocardial infarction did not differ between groups (4.4% in pre-treatment group versus 5.4% in no pre-treatment group, p=0.10).). Using direct multivariable Cox regression analysis, a statistically significant association between ticagrelor pre-treatment and lower rate of myocardial infarction at 30 days was seen (HR 0.73, 95% CI: 0.55-0.97, table 6). Propensity scoring and propensity score matching analyses showed similar trends towards favorable outcomes in MI, however did not reach statistical significance (table 6).

Table 6.
Summary of clinical end points at 30 days stratified by ticagrelor pre-treatment or not.

	Death	**MI**	**Stent thrombosis**
Unadjusted event rate (KM)	p = 0.86	p = 0.10	p = 0.80
- Ticagrelor pre-treatment	4.5%	4.4%	0.5%
- No ticagrelor pre-treatment	4.7%	5.4%	0.4%
Direct regressional multivariate analysis (Hazard ratio and 95% CI)	0.71 (0.48-1.06) p = 0.09	0.73 (0.55-0.97) p = 0.03	Not performed
Propensity score covariate adjustment (Hazard ratio and 95% CI)	0.76 (0.50-1.16) p = 0.20	0.73 (0.49-1.08) p = 0.11	0.94 (0.23-3.83) p = 0.93
Propensity score matching (Hazard ratio and 95% CI)	0.80 (0.50-1.27) p = 0.34	0.75 (0.49-1.14) p = 0.11	Not performed

No difference was observed in the rate of definite stent thrombosis at 30 days (0.5% in pre-treatment group versus 0.4% in no pre-treatment group, p = 0.80). The results remained similar after adjustment using propensity scoring. Direct multi-variable analysis and propensity score matching was not performed due to low event rates.

All results above remained similar after adjustment for cardiogenic shock in the statistical models.

No difference in major in-hospital bleeding rate was noted. Ejection fraction at discharge was similar between both groups. Subgroups analysis based on age, gender, hypertension, diabetes, etc showed no statistically significant p-value for interaction, suggesting no differential effect in any particular subgroup.

Paper V

A total of 13790 patients were included for analysis regarding delays in FMC-to-PCI and all-cause mortality at one year. Approximately 50% of these patients were diagnosed with a pre-hospital ECG. Median FMC-to-PCI delay was for the entire patient cohort 70 minutes (IQR 42-110 minutes). Patients outcomes were studied using time as a continuous variable as well as time delays divided into different 30 minute delays (0-30, 31-60, 61-90, 91-120 and >120 minutes). Patients with more than 360 minutes of FMC-to-PCI delay were excluded from analysis. Overall similar patient characteristics were noted between various strata of time delays, however patients with longer delays tended to have more pre-treatment with aspirin, clopidogrel and GPIIb/IIIa-inhibitors as well as being older. Patients excluded in the study tended to be similar in baseline characteristics, however they were to a lesser degree given pre-treatment with anti-thrombotic drugs.

Kaplen-Meier curves for mortality as a function of FMC-to-PCI delay are shown in figure 14, with a significant unadjusted association between FMC-to-PCI delay and one-year mortality. A statistically significant hazard ratio of 1.06 for one-year mortality was noted for every 30 minutes of time delay when studying the entire patient cohort. Dividing time into different time cohorts yielded a statistically significant increase in mortality after adjustment for covariates with FMC-to-PCI delays exceeding one hour (table 7). The longest time delay group showed an approximately 50% increase in mortality compared to the shortest time delay group. In patients with a pre-hospital ECG, the longest time delay group showed an approximately 70% increase in mortality compared to the shortest time delay group after adjustment for covariates. Analysis of patients with a 3-year follow-up showed a continued increase in long-term mortality with increasing FMC-to-PCI delay. FMC-to-PCI delay exceeding one hour also showed a significant association with severe heart failure at discharge.

Increasing symptom-to-PCI delays were associated with increased unadjusted one-year mortality as well as adjusted one-year mortality when using time as a continuous variable (table 7). However when dividing symptom-to-PCI into various time cohorts (0-60, 61-120, 121-180, 181-240 and 241-480 minutes), only the highest time delay showed a statistically significant association with increased mortality at one year, when compared to the lowest time delay group (table 7). In addition symptom-to-PCI did not show, after adjustment, the gradual and incremental increase in mortality that was observed for FMC-to-PCI. At 3 years, no association (adjusted or unadjusted) remained for increasing symptom-to-PCI time and increased mortality.

Patients with cardiogenic shock had a lower symptom-to-PCI time compared to non-cardiogenic shock patients. Also patients with LAD-infarctions tended to seek medical attention earlier.

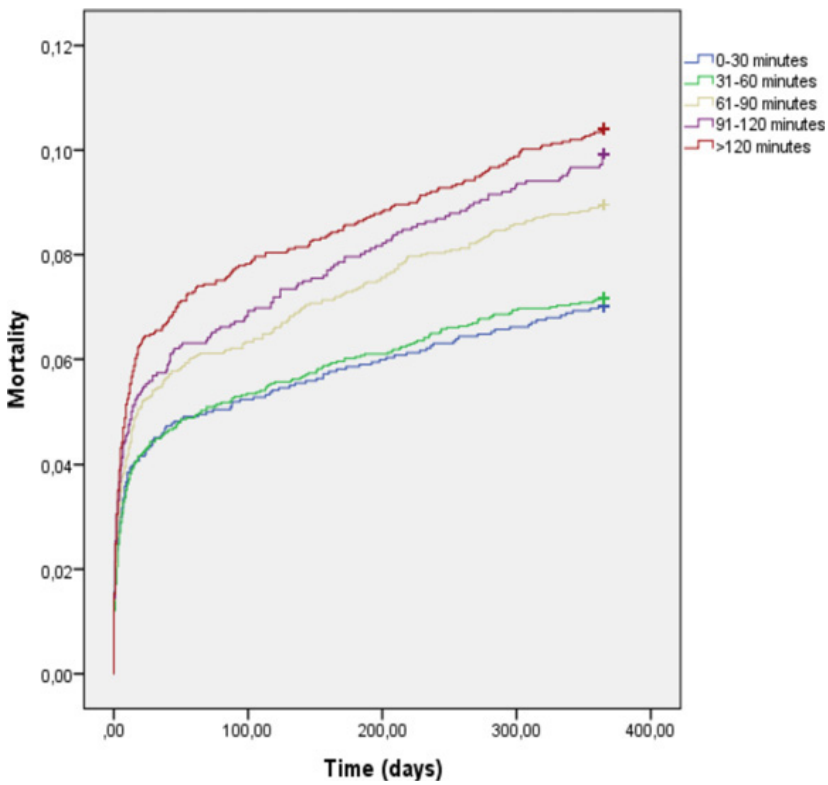

Figure 14.
Kaplan-Meier curve for mortality as function of time stratitied by various FMC-to-PCI cohorts.

Table 7.
Unadjusted and adjusted one-year mortality in various time delay cohorts

Patient group	Delay to PCI (mins)	Sample size	Unadjusted event rate	Adjusted hazard ratio (95% CI)
FMC-to-PCI, entire cohort				
	0-30	2237	7.0%	1.00
	31-60	3557	7.2%	1.08 (0.88-1.33)
	61-90	3238	9%	1.26 (1.03-1.55)
	91-120	1934	9.9%	1.41 (1.14-1.76)
	121-360	2824	10.4%	1.51 (1.23-1.86)
FMC-to-PCI, patients with a pre-hospital ECG				
	0-30	520	5%	1.00
	31-60	2379	7.1%	1.34 (0.88-2.05)
	61-90	2042	9.5%	1.57 (1.03-2.41)
	91-120	1015	10.2%	1.61 (1.03-2.53)
	121-360	1097	10.4%	1.71 (1.10-2.67)
Symptom-to-PCI, entire cohort				
	0-60	332	5.7%	1.00
	61-120	2567	7.2%	1.50 (0.93-2.43)
	121-180	3146	8.2%	1.47 (0.92-2.35)
	181-240	2142	8.5%	1.45 (0.90-2.35)
	241-480	3302	9.2%	1.68 (1.05-2.69)

Paper VI

The VALIDATE study has recently passed the halfway mark with 3061 patients currently included (1725 STEMI and 1336 NSTEMI). Figure 15 illustrates both the amount of patients that are theoretically possible to include as well as patients de facto included, showing that more than 60% of patients in an this all-comer ACS population are actually included in the study. No untoward side effects of note have been noted and the Data Safety and Monitoring Committee (DSMC) have so far not given any signal of any unusually large treatment effect or hazard with any of the treatment arms.

Baseline demographics for the first 3021 patients are presented in table 8. So far more STEMI than NSTEMI patients have been recruited. One-vessel disease was the most common angiographic finding (more than half the population). In the STEMI population, approximately 57% were given P2Y12-inhibitor treatment within one hour of PCI and 36% were given ticagrelor/prasugrel between 1-2 hours prior to PCI. In the NSTEMI population, 60% of patients received P2Y12-inhibitor treatment >12 hours prior to PCI. Evidence of thrombus (small, moderate or large) was more common in the STEMI population. A very low degree of coronary angiograms were completely normal, in line with the inclusion criteria of intention-to-treat with PCI. Almost 25% of the vessels with intention-to-treat with PCI had TIMI 0-1 flow in the NSTEMI group. Corresponding number for the STEMI group was nearly 68%. Few patients were on novel oral anticoagulants (NOACs).

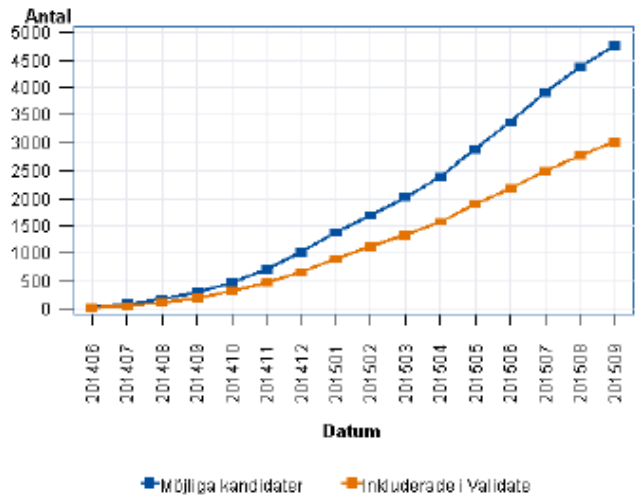

Figure 15.
Theoretically posible amount of patients that can be included (blue line) versus de facto randomized patients (orange line).

Table 8.
Baseline demographics for the first 3021 patients included in the VALIDATE study.

	NSTEMI (N=1316)	STEMI (N=1705)	Total (N=3021)
Male sex	981 (74.5%)	1253 (73.5%)	2234 (73.9%)
Age (median, IQR)	68 (60-76)	67 (58-74)	68 (59-75)
Previous MI	272 (20.7%)	219 (12.8%)	491 (16.3%)
Previous PCI	239 (18.2%)	201 (11.8%)	440 (14.6%)

Previous CABG	94 (7.1%)	38 (2.2%)	132 (4.4%)
Smoking status			
- **Previous smoker**	508 (38.6%)	487 (28.6%)	995 (32.9%)
- **Current smoker**	258 (19.6%)	490 (28.7%)	748 (24.8%)
Diabetes	280 (21.3%)	234 (13.7%)	514 (17.0%)
Hyperlipidemia	535 (40.7%)	387 (22.7%)	922 (30.5%)
Hypertension	758 (57.6%)	788 (46.2%)	1546 (51.2%)
Stroke	61 (4.6%)	52 (3.0%)	113 (3.7%)
Ambulance			
- No	595 (45.2%)	339 (19.9%)	934 (30.9%)
- Yes to ER	514 (39.1%)	292 (17.1%)	806 (26.7%)
- Yes to CICU/Cath lab	53 (4.0%)	951 (55.8%)	1004 (33.2%)
Prehospital CPR	5 (0.4%)	21 (1.2%)	26 (0.9%)
Killip klass			
- Killip I	1088 (82.7%)	1623 (95.2%)	2711 (89.7%)
- Killip II	18 (1.4%)	53 (3.1%)	71 (2.4%)
- Killip III	4 (0.3%)	13 (0.8%)	17 (0.6%)
- Killip IV	2 (0.2%)	11 (0.6%)	13 (0.4%)
Unknown	204 (15.5%)	5 (0.3%)	209 (6.9%)
Extent of coronary disease			
Normal/atheromatosis	12 (0.9%)	10 (0.6%)	22 (0.7%)
- 1VD no LM	683 (51.9%)	904 (53.0%)	1587 (52.5%)
- 2VD no LM	389 (29.6%)	496 (29.1%)	885 (29.3%)
- 3VD no LM	187 (14.2%)	242 (14.2%)	429 (14.2%)
- LM + 1VD	5 (0.4%)	4 (0.2%)	9 (0.3%)
- LM + 2VD	11 (0.8%)	20 (1.2%)	31 (1.0%)
- LM + 3VD	22 (1.7%)	24 (1.4%)	46 (1.5%)
- Isolated LM	1 (0.1%)	1 (0.1%)	2 (0.1%)
Time from administration of ticagrelor/prasugrel to PCI			
- 0 < 1 hours	125 (9.5%)	970 (56.9%)	1095 (36.2%)
- 1-2 hours	85 (6.5%)	612 (35.9%)	697 (23.1%)
- 2-3 hours	53 (4.0%)	63 (3.7%)	116 (3.8%)
- 3-6 hours	119 (9.0%)	27 (1.6%)	146 (4.8%)
- 6-12 hours	129 (9.8%)	13 (0.8%)	142 (4.7%)
- > 12 hours	798 (60.6%)	11 (0.6%)	809 (26.8%)
TIMI-flow prior to PCI			
- TIMI-0	247 (18.8%)	1005 (58.9%)	1252 (41.4%)
- TIMI-1	80 (6.1%)	152 (8.9%)	232 (7.7%)
- TIMI-2	230 (17.5%)	254 (14.9%)	484 (16.0%)
- TIMI-3	754 (57.3%)	289 (17.0%)	1043 (34.5%)
Thrombus estimation			
- No thrombus	789 (60.0%)	447 (26.2%)	1236 (40.9%)
- Possible thrombus	326 (24.8%)	490 (28.7%)	816 (27.0%)

-	Small thrombus	45 (3.4%)	109 (6.4%)	154 (5.1%)
-	Moderate thrombus	73 (5.5%)	288 (16.9%)	361 (11.9%)
-	Large thrombus	42 (3.2%)	234 (13.7%)	276 (9.1%)
-	Cannot be determined	33 (2.5%)	125 (7.3%)	158 (5.2%)
Medications at admission				
-	ACE-inhibitors	223 (16.9%)	225 (13.2%)	448 (14.8%)
-	AngiotensinII-inhibitors	222 (16.9%)	193 (11.3%)	415 (13.7%)
-	Aspirin	360 (27.4%)	309 (18.1%)	669 (22.1%)
-	Clopidogrel	39 (3.0%)	20 (1.2%)	59 (2.0%)
-	Ticagrelor	23 (1.7%)	22 (1.3%)	45 (1.5%)
-	Betablockers	346 (26.3%)	372 (21.8%)	718 (23.8%)
-	Ca^{2+}-inhibitors	230 (17.5%)	244 (14.3%)	474 (15.7%)
-	Warfarin	35 (2.7%)	19 (1.1%)	54 (1.8%)
-	Dabigatran	3 (0.2%)	4 (0.2%)	7 (0.2%)
-	Rivaroxaban	1 (0.1%)	2 (0.1%)	3 (0.1%)
-	Apixaban	7 (0.5%)	2 (0.1%)	9 (0.3%)
-	Statins	357 (27.1%)	282 (16.5%)	639 (21.2%)

LM = Lef main stem CICU = cardiac intensive care unit ER = emergency room

Discussion

Pharmacodynamic assessment of platetelet function to tailor treatment (paper II)

The main finding of paper II was that platelet function testing could not identify a particular threshold value that could be used to guide anti-platelet therapy with sufficient sensitivity and specificity. Concerning stent thrombosis, the VerifyNow system showed significantly higher PRU values for the stent thrombosis group compared to matched controls. Using a PRU value of ≥222 as a cut-off for poor clopidogrel response resulted in a sensitivity of 70% and specificity of 67%. Thus 33% of patients that would never experience a stent thrombosis on dual anti-platelet therapy would be labelled as clopidogrel resistant, and be subjected to intensified treatment using this cut-off. In addition, 30% of patients who had experienced a stent thrombosis would be labelled as good clopidogrel responders. Improving the sensitivity by establishing a cut-off at ≥123 PRU for poor clopidogrel response would identify 90% of stent thrombosis cases, however almost 80% of stent thrombosis controls would be identified as poor responders by this definition.

Concerning platelet inhibition and recurrent MI (not being a stent thrombosis), platelet function testing could not discriminate between patients with previous MI on dual anti-platelet therapy and matched controls. In fact there was a numerically higher degree of platelet inhibition (lower levels of VerifyNow PRU and VASP PRI) in patients with recurrent MI compared to matched controls. This probably reflects that recurrent myocardial infarction is a complex disease where in vitro measurements do not reflect the full biological interaction in vivo between platelets, atherosclerotic plaques and the inflammatory system. This has been indicated in a subgroup analysis of medically treated patients with ACS given prasugrel or clopidogrel.[132]

Results from large prospective and randomized trials

The two largest prospective and randomized trials that have studied platelet function testing to guide therapy are the *Gauging Responsiveness with A VerifyNow assay—Impact on Thrombosis And Safety* (GRAVITAS, n=2214) and *Assessment with a double Randomization of (1) a fixed dose versus a monitoring-guided dose of aspirin and*

Clopidogrel after DES implantation, and (2) Treatment Interruption versus Continuation, 1 year after stenting (ARCTIC, n=2440) trials.[123, 124]

In GRAVITAS, the majority of patients had stable angina and even in the ACS group, a majority of patients were troponin-negative. Platelet function testing was performed 12-24 hours after PCI. Patients that after treatment with clopidogrel showed a high platelet reactivity post-PCI (i.e. insufficient effect of clopidogrel), were randomized to a high loading dose clopidogrel (600 mg) followed by 150 mg daily versus a usual treatment group (continued 75 mg daily clopidogrel). The results indicated that a high dose clopidogrel regimen in clopidogrel non-responders, did not prevent cardiovascular outcomes (cardiovascular death, nonfatal myocardial infarction or definite/probable stent thrombosis) compared to non-responders receiving usual care.[123]

Whereas the GRAVITAS study included only patients that were considered poor responders to clopidogrel, the ARCTIC study was more clinically oriented and randomized patients with an intention-to-treat to either platelet function testing with subsequent drug adjustment, or to no platelet function testing with standard care. All patients underwent PCI treatment. In the platelet function testing group, a total of 34.5% of patients were labelled as clopidogrel poor responders and 7.6% as aspirin resistant and were subjected to drug adjustment. These drug adjustments included intravenous aspirin if aspirin resistance was noted. In patients with clopidogrel resistance, GPIIb/IIIa-inhibitor use followed by an additional loading dose of clopidogrel or a loading dose of prasugrel before PCI procedure was recommended. This was followed by a daily maintenance dose of 150 mg of clopidogrel or 10 mg of prasugrel after the procedure. The vast majority of clopidogrel poor responders were subjected to GPIIb/IIIa-inhibitor use during PCI and clopidogrel adjustment (renewed loading dose and increased maintenance dose, similar to GRAVITAS). Only 11.9% of patients were given prasugrel at discharge.[124]

The results of the ARCTIC study were similar to GRAVITAS with no improvement in the main composite end point (one-year rate of death, myocardial infarction, stent thrombosis, stroke or urgent revascularization) in the platelet function testing and drug adjustment group.[124]

Why have the results in large randomized platelet function trials been neutral?

There are several possible reasons behind the largely neutral results in the large and prospective randomized trials.

First, the populations enrolled have to a high degree been low-risk populations with stable angina. The event rate for the primary end point at 6 months in GRAVITAS was merely 2.3% in both arms. The pooled death rate at one year was approximately

2% in ARCTIC.[123, 124] In this low-risk group, conventional treatment already has a low event rate and expecting to lower this event rate further is both difficult to accomplish and would require much larger sample sizes. In comparison, reported cardiovascular event rates in clopidogrel poor responders that have STEMI are considerably higher than in stented stable angina populations.[133] However no large randomized interventional trial has studied this high-risk group selectively.

Second, the anti-platelet intervention has been criticized to be insufficient in the large randomized trials. In GRAVITAS and for the majority of patients in ARCTIC, the po medical intervention for clopidogrel resistance was a single extra loading dose of clopidogrel and adjustment of maintenance dose. Although this leads to further platelet inhibition in vitro, the intervention is quite weak compared to switching to more potent drug like ticagrelor or prasugrel.[123, 124] In the *Testing Platelet Reactivity In Patients Undergoing Elective Stent Placement on Clopidogrel to Guide Alternative Therapy With Prasugrel* (TRIGGER-PCI study, n=423), patients with stable coronary artery disease and clopidogrel resistance were randomized to prasugrel or continued clopidogrel.[134] The effect on platelet inhibition was highly efficacious, however again the patient population was a low-risk population consisting of stable angina patients with successful PCI and without major peri-procedural complications prior to randomization. The cardiovascular event rate was low in the study (<1%), which led to its premature termination after only 423 recruited patients.[134] In the recently published *The Responsiveness to Clopidogrel and Stent Thrombosis 3* (RECLOSE-3, n=302) trial, clopidogrel poor responders were prospectively identified and given prasugrel.[135] These patients were compared to clopidogrel poor responders from the previously published RECLOSE-2 trial, that were given clopidogrel high maintenance dose or ticlodipine to improve their platelet function status.[136] Despite the patients in RECLOSE-3 having more hypertension, previous MI, multi-vessel PCI or PCI against left main stenosis, there was a reduction in 2-year cardiac mortality in clopidogrel non-responders given prasugrel instead of higher dose clopidogrel or ticlodipine.[135]

Third, the timing of platelet function testing has in several studies been performed after stenting, thereby selecting an even more low-event group. In GRAVITAS, clopidogrel response was measured 12-24 hours after stenting and thus any complications during the procedure and 12-24 hours later would not be captured.[123] Similarly in TRIGGER-PCI, platelet function testing and randomization was performed several hours after ingestion of maintenance dose clopidogrel, thereby missing early events.[134] In contrast, Bonello et al (study outlined in detail under "introduction", n=162), administered repeated clopidogrel loading doses (up to 2.4 g) prior to PCI using the VASP assay and demonstrated improved cardiovascular outcomes.[119]

Finally, when designating specific cut-offs for clopidogrel resistance, thrombotic risk is considered an "all-or-nothing" phenomenon rather than a continuum of risk. For example using a clinically applied protocol of VerifyNow PRU ≥222 as clopidogrel

poor response would label a patient with a PRU of 222 as a "high-risk" patient with subsequent drug adjustment. A patient with a PRU of 221 would be considered clopidogrel responsive and placed in a "low-risk" group with no drug adjustment. From a biological perspective, both patients are nearly identical and the difference between their PRU values is far lower than the methodological variation of the measurement. Using platelet function testing as a continuum of risk with a division of platelet response into quartiles or more might provide more useful information rather than the "all-or-nothing" approach used in the large randomized trials.[133, 137] In our study we could identify a highly significant difference of 46.8 PRU units (<p.001) between stent thrombosis patients and matched controls. However the heterogeneity and subsequent overlap of platelet response meant that no cut-off value could be established with sufficient degree of sensitivity and specificity to be clinically feasible. Division of platelet response into quartiles, with comparison of first with last quartile might have rendered different results, but would have required a larger sample size.

Future of platelet function testing?

Although the negative results from the large randomized trials have discouraged routine use of platelet function testing, there are unaddressed questions that remain to be solved. As outlined above, there are smaller interventional trials suggestive of positive effects of platelet function testing in in certain groups of patients.[119, 135] However, the small sample sizes or non-randomized nature of these studies warrant careful interpretation. There is thus an unmet need for larger, prospective and randomized trials evaluating platelet function testing in high-risk populations (STEMI and NSTEMI), using potent interventions prior to PCI and with platelet function testing used as a continuum of risk (rather than in an "all-or-nothing" fashion).

Furthermore there is a need in clinical practice to better address the risk of bleeding versus thrombosis in patients with dual anti-platelet therapy. Traditional bleeding risk scores like CRUSADE, Mehran and ACTION are all limited in clinical use since multiple risk factors for bleeding are also predictors for future thrombotic events (age, kidney failure, biomarker positive ACS, etc).[138-140] Increasing evidence suggest that there might be a "sweet spot" or "therapeutic window" for platelet inhibition, where a sufficient anti-thrombotic effect is combined with a low risk of bleeding (figure 16).[110-113] This research area is less explored than platelet function testing and thrombotic risk. Several studies have been published in recent years evaluating long-term dual anti-platelet therapy compared to standard duration in patients with ACS or with DES implantation.[141-144] The results have been conflicting, however only two of these trials can be considered sufficiently large enough for adequate statistical power. Both in the *Dual Antiplatelet Therapy* (DAPT, n=9961) trial and in the *Prevention of Cardiovascular Events in Patients With Prior Heart Attack Using Ticagrelor Compared to Placebo on a Background of Aspirin* (PEGASUS-TIMI 54,

n=21162) trial, a reduction of ischemic events was noted with long term dual anti-platelet therapy, however at the cost of more major bleeding events.[143, 144] Both of these trials highlight the need for a tailored platelet inhibition strategy based upon a therapeutic window. This has not yet been evaluated in a randomized prospective study.

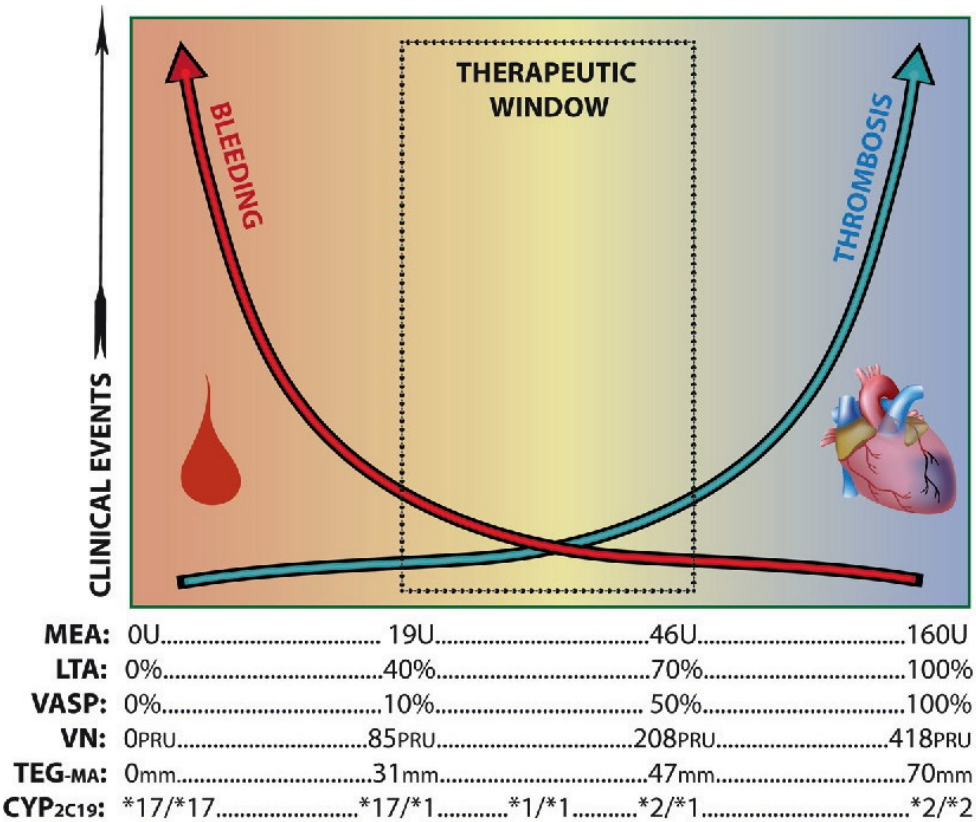

Figure 16.
Bleeding and thrombotic risk on different ends of the platelet inhibition spectrum. Reprinted with permission from Thrombosis and Hemostasis.

Pre-treatment and switching anti-platelet drugs

Clopidogrel

Paper I evaluated the effect of clopidogrel pre-treatment in patients with STEMI undergoing primary PCI and is (to our knowledge) the largest study on this subject.

Our results indicated that clopidogrel pre-treatment was associated with a lower rate of death/MI at one year compared to clopidogrel given peri-procedurally. Mortality alone was also reduced by clopidogrel pre-treatment, both at 30 days and at one year. No reduction of MI alone or stent thrombosis was noted. The effect was consistent in patients discharged with dual anti-platelet therapy as well as in all other subgroups investigated with no p-value of interaction <0.05 noted. Whether these findings represent a true association cannot be established due to the non-randomized nature of the study. Previous data regarding clopidogrel pre-treatment in the setting of primary PCI was, prior to our study, limited to smaller register studies with low power for detection of hard clinical end points.[80-82] A meta-analysis by Vlaar et al had indicated improved clinical outcomes with clopidogrel pre-treatment. However the meta-analysis included studies with widely different PCI strategies, adjunctive medical therapies and different trial designs making results difficult to interpret.[145] In the same issue of European Heart Journal as paper I was published in, an Austrian study (n=5955) corroborated our results, with reduced mortality with clopidogrel pre-treatment.[79] One year later, the *Clopidogrel to Improve Primary percutaneous coronary Intervention in Acute Myocardial Infarction* (CIPAMI, n=337) trial was published. To date this is the only prospective randomized trial to have studied the impact of clopidogrel pre-treatment on clinical outcomes in STEMI patients undergoing primary PCI. The study was underpowered due to slow inclusion rate (since many centers during the study period found it unethical to withhold clopidogrel pre-treatment). However despite the small study sample there was a clear trend towards favorable outcomes with clopidogrel in STEMI patients undergoing primary PCI, however not reaching statistical significance (figure 17).[84] Recently a Spanish register study in a mixed ACS population (n=9621) was published where clopidogrel pre-treatment was studied. STEMI patients undergoing primary PCI showed improved cardiovascular outcomes, including mortality, in accordance with our data. NSTEMI patients did not benefit from pre-treatment.

The data above suggest that in case a clopidogrel based therapy is being used for STEMI and primary PCI, then pre-treatment might improve clinical outcomes. A definite answer would require a large prospective and randomized study which most likely will never be performed in the current era of widespread P2Y12-inhibitor pre-treatment worldwide.

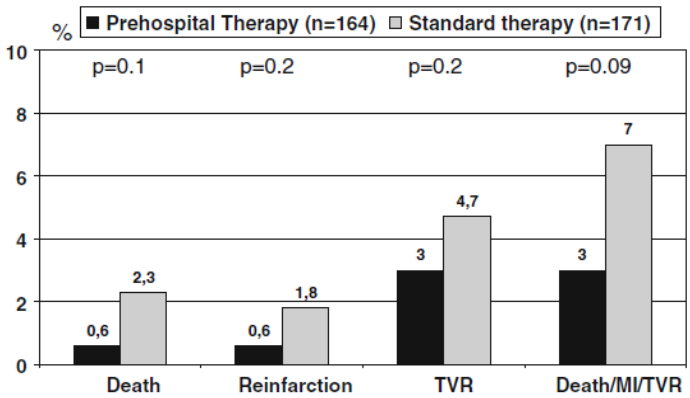

Figure 17.
Clinical outcomes in the CIPAMI trial. Reprinted with permission from Clinical Research in Cardiology.

Ticagrelor

The purpose of paper IV was to evaluate the effects of ticagrelor pre-treatment in patients with STEMI undergoing primary PCI and is (to our knowledge) the largest study to date investigating this subject. Our results indicated that ticagrelor was safe in this setting with no excess mortality at 30 days. In one of three statistical models, a beneficial effect on myocardial infarction at 30 days was seen. No effect was seen on the rate of stent thrombosis or major in-hospital bleedings. Whether these findings represent a true association cannot be established due to the non-randomized nature of the study.

In the PLATO study, ticagrelor was allowed prior to arrival at the cath lab, however the average time duration between ticagrelor administration and admission into the cath lab was only 15 minutes. Therefore any beneficial or deleterious effects of ticagrelor pre-treatment is difficult to ascertain.[8] To address this issue, the ATLANTIC study was performed where STEMI patients undergoing primary PCI were randomized to pre-treatment with ticagrelor versus ticagrelor administration in the cath lab.[88] The results were neutral concerning the primary composite end point of ST-resolution and infarct vessel blow flow at initial angiography. However the rate of definite stent thrombosis at 30 days was markedly reduced with ticagrelor pre-treatment (0.2% versus 1.2%, p=0.02). On the other hand mortality trended unfavorably with ticagrelor pre-treatment (3.3% vs 2.0%, p=0.07).[88] Whether both these findings represent chance or signal potential benefit/harm with pre-treatment is not known due to low overall absolute event rates. Our results did not show any effect on definite stent thrombosis with ticagrelor pre-treatment. However the pooled rate of stent thrombosis was lower in our study than in the ATLANTIC study. The study

populations differed in that more drug eluting stents were used in our study and different degrees of various anti-thrombotic drugs were used in the studies, making direct comparisons difficult. In addition the rate of the composite end point of definite or probable stent thrombosis at 30 days did not differ in the ATLANTIC study between the two study arms (2.3% versus 2.1%, p=0.75), thus further complicating interpretation of stent thrombosis.[88] The observed trend towards increased mortality in ATLANTIC have spurred certain centers to question the safety of prehospital ticagrelor.[69] In a study of operated patients with aortic dissection, a substantial degree of patients had received dual anti-platelet therapy prior to surgery, with only 29% of these cases having received treatment according to guidelines. Dual anti-platelet therapy in the setting of acute aortic dissection was associated with worse clinical outcomes, including mortality.[146] Our results suggest that in patients with a correct indication for STEMI (i.e. undergoing primary PCI), pre-treatment was not associated with increased mortality. However, one limitation with our study is that patients not undergoing PCI could not be included since information on pre-treatment is only available through registers for patients undergoing PCI. The results therefore need to be interpreted with a certain degree of caution. The usage of the reversible and intravenous P2Y12-inhibitor cangrelor in combination with clopidogrel given at the cath lab, has been shown to decrease ischemic end points, including stent thrombosis, without excess bleeding in a mixed coronary population.[147] If these results can be reproduced with ticagrelor given at the cath lab, it might represent a clinically feasible future anti-platelet combination.

Pharmacodynamic comparison of anti-platelet protocols including switching

Paper IV evaluated the pharmacodynamic response (measured by the VASP assay) of 5 different anti-platelet protocols in patients with STEMI undergoing primary PCI. The 5 treatment cohorts represented changing treatment patterns of anti-platelet drugs from 2009 to 2012. At the time of study initiation, pre-treatment with clopidogrel was the default option for patients with STEMI undergoing primary PCI. With the introduction of prasugrel after the *TRial to assess Improvement in Therapeutic Outcomes by optimizing platelet InhibitioN with prasugrel Thrombolysis In Myocardial Infarction 38* (TRITON) trial, there arose difficulties in patients with STEMI. In the TRITON study, pre-treatment with prasugrel/clopidogrel was not allowed for NSTE-ACS, however provision for this was given for STEMI patients but only a minority of these (25% of STEMI patients) received pre-treatment. With the high rate of major bleeding in CABG patients receiving prasugrel, as well as the increased rate of non-CABG major bleeding in the overall study population, there was a fear that introduction of prasugrel in a prehospital setting without knowledge of the coronary anatomy could induce potential harm.[7, 148] Data at that time on switching between clopidogrel and prasugrel was scarce and the results from paper I discouraged removing pre-treatment of clopidogrel. Therefore patients with STEMI and primary

PCI were given clopidogrel pre-treatment and after coronary angiography and decision of primary PCI, a prasugrel loading dose (60 mg was given). The concern with this protocol of 600 mg clopidogrel followed by 60 mg of prasugrel was that an "overshoot" of platelet inhibition could occur with too potent platelet inhibition and subsequent increased bleeding risk. Patients were thus included in the Lund Platelet Register with platelet function testing performed at three intervals (as defined under "results"). In a subset of patients, the time to cath lab was sufficiently small that no clopidogrel pre-treatment was given and instead prasugrel was administered as lone therapy in the cath lab. As ticagrelor became available, there was a changing pattern with continued clopidogrel pre-treatment, followed by a ticagrelor switch in the cath lab (180 mg). The same concern of too potent platelet inhibition was also shared for this protocol. However as ticagrelor became available in the ambulances, pre-treatment with ticagrelor was introduced, both in ambulances as well as referring hospitals and is the current therapy of choice at Skåne University Hospital Lund, Sweden.

The results from paper IV indicated that switching (clopidogrel to prasugrel or clopidogrel to ticagrelor) was not associated with a pharmacodynamic overshoot of platelet inhibition. The comparator groups were prasugrel and ticagrelor monotherapy groups. Similar results of switching from a clopidogrel loading dose to a prasugrel loading dose have been corroborated.[149] Prasugrel and ticagrelor had considerably faster onsets than clopidogrel, however both their onsets (1.5 hours and 2.2 hours) were slower compared to stable patients without STEMI.[150, 151] Clopidogrel showed an even more marked slowness in onset (16.7 hours) compared to stable patients.[150, 152] These findings of delayed effect with all three drugs are probably due to the increased physiologic stress STEMI patients are subjected to as well as opiate use with subsequent decreased gastrointestinal motility and absorption.[153, 154] In fact in ATLANTIC, the primary end point was significantly in favor of prehospital ticagrelor treatment when ticagrelor was given to patients not having received morphine.[88] Both prasugrel and ticagrelor showed a very high degree of platelet inhibition the day after PCI in comparison to clopidogrel. A total of 50% of patients receiving ticagrelor pre-treatment were good responders post-PCI. This result is very similar to ticagrelor pre-treatment patients in an ATLANTIC platelet sub-study.[88] Prasugrel patients showed similar platelet inhibition data as in a platelet sub-study of the TRITON trial (however being a mixed ACS population).[155] Similar ticagrelor and prasugrel pharmacodynamic data as ours have been reproduced in other studies as well.[149, 156, 157]

Treatment delays to PCI and mortality

In paper V we wanted to investigate the relationship between FMC-to-PCI delay and mortality in STEMI patients undergoing primary PCI. The study is to our knowledge

the largest published study using FMC-to-PCI as time to intervention metric in patients with STEMI and primary PCI. Our results suggest an increase in one-year mortality with an adjusted HR of 1.06 for every additional 30 minute of FMC-to-PCI delay. Dividing time into different 30 minute cohorts yielded a significant increase in mortality observed after 1 h, suggestive of a "golden hour" in primary PCI (previously shown for thrombolysis).[158] Symptom-to-PCI showed a significant association with mortality with an adjusted HR of 1.05 for every 1 h of time delay. However when dividing time into different time cohorts, the association could only be proven for the highest symptom-to-PCI delay. In addition, an overall incremental increase in mortality with increasing symptom-to-PCI delays could not be shown after statistical adjustment.

Multiple studies have evaluated the impact of increasing symptom-to-PCI delays and its effect on cardiovascular outcomes. Results are quite divergent with some studies showing very clear correlations with worse cardiovascular outcomes with increasing delays, whereas others studies show more intricate relationships and some even failing to show any clear association.[67, 159-165] Symptom-to-PCI due to its multiple deficiencies and biases might thus not be an ideal benchmark in this context. Patients might have difficulty in recalling the exact time of symptom onset. Furthermore symptom onset can be preceded by recurrent unstable angina making the symptom onset difficult to ascertain, both for the patient as well as for the physician. Finally patients that seek medical attention earlier, might be sicker than those presenting late which in a statistical model would cancel out any beneficiary effects of early revascularization.[61, 62, 166] The results from our study indicate that patients with LAD-infarctions as well as cardiogenic shock tend to seek medical attention earlier, supporting the notion of sicker patients being early presenters. The most compelling evidence for this are the 3-year outcome data in our study. As mentioned, using time as a continuous variable yielded a significant overall association between symptom-to-PCI and mortality at one year. However this association was completely abolished at 3 years (HR 1.007 per hour of treatment delay). This suggests that early reperfusion is to a greater extent performed in sicker patients, and given sufficient time, the comorbidities/severity of coronary disease of this sicker but early presenter group will cancel out any potential benefit of early reperfusion. The late presenters have limited effect of reperfusion, however long-term mortality wise they catch up with the early presenters, probably due to less comorbidities/severity of coronary disease. In contrast, FMC-to-PCI showed a robust association with mortality even at 3 years.

The door-to-balloon time metric is often used as a measure of health care delay.[167] A decreased door-to-balloon time is always preferable, however the metric is also flawed since the definition only includes the final part of the interventional chain between symptom onset to PCI.[167] Furthermore improving patient flows to primary PCI capable hospitals with decreasing overall treatment delay could actually worsen door-to-balloon times. The reason behind this is that time is required for staff mobilization and preparation of the cath lab. If patient arrival is swift (by ambulance bypassing

local hospitals), there could be a waiting time for admission to the cath lab and thus increased door-to-balloon time, despite an overall reduction in FMC-to-PCI time. This has been shown in a Danish study.[63]

The accumulated data from our study and previously published studies suggest that FMC-to-PCI (system delay) might be the optimal measure when assessing the impact of time delay on cardiovascular outcomes.[61, 62] FMC-to-PCI also encompasses the entire chain of medical events that the health care system can directly influence.[61, 62] System delays exceeding one hour were associated with increased rates of severe heart failure at discharge in our study which could partially explain our findings. In another study, increased system delay was associated with increased re-hospitalization or outpatient contact due to heart failure, in accordance .[168] Reducing patient delay (symptom-to-FMC) would require community information and education. Several randomized studies have been conducted with disappointing results regarding efforts to reduce patient delay in myocardial infarction.[169, 170]

Organizing STEMI networks

It is vital to ensure that primary PCI capable hospitals have a sufficient volume of STEMI patients which has been associated with decreased mortality.[64, 65] This is probably multifactorial, including increased PCI operator skill, increased skill of cardiac intensive care physicians and having access to round the clock advanced coronary care. In a recently published study, out of hospital cardiac arrest survivors without STEMI that were referred to a tertiary center, showed improved survival compared to patients referred to non-tertiary centers. Indicators for a good level of care were higher in the tertiary centers compared to non-tertiary centers.[171] These results indicate that high risk cardiac patients might benefit from centralized care in dedicated hospital units. Furthermore having multiple hospitals running primary PCI round the clock would also increase health care costs.

However this has to be balanced by the notion of potentially increasing treatment delays with subsequent less myocardial salvage, when care is centralized to a select few primary PCI capable hospitals.[67, 159-161] Outmost care should thus be taken to keep transportation time as low as possible. Usage of ambulance-ECGs with direct triage to a primary PCI capable hospital and bypassing local hospitals is essential and has been shown to decrease treatment delays.[172] Usage of helicopters for patients with long transportation distances has been shown to decrease system delay with timely intervention, despite transportation distances up to 150 km.[173]

The RRCT concept

Registers can be used to include patients in a clinically controlled, prospective and randomized fashion. This RRCT concept is being utilized in the VALIDATE study (paper VI) where patient randomization, background characteristics as well as secondary clinical outcomes are all taken from the national registers. The advantages are several. First, the costs of performing an RCT in the modern era have become prohibitive and several medical companies are hesitant in pursuing the cardiovascular research field due to these high costs of phase III clinical trials.[127, 130] Second, RRCTs have the potential of having a very high inclusion rate (included patients/possible patients to be included). Third, RRCTs might be able to include patients that traditionally are not included in regular RCTs (advanced age, multiple comorbidities, etc).[130] When comparing background demographics of currently enrolled patients in VALIDATE (table 8) and comparing to other contemporary RCTs, the patients are quite similar in background demographics.[7, 8, 105] However the main striking difference is age, where patients enrolled in the VALIDATE study are considerably older than in other contemporary ACS trials, suggesting that VALIDATE includes a more real-life cohort rather than a "healthier" RCT cohort.[7, 8, 105]

The Thrombus Aspiration in ST-Elevation myocardial infarction in Scandinavia (TASTE) trial, evaluating routine thrombectomy in STEMI patients, was one of the first nationwide and currently the largest RRCT to be published.[174] It was about seven times larger in sample size than the previous published RCT in the field of thrombectomy.[175] About 60% of all patients that theoretically could be included were de facto included and the cost of the study did not exceed one million dollars, an extremely low sum for a clinically controlled, prospective and randomized trial.[130, 174, 176] VALIDATE has so far shown a similar inclusion rate as the TASTE study (figure 15). Besides VALIDATE, there are several RRCTs ongoing using the SWEDEHEART register including DETermination of the role of OXygen in suspected Acute Myocardial Infarction trial (DETOX)[177] and Instantaneous Wave-Free Ratio versus Fractional Flow Reserve guided intervention (IFR-SWEDEHEART).

Heparin versus bivalirudin in ACS

Heparin monotherapy has not been compared to bivalirudin in a pure NSTEMI population. In mixed populations of ACS patients or in ACS combined with stable angina, the results have been somewhat divergent. However none of these studies have had sufficient sample size of NSTEMI populations to adequately determine

outcomes in this ACS subgroup (NAPLES III data currently unpublished, presented at ACC 2014).[75, 178] The VALIDATE study will have the largest NSTEMI cohort randomized to heparin or bivalirudin during PCI.

In comparison, there are several heparin versus bivalirudin studies in STEMI patients undergoing primary PCI. However the different trial designs have led to conflicting results and there still is no world-wide consensus on optimal anti-coagulant therapy during primary PCI.[75, 104, 105]

Heparin compared to bivalirudin monotherapy in STEMI patients undergoing primary PCI has been studied in the *Unfractionated heparin versus bivalirudin in primary percutaneous coronary intervention* (HEAT-PPCI, n=1812) study. The results showed a beneficial effect of heparin monotherapy compared to bivalirudin with respect to ischemic events, with no increased bleeding rate.[104] However HEAT-PPCI has several drawbacks. First, it was a uni-center trial and results have to be interpreted with caution. Second, the HEAT-PPCI study had no pre-treatment with heparin (common practice in many countries) which has been associated with a decreased rate of stent thrombosis in post-hoc analyses.[179] Third, prolonged infusion of bivalirudin was not allowed. Fourth, P2Y12-inhibitors were not given early upstream at first medical contact.[104] In contrast, the *Bivalirudin vs Heparin With or Without Tirofiban During Primary Percutaneous Coronary Intervention in Acute Myocardial Infarction* (BRIGHT, n=2194) study showed that bivalirudin was superior to heparin in ACS patients (90% STEMI) undergoing primary PCI. However the extensive delay between symptom onset and hospital admission of more than 6 hours (with an additional more than one hour door-to-balloon time) obscures the data. Furthermore only clopidogrel was used as P2Y12-inhibitor and almost 30% of patients did not get a 600 mg loading dose (300 mg instead).[75] The recently published Minimizing *Adverse haemorrhagic events by TRansradial access site and systemic Implementation of angioX* (MATRIX, n=7213) study compared bivalirudin and heparin in a mixed ACS population with no significant difference in the primary end point (major cardiovascular events). However a reduction of bleedings as well as all-cause mortality was noted in favor of bivalirudin, at the cost of an increase in definite stent thrombosis. Unfortunately this study did not test pure heparin monotherapy compared to bivalirudin, with approximately 25% of heparin patients receiving GPIIb/IIIa-inhibitors.[105] More than 80% of patients received P2Y12-inhibitor pre-treatment, however most of these patients received upstream clopidogrel instead of modern P2Y12-inhibitors.

In VALIDATE, a low dose of heparin (max 3000 U for NSTEMI and 5000 U for STEMI) is allowed during the procedure prior to randomization. We believe that a small dose of early heparin might be beneficial in STEMI patients undergoing primary PCI. The lack of heparin, prior to randomization, might partially explain the high rate of stent thrombosis in the bivalirudin arm in HEAT-PPCI.[104, 179] Post-PCI infusion of bivalirudin did not improve outcomes compared to no post-PCI infusion, in the MATRIX study. However a post-hoc analysis showed significantly better

results with a high-dose post-PCI infusion (as used in the current VALIDATE trial) compared to a low-dose infusion.[105] In the BRIGHT study, where bivalirudin was shown to be beneficial compared to heparin, bivalirudin patients were given a high-dose post-PCI infusion.[75] We believe that a high-dose post-PCI infusion of bivalirudin might be beneficial and recommend it in the study, although it is not mandatory. Finally all patients in the VALIDATE study are required to have received pre-treatment with either ticagrelor or prasugrel. Both of these drugs have been shown to be superior to clopidogrel in ACS patients[7, 8] and we believe that a true test of the optimal anti-coagulant during PCI, should be performed on a background of optimal anti-platelet medication.

Conclusions

- Pre-treatment with clopidogrel in patients with STEMI and primary PCI was associated with a reduction in both 30-day and one-year mortality/myocardial infarction, compared to clopidogrel given in hospital. A reduction in mortality alone was also seen, both at 30 days and at one year. No effect on myocardial infarction alone or on stent thrombosis was seen. The results were consistent in different subgroups, including in patients with dual anti-platelet therapy at discharge. These results suggest that in case a clopidogrel based treatment is intended in STEMI patients undergoing primary PCI, pre-treatment might be of significant value.
- Platelet function testing with VerifyNow or with the VASP assay could not identify clinically applicable threshold values to predict stent thrombosis or new onset MI (not stent thrombosis) in patients with dual anti-platelet therapy. Although an association between increased platelet reactivity on treatment and stent thrombosis was seen, measured cut-off values to discriminate good from poor clopidogrel response did not have adequate sensitivity or specificity to be implemented in a clinical protocol.
- Platelet function testing in patients with STEMI and primary PCI showed that a loading dose of either prasugrel (60 mg) or ticagrelor (180 mg) in the cath lab, on top of previously administered clopidogrel (600 mg), did not lead to an overshoot of platelet inhibition. Both prasugrel and ticagrelor had significantly slower onset in STEMI patients compared to more stable patients, however the onset was more rapid compared to clopidogrel. Pre-hospital treatment with ticagrelor resulted in 50% of patients being good responders at the completion of PCI.
- Pre-treatment with ticagrelor in patients with STEMI and primary PCI was not associated with improved or worse mortality compared to ticagrelor given in hospital. A signal for a reduction in myocardial infarction was seen in one of 3 statistical models. No adverse effect on major bleeding was seen. The results suggest that ticagrelor pre-treatment is safe in STEMI patients undergoing primary PCI.
- A treatment delay exceeding one hour from first medical contact to PCI (first medical contact defined by time point of first ECG registration) was associated with increased mortality at one year. Treatment delays exceeding one hour were also associated with severe heart failure at discharge, offering a partial plausible

explanation for the findings. Symptom-to-PCI was not equally well associated with increased mortality and due to various confounding and biases, might not be the ideal metric for studying treatment delay and mortality.

- Using a nationwide register for prospective inclusion and randomization evaluating two established anti-thrombotic drugs during PCI (bivalirudin versus heparin) is feasible. The inclusion rate so far has been high with no reported unexpected incidents. Patients included so far using this register based concept have been older than in other ACS trials and might be more representative of a real-world setting, compared to patients included in classic randomized clinical trials.

Summary in Swedish (populärvetenskaplig sammanfattning)

Hjärt och kärlsjukdomar utgör idag den vanligaste orsaken till död världen över. I denna avhandling studerades patienter med hjärt-kärlsjukdom inom svenska register för att analysera betydelsen av olika komponenter av behandlingen vid hjärt-kärlsjukdom. Komponenter som utvärderades var tid till primär perkutan (endovaskulär) kranskärlsintervention (PCI), dvs ballongvidgning av kranskärl med eller utan implantation av kärlprotes (stent), och olika strategier för hämning av blodproppsbildning (trombocythämning och antikoagulation) i anslutning till PCI. De metoder som använts i avhandlingen är studier inom olika register samt design av en randomiserad klinisk prövning inom ramarna för ett register.

Det huvudsakliga registret som använts inom avhandlingsarbetet är det nationsövergripande kvalitetsregistret SWEDEHEART. I detta ingår samtliga patienter som vårdas på hjärtintensivvårdsavdelning eller genomgår PCI i Sverige. I de första arbetet analyserades förbehandling (pre-hospitalt) med läkemedlet clopidogrel jämfört med behandling först i anslutning till PCI vid hjärtinfarkt med ST-höjning på EKG (STEMI). Clopidogrel är ett s k trombocythämmande läkemedel som påverkar kroppens blodplättar och därmed förmågan att bilda blodproppar. Det vetenskapliga underlaget för prehospital behandling är att åstadkomma så tidig trombocythämning som möjligt för att optimera förhållanden vid PCI. Nackdelen med detta förfarande är risken för blödning med kraftigt blodförtunnande behandling, speciellt när diagnosen akut hjärtinfarkt inte är helt säkerställd. Studien utgjorde den största registeranalysen av denna frågeställning, och visade att pre-hospital behandling med clopidogrel minskade risken för död och hjärtinfarkt jämfört med behandling först vid PCI.

I det andra arbetet studerades en fruktad komplikation vid PCI-behandling, s k stenttrombos. De stentar som implanteras i kranskärlen efter en ballongvidning kan potentiellt stängas igen pga blodproppsbildning, framförallt under den första tiden efter PCI. Detta innbär att potent trombocythämmande behandling bör ges till samtliga patienter som erhåller stentar. Även om stenttrombos minskat betydligt på senare år är dödligheten vid akut stenttrombos hög. Flera patienter som fått PCI-behandling drabbas även av förnyade hjärtinfarkter i andra kärlområden som inte är stentade. I arbete II, erbjöds patienter delta i en studie där deras trombocytfunktion mättes, i hopp om att hitta kliniskt användbara tröskelvärden i trombocytfunktion

där risk för stenttrombos/hjärtinfarkt ökar påtagligt. Ett samband mellan dålig hämning av trombocyter och risk för stenttrombos eller annan hjärtinfarkt förelåg, men något tröskelvärde med tillräcklig grad av känslighet och precision för att möjliggöra tillämpning i klinisk vardag kunde ej identifieras.

I det tredje arbetet analyserades trombocytfunktionen hos patienter med STEMI med täta intervall det första dygnet. Sammanlagt studerades 5 olika trombocythämmande behandlingsstrategier. Effekten av trombocythämmande läkemedel var markant långsammare hos patienter med akut hjärtinfarkt jämfört med tidigare studier på friskare patienter, och var långsammare med clopidogrel än den nyare generationen av trombocythämmare (prasugrel eller ticagrelor). Hos patienter som tidigare fått clopidogrel pre-hospitalt, medförde ytterligare en dos av läkemedelena prasugrel eller ticagrelor ingen extra sänkning av trombocytfunktionen.

I det fjärde arbetet studerades effekten av pre-hospital behandling med det trombocythämmande läkemedelet ticagrelor. Detta farmaka har i tidigare randomiserade studier visat på bättre effekt än clopidogrel men även mer blödningsbiverkningar. Syftet med studien var, i likhet med arbete I, att jämföra pre-hospital behandling med behandling på sjukhuset. Förbehandling med ticagrelor vid STEMI har analyserats i en tidigare randomiserad studie där man såg mindre incidens av stenttrombos men en trend till ökad dödlighet med ticagrelor. Data från arbete IV påvisade inga skillnader i dödlighet med förbehandling. I en av 3 statistiska modeller sågs en minskad risk för ny hjärtinfarkt. Resultaten antyder att förbehandling med ticagrelor förefaller tryggt hos patienter med akut hjärtinfarkt som genomgår ballongvidning.

I det femte arbetet studerades sambandet mellan tid från första medicinska kontakt till PCI och överlevnad vid STEMI. Arbete V, som utgör den största publicerade studien av denna frågeställning i dagsläget, visade att varje 30 min fördröjning efter en timme från första medicinska kontakt var associerad med ökad dödlighet. Effekten kunde delvis förklaras av att tidsfördröjning över en timme från första medicinska kontakt var associerat med ökad risk för uttalad hjärtsvikt.

I det sista arbetet designades en randomiserad studie inom ramarna för ett register, s.k. register based randomized clinical trial (RRCT). Studien som heter, VALIDATE-studien, kommer prospektivt att inkludera 6000 patienter med hjärtinfarkt. Syftet med studien är att jämföra två intravenösa blodförtunnande läkemedel som ges i samband med PCI - vanligt heparin och trombinhämmaren bivalirudin. Båda preparaten används idag men jämförande studier mellan dessa har visat på olika resultat pga olika studiedesign. Studien utnyttjar nationella register för inklusion, randomisering och insamling av baslinjevariabler vilket reducerar den nödvändiga budgeten från åtskilliga 100-tals miljoner kronor till drygt 15 miljoner kr. I arbete VI beskrivs designen av studien med avseende på powerberäkning, inklusionskriterier, exklusionskriterier, utfallsmått, statistiska metoder och övriga delar av protokollet.

VALIDATE-studien pågår för närvarande och är nu mer än halvvägs igenom med över 3000 inkluderade patienter.

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