Improving management of STEMI patients treated with primary PCI: Pharmacotherapy, renal function estimation and gender perspective.

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Linköping 2017
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Printed in Sweden by LiU-Tryck, Linköping, Sweden, 2017

ISSN 0345-0082
To my father

To Despina, Silia and Anastasia.

“Ignoramus et ignorabimus”

(“we do not know and will not know”, “On the limits of our understanding of nature”, Emil Du Bois Reymond, 1872)

or

“wir müssen wissen wir werden wissen”

(“We must know. We will know”, David Hilbert, 1930)
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Abstract

This thesis focused on the acute management of patients with ST-segment elevation myocardial infarction (STEMI) in an effort to provide information that may improve outcome. The aim was to evaluate the efficacy and safety of bivalirudin versus unfractionated heparin (UFH) in STEMI patients during primary PCI. Furthermore, to provide pharmacodynamic data of novel ways of ticagrelor administration. Additionally, to identify subgroups of patients, such as women who may derive greater benefit from specific antithrombotic strategies due to their risk/benefit profile. Finally, to evaluate current formulas for estimation of renal function in the acute phase of STEMI, due to the importance of renal function estimation for dose adjustments of antithrombotic agents.

In Paper I, all STEMI patients in Sweden between 2008 and 2014, presenting within 12 hours from symptom onset, treated with primary PCI and UFH or bivalirudin (both with or without glycoprotein IIb/IIIa inhibitors, GPI) were included in our analysis.

Of the total population of 23 800 patients, 8783 (36.9%) were included in the UFH group and 15 017 (63.1%) in the bivalirudin group. Concomitant GPI administration was 68.5% in the UFH arm compared to 3.5% in the bivalirudin arm (p<0.01). The adjusted incidence of 30-day mortality was not significant different between the two groups (UFH vs bivalirudin, adjusted HR 0.94; 95% CI 0.82 -1.07). The adjusted risk for 1-year mortality, 30-day and 1-year stent thrombosis and re-infarction did not differ significantly between the two groups. In contrast, patients treated with UFH had a significantly higher incidence of major in-hospital bleeding (adjusted OR 1.62; 95%CI 1.30 -2.03).

In Paper II pharmacodynamic data of chewed or crushed ticagrelor compared to standard ticagrelor loading dose (LD) was assessed in 99 patients with stable angina. Platelet reactivity (PR) was assessed with VerifyNow before, 20 and 60 minutes after LD. High Residual platelet reactivity (HRPR) was defined as > 208 P2Y12 reaction units (PRU).

Chewed ticagrelor tablets resulted in significantly lower PRU values compared to crushed or integral tablets at 20 and 60 minutes. Crushed ticagrelor LD resulted in significantly lower PRU values compared to integral tablets at 20 minutes whereas no difference was observed at 60 minutes. At 20 minutes, no patients had HRPR with chewed ticagrelor compared to 68% with integral and 30% with crushed ticagrelor LD (p<0.01).

In Paper III we presented a pre-specified gender analysis of the ATLANTIC trial including 1 862 STEMI patients that were randomly assigned to pre-hospital versus in-hospital administration of 180mg ticagrelor.

Women were older and had higher TIMI risk score. Women had a 3-fold higher risk for all-cause mortality compared to men (5.7% vs 1.9%, HR 3.13, 95% CI 1.78 – 5.51). However, after adjustment for baseline characteristics, the difference was lesser and no longer significant (HR 1.98, 95% CI 0.97 – 4.04). Female gender was not an independent predictor of risk for bleeding after multivariable adjustments (BARC type 3-5 HR 1.52, 95% CI 0.74-3.09). There was no interaction between gender and efficacy or safety of randomised treatment.

In Paper IV, forty patients with PCI- treated STEMI were included between November
Abstract

2011 and February 2013. We validated the performance of the Cockcroft-Gault (CG), the Modification of Diet in Renal Disease (MDRD-IDMS), the Chronic Kidney Disease Epidemiology (CKD-EPI) and the Grubb relative cystatin C (rG-CystC) equations for estimation of GFR against measured GFR (mGFR) during the index hospitalisation for STEMI. MDRD-IDMS and CKD-EPI demonstrated a good performance to estimate GFR with accuracy within 30% (P30) 82.5% vs 82.5%, respectively. CKD was best classified by CKD-EPI (Kappa 0.83). CG showed the worst performance with the lowest P30. The rG-CystC equation had a marked bias of -17.8% and significantly underestimated mGFR (p=0.03).

Conclusions – In STEMI patients treated with primary PCI, bivalirudin should be preferred in patients at high risk for bleeding. With crushed or chewed ticagrelor tablets a more rapid platelet inhibition may be achieved, compared with standard integral tablets. In STEMI patients, fast and potent platelet inhibition with chewed ticagrelor may reduce the risk of early stent thrombosis and patients treated with a less aggressive antithrombotic strategy, such as UFH or bivalirudin monotherapy, may derive a greater benefit. Although gender differences in adverse outcomes could mainly be explained by older age and clustering of comorbidities in women, a bleed-reduction strategy in women with high risk characteristics is warranted in order to improve their outcome. Regardless the choice of antithrombotic strategy, dose adjustment of drugs cleared by kidneys based on GFR estimation is of crucial importance. MDRD and CKD-EPI should be the formulas used for estimation of GFR in STEMI patients.
LIST OF PAPERS

**Paper I**
D. Venetsanos, S. Sederholm Lawesson, Stefan James, Sasha Koul, David Erlinge, E Swahn, J. Alfredsson

*Bivalirudin versus heparin with or without GP IIb/IIIa inhibitors in ST-elevation myocardial infarction patients treated with primary PCI. A report from the SWEDEHEART registry.*
Submitted

**Paper II**
D. Venetsanos, S. Sederholm Lawesson, E. Swahn, J. Alfredsson

*Chewed ticagrelor tablets provide faster platelet inhibition compared to integral tablets.*

The Inhibition of Platelet Aggregation after Administration of three Different Ticagrelor formulations (IPAAD-Tica) Study, a randomised controlled trial. Thrombosis research. 2017 Jan; 149:88-94.

**Paper III**

*Association between gender and short term outcome in STEMI patients planned for primary PCI and treated with novel antiplatelet therapy.*

A pre-specified gender analysis of the Administration of Ticagrelor in the Cath Lad or in the Ambulance for New ST Elevation Myocardial Infarction to Open the Coronary artery (ATLANTIC) trial – a multicenter, randomised, placebo-control study.
Submitted

**Paper IV**
Dimitrios Venetsanos, MD, Joakim Alfredsson, MD, PhD, Mårten Segelmark, MD, PhD, Eva Swahn, MD, PhD, Sofia Lawesson, MD, PhD

*Glomerular Filtration Rate (GFR) during and after STEMI – A single-centre, methodological study comparing estimated and measured GFR.*

BMJ open. 2015 Sep 23; 5(9):e007835.
Kranskärlssjukdom förorsakar ca 25 % av alla dödsfall i Sverige och är en vanligare dödsorsak än alla cancersjukdomar. Dessutom är det en av de vanligaste orsakerna till läkemedelsanvändning, sjukhusinläggningar, sjukbidrag och förtidspension. Akuta manifestationer av kranskärlssjukdom består av ST-höjningsinfarkt (STEMI), icke-ST-höjningsinfarkt (NSTEMI) och instabil kärlkramp. Den underliggande orsaken är oftast en bristning i ett åderförfettningssystem i något av hjärtats kranskärl, följt av en pålagrad blodpropp (trombos) som förtränger blodflödet till den del av hjärtmuskeln som försörjs av det kärl som ledde till en hjärtmuskelskada på grund av blodbrist (hjärtinfarkt).

Vid STEMI är orsaken så gott som alltid att trombosen orsakat en total igentäppning av kranskärellet. Därför är den akuta behandlingen inriktad på att omedelbart öppna det tilltäppta kranskärlet (reperfusion) och återställa det normala blodflödet för att begränsa utbreddningen av infarkten. Detta kan åstadkommas med antingen kateterburet krankärlsinsprengrepp (PCI) eller läkemedel som kan lösa upp proppen (trombolys). De senaste åren har studier visat att omedelbar behandling med PCI plus inläggning av ett ställdräsmätt (stent) förbättrar överlevnad och minskar risken för allvarliga blödningsjämfört med trombolys. Därför har PCI blivit den dominerade behandlingen av STEMI i Sverige. Blodförtunnande behandling som ges i injektion direkt i blodet eller i tabletform under PCI hämmar fortsatt trombosutveckling och minskar risken för återbildning av proppar i framtiden men ökar samtidigt risken för blödningar.

Denna avhandling fokuserar på STEMI patienter och deras akuta omhändertagande och farmakologiska behandling. I arbete I jämförde vi effektivitet och säkerhet hos de två vanligaste blodförtunnande läkemedlen som ges under PCI ingreppet, bivalirudin och heparin med eller utan glycoprotein IIb/IIIa hämmare (GPI) i båda grupper. Vi använde data från det nationella hjärt-registret (SWEDEHEART). Studien visade att behandling med bivalirudin ± GPI gav färre blödningskomplikationer under vårdtillfället jämfört med heparin ± GPI. Dödlighet, återinsjuknande i hjärtinfarkt och stent trombos skiljde sig inte mellan grupperna vare sig vid kort eller lång uppföljning.

I arbete II testade vi och studerade farmakodynamiken av två nya sätt att ge ticagrelor, en stark och snabbverkande blodförtunnande tabletträtt. Patienterna lottades slumptillfället till engångs behandling med 180 mg tuggade, krossade eller hela ticagrelor tabletter. Tuggad och krossad ticagrelor gav en snabbare och effektivare blockering av blodplättarna jämfört med hel tablet 30 minuter efter intag av tabletterna. Dessutom, visade sig tuggade ticagrelor-tabletter vara effektivare än krossade ticagrelor-tabletter i hämning av blodplättar.

I arbete III använde vi data från den internationella, randomiserade studien som kallas ATLANTIC. Studien inkluderade patienter med STEMI och jämförde effektivitet och säkerhet av ticagrelor-behandling som påbörjades i ambulansen jämfört med start av behandlingen på sjukhuset strax före PCI. Vårt syfte var att se om könsskillnader i dödlighet och blödningskomplikationer existerar och om behandling med ticagrelor är lika effektiv och säker hos båda könen. Vår studie visade att kvinnor hade betydligt högre risk för död och blödningsinom 30 dagar efter STEMI men dessa skillnader kunde förklaras av åldersskillnaden mellan män och kvinnor och högre samtidig förekomst av andra sjukdomar hos kvinnorna. Behandling med ticagrelor i ambulansen var lika säkert hos båda könen.
I den sista studien, arbete IV, utvärderade vi de fyra vanligaste ekvationerna som används för att beräkna njurfunktion (eGFR), i förhållande till mätt njurfunktion hos STEMI patienter. Njurfunktionen är mycket viktig för att kunna justera dosen av blodförtunnande läkemedel, t.ex. bivalirudin, GPI eller heparin. Överdosering av dessa läkemedel ökar kraftigt risken för blödningar. Våra resultat visade att ”MDRD-IDMS” och ”CKD-EPI” ekvationerna presterade mycket bra och bör företrädesvis användas i klinisk praxis medan CG-ekvationen visade sig vara särskilt på att bedöma njurfunktionen och borde därmed inte användas. Ett intressant fynd var att cystatin C steg kraftigt under vårdtillfälle och ledde till en felaktig bedömning av njurfunktionen när cystatin C ekvation användes.

**Sammanfattning**

ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ACC/AHA</td>
<td>American College of Cardiology/American Heart Association</td>
</tr>
<tr>
<td>ACE-I</td>
<td>Angiotensin Converting Enzyme Inhibitors</td>
</tr>
<tr>
<td>ACS</td>
<td>Acute Coronary Syndrome</td>
</tr>
<tr>
<td>ADP</td>
<td>Adenosine Diphosphate Receptor</td>
</tr>
<tr>
<td>AF</td>
<td>Atrial Fibrillation</td>
</tr>
<tr>
<td>BARC</td>
<td>Bleeding Academic Research Consortium</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>CAD</td>
<td>Coronary Artery Disease</td>
</tr>
<tr>
<td>CCU</td>
<td>Coronary Care Unit</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence Interval</td>
</tr>
<tr>
<td>CKD</td>
<td>Chronic Kidney Disease</td>
</tr>
<tr>
<td>CKD-EPI</td>
<td>Chronic Kidney Disease-Epidemiology Collaboration</td>
</tr>
<tr>
<td>COPD</td>
<td>Chronic Obstructive Pulmonary Disease</td>
</tr>
<tr>
<td>DAPT</td>
<td>Double Antiplatelet Therapy</td>
</tr>
<tr>
<td>DES</td>
<td>Drug Eluting Stent</td>
</tr>
<tr>
<td>DM</td>
<td>Diabetes Mellitus</td>
</tr>
<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
</tr>
<tr>
<td>eGFR</td>
<td>Estimated Glomerular Filtration Rate (mGFR)</td>
</tr>
<tr>
<td>GPI</td>
<td>Glycoprotein IIb/IIIa receptor Inhibitors</td>
</tr>
<tr>
<td>GPIb</td>
<td>Glycoprotein Ib</td>
</tr>
<tr>
<td>GPIIb/IIIa</td>
<td>Glycoprotein IIb/IIIa receptors</td>
</tr>
<tr>
<td>HF</td>
<td>Heart Failure</td>
</tr>
<tr>
<td>HR</td>
<td>Hazard Ratio</td>
</tr>
<tr>
<td>HRPR</td>
<td>High Residual Platelet Reactivity</td>
</tr>
<tr>
<td>IHD</td>
<td>Ischemic Heart Disease</td>
</tr>
<tr>
<td>IRA</td>
<td>Infarct Related Artery</td>
</tr>
<tr>
<td>LD</td>
<td>Loading Dose</td>
</tr>
<tr>
<td>MACE</td>
<td>Major Adverse Cardiovascular Events</td>
</tr>
<tr>
<td>MDRD-IDMS</td>
<td>Modification of Diet in Renal Disease</td>
</tr>
<tr>
<td>mGFR</td>
<td>Measured Glomerular Filtration Rate</td>
</tr>
<tr>
<td>MI</td>
<td>Myocardial Infarction</td>
</tr>
<tr>
<td>MVD</td>
<td>Multivessel Disease</td>
</tr>
<tr>
<td>NACE</td>
<td>Net Adverse Clinical Event</td>
</tr>
<tr>
<td>NPR</td>
<td>National Patient Registry</td>
</tr>
<tr>
<td>NSTE ACS</td>
<td>Non-ST Segment Elevation Acute Coronary Syndromes</td>
</tr>
<tr>
<td>NSTEMI</td>
<td>Non-ST Segment Elevation Myocardial Infarction</td>
</tr>
<tr>
<td>OCT</td>
<td>Optical Coherence Tomography</td>
</tr>
<tr>
<td>OR</td>
<td>Odds Ratio</td>
</tr>
<tr>
<td>PPCI</td>
<td>Primary Percutaneous Coronary Intervention</td>
</tr>
<tr>
<td>PR</td>
<td>Platelet Reactivity</td>
</tr>
<tr>
<td>rG-CystC</td>
<td>relative Grubb cystatin C</td>
</tr>
<tr>
<td>RR</td>
<td>Relative Risk</td>
</tr>
<tr>
<td>SAP</td>
<td>Stable Angina Pectoris</td>
</tr>
<tr>
<td>STEMI</td>
<td>ST segment Elevation Myocardial Infarction</td>
</tr>
<tr>
<td>SWEDEHEART</td>
<td>Swedish Web-system for Enhancement and Development of</td>
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**Abbreviations**

Evidence-based care in Heart Disease Evaluated According to Recommended Therapies

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>TCFA</td>
<td>Thin-Cap Fibroatheroma</td>
</tr>
<tr>
<td>TLR</td>
<td>Target Lesions Revascularisation</td>
</tr>
<tr>
<td>UA</td>
<td>Unstable Angina</td>
</tr>
<tr>
<td>UFH</td>
<td>Unfractionated Heparin</td>
</tr>
<tr>
<td>vWF</td>
<td>Von Willebrand factor</td>
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INTRODUCTION

The term acute coronary syndrome (ACS) includes the three different types of acute clinical manifestations of ischemic heart disease (IHD), i.e. unstable angina (UA), non-ST segment elevation myocardial infarction (NSTEMI) and ST segment elevation myocardial infarction (STEMI). These clinical manifestations share a common pathophysiologic mechanism but have different diagnostic criteria and management. Symptoms suggesting myocardial ischemia and ST segment elevation on the electrocardiogram (ECG), obtained soon after the first medical contact, set the diagnosis of STEMI and mandate immediate reperfusion therapy. When the diagnosis of STEMI has been excluded, clinical assessment of symptoms, repeated ECGs and analysis of cardiac biomarkers, preferably high sensitive troponin, are necessary to rule out or to confirm the diagnosis of an ACS.\textsuperscript{1,2}

The incidence rate of STEMI has slightly declined over the last decades, but has remained stable the last years, and a concomitant rise in the incidence rates of NSTEMI has been observed. STEMI still represents around 30\% of patients with ACS with 66 hospital admissions/100,000 inhabitants/year in Sweden.\textsuperscript{3,4} Coronary artery disease (CAD) remains the leading cause of death, worldwide, and accounts for 12.8\% of all deaths.\textsuperscript{5} In STEMI patients, age, time delay to treatment, mode of reperfusion therapy, history of prior myocardial infarction (MI), diabetes mellitus (DM), chronic kidney disease (CKD) and number of diseased coronary arteries, are some of the most powerful predictors of adverse outcome. Female gender has also emerged as an independent predictor of early mortality and bleeding complications.\textsuperscript{6,7}

The management of patients with ACS has undergone dramatic changes the last three decades. At the same time, a significant decrease of IHD mortality in older patients and to a lesser extent in subgroups such as young women has been observed.\textsuperscript{8} However, mortality remains substantial with approximately 6\% of STEMI patients dying within the first 6 months after the index event. Therefore, additional efforts to optimise the management of STEMI patients are needed.

This thesis focuses on the acute management of STEMI patients in an effort to improve outcome and potentially lead to an individualised treatment based on patients risk profile. Especially, this thesis will focus on 1) the pharmacologic therapy during primary percutaneous coronary intervention (PPCI) 2) the effectiveness and safety of current treatment in both genders as well as on the potential importance of gender as a predictor of outcome and 3) methods to estimate renal function in STEMI patients during the index hospitalisation.

Background

Pathogenesis of Acute Coronary Syndromes

In Greek, \emph{Athére} means gruel or porridge and refers to the lipid-rich core, and \emph{scleros} means hard, which describes the fibrotic and often calcified encapsulating tissue. In 1786, Edward Jenner proposed atherosclerosis in coronary vessels as the cause of angina pectoris, a disease described some years before by William Heberden. It took
many years before Constantinides, Chapman, and Friedman described the mechanism linking coronary atherosclerosis to myocardial infarction. They found that plaque rupture exposing thrombogenic plaque components to the blood flow led to thrombus formation in the coronary vessels and to myocardial infarction.\(^9,10\) Furthermore, non-ruptured plaques with surface irregularities may have superimposed thrombus, that was later recognised as plaque erosion and as an alternative mechanism to myocardial infarction.\(^11\)

It is now well recognised that ACS is caused by coronary artery thrombosis. Thrombus is usually occlusive and sustained in STEMI, leading to transmural ischemia and typical ECG changes whereas it is non-occlusive, dynamic or even absent in UA and NSTEMI.\(^12\) Rupture of an atherosclerotic plaque remains the main cause of coronary thrombosis and occurs in the presence of a thin fibrous cap, abundant inflammatory cells and a large lipid core in the plaque. These observations led to the definition of the vulnerable plaque or thin-cap fibroatheroma (TCFA) which can thus be assumed to encompass the majority of plaques at risk for rupture.\(^13\) Although the absence of a TCFA in a patient indicates a low imminent risk for plaque rupture and thrombosis, the presence of a TCFA does not inevitably lead to plaque rupture and to a thrombotic event. Studies using intravascular imaging, intravascular ultrasound or optical coherence tomography (OCT) have clearly shown that only 5% of TCFA caused coronary events over a 3.4 years follow-up, weaken the rationale for an interventional targeted therapeutic approach to rupture-prone plaques.\(^14,15\) Additionally, a significant proportion of thrombotic lesions found on autopsy are not associated with a plaque rupture.\(^13\) Superficial erosion and calcified nodules have emerged as underlying mechanism of ACS with increasing frequency.\(^16\) Lesions underlying superficial erosions differ in their histological characteristics compared to lesions associated with plaque rupture (figure 1). They lack thin cap, large lipid pool and inflammatory cells but accumulate abundant extracellular matrix, notably proteoglycans and glycosaminoglycans.\(^17\)

**Figure 1.** Contrasts between superficial erosion and fibrous cap rupture as causes of arterial thrombosis. LDL, low-density lipoprotein (Libby, Requiem for the ‘vulnerable plaque’, European Heart Journal (2015) 36, 2984–2987. Reprinted with permission)
The mechanism leading to thrombus formation without rupture remains an unsolved question and different hypotheses have been provided, e.g. vasospasm that may be a cause of the endothelial damage and subsequent thrombosis. Calcified nodules are pathologically defined as the presence of fracture of a calcified plate, interspersed fibrin, and a disrupted fibrous cap with an overlying thrombus and are the less common identifiable mechanism of coronary thrombosis with an incidence of around 8% (figure 2, 3 and figure 4).

Figure 2. Plaque rupture is identified on cross-sectional optical coherence tomography (OCT) images by the disrupted fibrous-cap (arrowheads) and a cavity (*) formation inside the plaque (Haibo Jia, J Am Coll Cardiol 2013; 62:1748–58. Reprinted with permission from Elsevier).

Figure 3. Superficial erosion. Serial optical coherence tomography (OCT) cross-sectional images from proximal to distal of the culprit lesion indicate that no rupture is detected. Cross-sectional images indicate fibrous plaque (homogeneous high signal region) proximal (A) and distal (D) to thrombus. OCT-erosion is identified as an irregular lumen surface with attached mural thrombus (arrows) overlying a fibrous plaque (B and C) (Haibo Jia, J Am Coll Cardiol 2013; 62:1748–58. Reprinted with permission from Elsevier).

The increasing frequency of superficial erosions as the underlying mechanism of ACS may be explained by the increasing use of statin treatment and less active smoking that may lead to stabilisation of vulnerable plaques and reduce their risk for rupture. Erosions are more frequently associated with NSTEMI whereas plaque rupture is more common in STEMI. The increasing frequency of plaque erosions may have contributed to the increasing frequency of NSTEMI. Noteworthy, plaque rupture is particularly infrequent in premenopausal young women whereas superficial erosions occur more frequently in women, in diabetics and the elderly.
Background

Figure 4 Optical coherence tomography (OCT)-calcified nodule is identified as a nodular calcification (A) protruding into lumen through a disrupted fibrous cap (arrowheads) overlying superficial calcification with red thrombus (B and C, arrows) attached to the disrupted site. *Guidewire (Haibo Jia, J Am Coll Cardiol 2013; 62:1748–58. Reprinted with permission from Elsevier).

Thrombosis – the fundamental role of platelets and coagulation system

The thrombotic response following the plaque rupture or erosion varies considerably and may range from a small mural thrombus sealing the plaque to a large occlusive thrombus causing a STEMI. Determinants of the thrombotic magnitude are probably those of the classic triad of Virchow: 1) thrombogenicity of the exposed plaque material; 2) local flow disturbances due to coronary stenosis; and 3) systemic thrombotic propensity. In the setting of plaque rupture, thrombogenicity of the exposed plaque material is probably the main determinant. Collagen from the cap and the thrombogenic lipid-rich core are exposed to blood flow and initiate the thrombotic process.20 In the case of plaque erosion, endothelial denudation is a weak thrombogenic stimulus. Therefore flow disturbances and systemic thrombotic factors such as platelet hyperaggregability and hypercoagulability or depressed fibrinolysis may play the most important role.21, 22

Normally, platelets do not interact with other cells or the intact vessel wall. In case of vessel injury a cascade of biochemical and cellular processes leads to thrombus formation that, depending on the initiating event, may represent protective hemostasis or an ACS. This process can be divided into five steps: platelet translocation, activation, secretion, adhesion and aggregation (figure 5).
The initial adhesion of platelets (tethering, A) is mediated by the binding of the glycoprotein (GP) Ib-V-IX receptor complex to the A1 domain of the von Willebrand factor (VWF) on endothelial cells. Additionally, binding to P-Selectin can enhance platelet recruitment to the intact vessel wall. In a second step (B) interactions between GPVI and collagen stabilise the thrombus. Moreover, translocation is followed by platelet activation, mainly initiated by collagen and thrombin, that produces a platelet monolayer that promote further thrombin generation and adhesion of new platelets. Platelet activation prompts secretion of platelet storage granules that leads to the release of ADP, thrombin and other activating factors. Through this sustaining autocrine circuit further platelet activation is achieved. The two final steps towards stable thrombus formation and growth are adhesion and aggregation. Binding of fibrinogen and VWF via activated GPIIb/IIIa receptors are the key components of this process (figure 6).
Despite the crucial role of platelets in thrombosis, the importance of the coagulation system should not be overlooked (figure 7). Exposure of tissue factor activates coagulation cascade that leads to a burst of thrombin generation. Thrombin has a critical role by converting soluble fibrinogen into a network of fibrin, by activating platelets through PAR receptors and contributing to vessel constriction.

Figure 7. The cell-based model of coagulation highlights the initiation of events on tissue-factor bearing cells, followed by an amplification step wherein events transition to activated platelets. The propagation state is characterised by a burst of thrombin generation. (From Hoffman M: A cell-based model of hemostasis. Thromb Haemost 2001; 85:958-965. Reprinted with permission)
Management of STEMI patients - Timely reperfusion therapy

Patients with STEMI usually have an occluded coronary artery. Therefore reperfusion therapy in order to restore patency and to reduce infarct size is the cornerstone of the management. Timely diagnosis and reperfusion are key components since the greatest benefit gained from reperfusion therapy occurs within 2-3 hours from symptom onset. In order to reduce time between symptom onset and provision of reperfusion therapy and improve outcome, organisation of local networks including ambulances, referral hospital or emergency departments and the receiving hospital is crucial. Still, one third of STEMI patients do not receive reperfusion therapy within 2 hours after the first medical contact. Early diagnosis, in a prehospital setting, triage and initiation of pharmacologic reperfusion therapy or transfer to a PCI capable center have been shown to reduce delays and improve clinical outcomes.

Two main reperfusion therapies exist, fibrinolytic therapy and primary PCI. Fibrinolytic therapy was a major advance in the treatment of STEMI. In the late 1980s thrombolytic therapy with streptokinase significantly reduced mortality compared to placebo. Results were further improved when recombinant tissue-type plasminogen activator, such as alteplase, was used. The main benefit was observed when thrombolytic therapy was administrated within 2-3 hours after symptom onset and diminished with time. Concerns about risk for bleeding complications, mainly intracranial bleeding and absolute or relative contraindications to treatment, resulted in relatively low rates of provided reperfusion therapy in STEMI.

In 1974, at the Medical Policlinic of the University of Zürich, Andreas Grünzig (1939–1985) for the first time used a balloon-tipped catheter to re-open a severely stenosed femoral artery, a procedure, which he called “percutaneous transluminal dilatation”. In 1977, Dr. Grünzig performed the first coronary angioplasty on a 38-year-old man suffering from angina due to a stenosis in the LAD. Balloon angioplasty became one of the most successful examples of translational medicine in the twentieth century for which Grünzig and Charles T. Dotter (1920–1985) received a nomination for the Nobel Prize in Physiology or Medicine in 1978. Since then PCI has undergone continued advances and has become one of the most frequently performed therapeutic interventions. Primary PCI is defined as PCI in the setting of STEMI, without previous fibrinolysis. Compared to fibrinolysis, reperfusion with PPCI significantly reduced the risk for mortality, re-infarction and intracranial bleeding. These results led to an international shift from fibrinolytic therapy to PPCI and an overall increase in reperfusion rates. PPCI is the preferred reperfusion strategy in STEMI patients and is used in Sweden in more than 80% of patients under the age of 80. Thrombolytic therapy still remains an option due to limited availability of timely PPCI in some areas. Only 5% of STEMI patients are treated with fibrinolytic therapy in Sweden. (figure 8)
Anticoagulation and antiplatelet therapy in STEMI patients during PPCI

STEMI represents a highly thrombotic state and therefore adjunctive antithrombotic therapy as a complement to PPCI is of crucial importance. The ideal antithrombotic therapy should reduce the risk for ischemic complications such as stent thrombosis and distal embolisation and at the same time minimise the risk of bleeding events, providing a net clinical benefit for the patient.

The glycoprotein IIb/IIIa receptor inhibitors (GPI) are potent antiplatelet agents that after intravenous administration, rapidly inhibit platelet aggregation and thrombus formation and may dissolve fresh thrombus already formed (figure 6). 37 For many years, unfractionated heparin (UFH) with upfront or peri-procedural administration of GPI during PPCI was the standard treatment, as early reports showed a significant reduction in mortality. 38, 39 However, in the era of potent P2Y12 receptor blockers including high loading dose of clopidogrel the role of adjunctive GPI administration is controversial. The ON-TIME-2 trial showed a lower incidence of 30 day death, recurrent MI or urgent vessel revascularisation with pre-hospital administration of tirofiban compared with placebo (5.8% vs 8.6%, p=0.043) without a significant increase in bleeding (3.4% vs 2.9%, p=0.58). 40 Conversely, the ASSIST trial and the BRAVE 3 showed no benefit with routine eptifibatide or upstream abciximab, respectively. 41, 42 A significantly higher risk for major or minor bleeding was observed in the ASSIST trial but not in BRAVE. The randomised FINESSE trial included almost 2 500 STEMI patients presented within 6 hours from symptom onset and compared the efficacy and safety of facilitated PPCI with upfront administration of abciximab, abciximab plus
reteplase versus PPCI only. Although the combination of abciximab and reteplase was associated with significantly higher rates of ST-segment resolution, the primary outcome, a combination of death, ventricular fibrillation, cardiogenic shock and congestive heart failure, did not significantly differ between the three groups. The rate of bleeding, intracranial haemorrhage and transfusion was significantly higher in the facilitated PPCI groups. However, a sub study showed a benefit in the primary outcome in high risk patients.

The HORIZON-AMI trial, published almost 10 years ago, was a landmark trial comparing the efficacy and safety of UFH + routine GPI versus bivalirudin monotherapy with bailout administration of GPI in 3,602 STEMI patients. At 30 days and 1 year the rate of net adverse clinical outcome (NACE) (death, re-infarction, target lesion revascularisation (TLR), stroke or major bleeding) as well as the individual endpoint of cardiac mortality and major bleeding were significantly lower with bivalirudin (30 day NACE rate 9.2% versus 12.1% with UFH+GPI, RR 0.76, p<0.01). While there was no difference in major adverse cardiovascular events (MACE), the superiority in NACE was mainly driven by a reduction in major bleeding (4.9% versus 8.3%, p=0.01), cardiac mortality (1.8% versus 2.9%, p=0.03) and all-cause mortality (2.1% versus 3.1%, p=0.046). Notably, a significantly higher risk of acute stent thrombosis was noted with bivalirudin (1.3% versus 0.3%, p<0.01). The benefits were sustained and continued to increase through 3 years follow-up. This study caused an international shift in the antithrombotic strategy during PPCI towards increased bivalirudin use (figure 9).

Figure 9. Anticoagulation and proportion of GPI and for bivalirudin before/during PPCI in STEMI patients in Sweden, 2006-2015 (SWEDEHEART annual report 2015).

In the era of potent P2Y12 receptor blockers and early mechanical reperfusion, GPI is no longer assumed as a frontline therapy but only as an adjunctive therapy in selected cases. This is reflected by current recommendations for GPI use from the European society of cardiology and ACC/AHA guidelines. GPI use is mainly recommended as bailout treatment in patients with giant thrombus, no reflow or slow flow and as upstream treatment in high risk patients undergoing transfer for primary PCI. Recent trials comparing different antithrombotic strategies in STEMI patients have reported very low rates of GPI use, when GPI was restricted to bailout use by the study protocol but considerably higher rates when the decision was left to the operators’ discretion. However, GPs may still have an important role in our armamentarium of contemporary PCI and the optimal rate of administration certainly depends on the population studied.
The initial enthusiasm for bivalirudin following HORIZON-AMI trial, has been dampened by subsequent studies comparing bivalirudin with UFH and provisional or bailout GPI showing conflicting results. The HEAT-PCI, was a single centre trial of 1 812 patients randomised to UFH versus bivalirudin monotherapy without post-PCI infusion. This trial reflected current PCI techniques, with high rate of DES implantation (80%) and radial access (81%) Furthermore novel P2Y12 blockers were used in 89% of the patients. The GPI use was 15% in both groups. In contrast with previous studies, the composite outcome of death, re-infarction, stroke or unplanned TLR was higher in the bivalirudin group (8.7% versus 5.7%, p=0.01) mainly driven by higher re-infarction and TLR, where both were attributed to the higher rate of stent thrombosis in the bivalirudin arm (2.9% versus 0.9%, p<0.01). The incidence of major bleeding, according to Bleeding Academic Research Consortium (BARC) definition, did not significantly vary between the two groups (3.5% versus 3.1%, p=0.59). The study was criticised for the single centre design and the activated clotting time monitoring system that was utilised and generating the hypothesis of potential under dosing of bivalirudin used. Following this study the BRIGHT study included patients with ACS (88% STEMI) randomly assigned to three different treatment strategies: bivalirudin monotherapy (n=735), UFH monotherapy (n=729) and UFH plus tirofiban (n=730). Compared to HEAT-PCI trial, the UFH dose was higher in the UFH-monotherapy arm (100U/kg versus 70U/kg) but the GPI use was lower (only 5%). Clopidogrel was used and bivalirudin infusion was continued after PCI. Bivalirudin was associated with a significantly lower rate of BARC major bleeding compared to UFH monotherapy or UFH+ tirofiban (0.5% versus 1.5% versus 2.1%). The incidence of death, myocardial infarction or TLR did not differ between the three groups whereas the incidence of stent thrombosis was very low (0.3%) in all the groups. The most recent MATRIX trial included 7 000 ACS patients (56% STEMI) and compared radial versus femoral access and bivalirudin versus UFH and provisional GPI administration. The use of GPI significantly differed between the two groups and was 4.6% with bivalirudin and 25.8% with UFH. Bivalirudin use was associated with significantly lower incidence of BARC major bleeding (1.4% versus 2.5%) and all-cause mortality (1.7% versus 2.3%) whereas the rate of stent thrombosis was significantly higher in the bivalirudin group (1.0% versus 0.6%).

Oral antiplatelet therapy in STEMI patients

In STEMI patients, dual antiplatelet therapy with aspirin and an adenosine diphosphate receptor (ADP) antagonist at the time of first medical contact is highly recommended (Ib recommendation according to ESC guidelines on myocardial revascularisation) to prevent ischemic complications. Clopidogrel, a thienopyridine ADP receptor blocker was the drug of choice in ACS care for more than a decade. However, clopidogrel has some important limitations: a slow onset of action due to the need of metabolism to produce the active thienopyridine metabolites, mild potency and large inter-individual variability in response. A 75-mg o.d. clopidogrel maintenance dose requires at least 5 days and a 600-mg loading dose (LD) of clopidogrel requires up to 8 hours to achieve around 50% steady state of inhibition of ADP-induced platelet aggregation in stable patients. Furthermore, approximately 15- 30% of patients have been reported to be nonresponsive. Of importance, several studies have highlighted a link between high residual platelet reactivity (HRPR) or suboptimal inhibition of platelet reactivity (PR), as measured by platelet assays after a clopidogrel
Background

LD, and the occurrence of thrombotic events after PCI. Those limitations could only partially be overcome by high LD or maintenance dose of clopidogrel that improves pharmacodynamics properties of the drug. In the pre-specified analysis of CURRENT-OASIS 7 trial including 17,263 individuals with ACS who underwent PCI, high LD clopidogrel (600mg) was associated with a significant reduction in cardiovascular events and stent thrombosis compared to standard LD. The rate of definite or probable stent thrombosis was 31% lower and definite stent thrombosis was 46% lower with double dose clopidogrel. CURRENT-defined major bleeding was more common with high LD than with the standard dose clopidogrel but the incidence of TIMI-major bleeding was similar between the two groups. Furthermore, upstream administration of clopidogrel prior to arrival at the catheterisation laboratory compared to administration after coronary angiography have shown to significantly reduce the risk of re-infarction and stent thrombosis and improved survival.

Ticagrelor is a direct acting and reversibly binding P2Y12 receptor inhibitor that is highly recommended in clinical guidelines for treatment of patients with ACS. In patients with stable angina pectoris (SAP), administration of 180 mg LD of ticagrelor resulted in a more rapid and stronger inhibition of PR compared to clopidogrel. Within 30 minutes, ticagrelor administration led to the same degree of inhibition of PR as that achieved 8 hours after a 600 mg LD of clopidogrel. In the PLATO trial, including 18,624 patients with ACS, ticagrelor as compared with clopidogrel was associated with a reduction of cardiovascular death (4% versus 5.1%, p<0.01) and MI (5.8% versus 6.9%, p=0.01) without increasing the rate of overall major bleeding but with an increase of non-CABG-related major bleeding (4.5% versus 3.8%, p=0.03). In the pre-specified analysis of the PLATO trial in patients with STEMI, a significant reduction of definite stent thrombosis was observed (HR 0.58; 95% CI 0.37-0.89) without any increase in the incidence of major bleeding events. The faster and more potent platelet inhibition with ticagrelor compared to clopidogrel is the main explanation for the lower occurrence of ischemic events observed in the PLATO trial. The last years, ticagrelor has become the main oral antiplatelet therapy for treatment of patients with ACS in Sweden. About 90% of STEMI patients are treated with ticagrelor (figure 10).

Figure 10. Trend in the use of P2Y12 receptor blockers at discharge, in patients with STEMI <80 years in Sweden (SWEDEHEART annual report 2015).
Despite the predictable pharmacodynamics properties of ticagrelor in patients with SAP, in patients with STEMI, where fast and effective platelet inhibition is even more important, a delayed onset of action of platelet inhibitors and a wider variability of drug response have been demonstrated.70-72 Beside the higher baseline PR in STEMI patients, a limited or delayed intestinal absorption of orally administered drugs is another major contributor to this observation.71,72 More than half of the STEMI patients treated with LD of integral ticagrelor still have HRPR up to 4 hours after administration of the drug71 and are at increased risk of ischemic complications.73 In the ATLANTIC trial, despite a short median time between pre-hospital and in-hospital administration of ticagrelor, prehospital administration significantly reduced the risk of stent thrombosis, suggesting that fast and strong platelet inhibition at the time of PCI is clinically important.74 Previous pharmacokinetic studies have demonstrated that chewable aspirin and crushed clopidogrel administration increased the rate of drug absorption compared to integral tablets, when administered orally.75,76 Recently, crushed ticagrelor tablets, administered orally or via a naso-gastric tube, has been shown to be feasible and resulted in increased plasma concentration of ticagrelor and its active metabolite at an earlier time point compared to integral tablets.77,78 As the plasma concentration of ticagrelor and its active metabolite is linearly associated with the degree of platelet inhibition, administration of crushed or chewed ticagrelor may provide a more rapid onset of drug action. The MOJITO trial showed that crushed ticagrelor tablet administration in STEMI patients is feasible and provides faster platelet inhibition compared with standard integral tablets.79 However, data about the pharmacodynamics properties of novel ways of ticagrelor administration are limited.

Bleeding events – the Achilles heel of invasive management and antithrombotic treatment

Advances in the care, increasingly efficacious antithrombotic therapy and early invasive management have led to a significant improvement in outcomes in patients with ACS.8 However, the anti-ischemic benefit obtained with a more aggressive antithrombotic therapy has been associated with a concomitant risk for bleeding and blood transfusion. The addition of clopidogrel to aspirin for the treatment of ACS in the CURE trial was associated with reductions in ischemic events but at the expense of an increased risk of bleeding (3.7% versus 2.7%, RR, 1.38; p =0.001).80 Compared to clopidogrel, the use of more potent antiplatelet therapy such as prasugrel or ticagrelor resulted in significant improvement in ischemic outcomes in the TRITON—TIMI 38 and PLATO studies.68,81 That improvement came at a cost, as prasugrel increased major bleeding (HR 1.32; 95% CI 1.03 – 1.68, p=0.01) and life-threatening bleeding (1.4% versus 0.9%, p=0.01). Similarly, but to a less extent, ticagrelor increased the risk of non-CABG major bleeding compared to clopidogrel in the PLATO trial. In a meta-analysis enrolling 31 402 patients with ACS, treatment with GPI significantly reduced the occurrence of death and myocardial infarction (10.8% versus 11.8%, p=0.02) and the greatest benefit was observed in patients at high risk for thrombotic complications.82 However, major bleeding complications were significantly increased with GPI (2.4% versus 1.4%, p<0.01).

Recent analyses and clinical trials have clearly shown a strong independent association between bleeding complications, blood transfusion and poor outcomes in patients with ACS (figure 11).83-87 Given the high efficacy of the current antithrombotic treatment to
reduce ischemic complications, strategies reducing the risk of bleeding have the potential to further improve outcome in patients with ACS.

**Figure 11.** Independent predictors of mortality in patients with ACS (Manoukian et al. Major Bleeding in ACS. JACC Vol. 49, No. 12, 2007. Reprinted with permission)

The incidence of major bleeding significantly varies in clinical trials between 1 and 10%. Variations in the utilisation of invasive treatment, combination of various antiplatelet and anticoagulation agents at different doses and differences in underlying risk of bleeding are some plausible explanations for the disparity of the reported rate of bleeding. Utilisation of different bleeding definitions in ACS trials is another explanation. Multiple bleeding definitions exist with considerable differences in their bleeding severity classification criteria (**Figure 12**). A main difference between various definitions is the use of clinical or laboratory parameters for classification of bleeding severity. The GUSTO bleeding definition uses clinical outcome whereas the TIMI definition is based on laboratory parameters such as haemoglobin drop for discrimination of bleeding. The BARC definition arose from the need to overcome the drawbacks of the myriad of the bleeding definitions currently in use in clinical trials, including TIMI and GUSTO and combined both clinical and laboratory criteria. The PRODIGY trial was the first prospective study to use the BARC bleeding scale and provided evidence that BARC >2 bleeding events carry prognostic implications with respect to overall mortality at 2 years to a similar range compared to TIMI major or minor as well as GUSTO moderate to severe events. Although a significant association between different definitions and adverse clinical outcome has been demonstrated, data indicate that bleeding defined by clinical events is more important in terms of prognosis rather than bleeding defined solely on the basis of reductions in haemoglobin concentration.
### Background

**Figure 12a.** Bleeding Academic Research Consortium (BARC) definition for bleeding

<table>
<thead>
<tr>
<th>Type</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No bleeding</td>
</tr>
<tr>
<td>1</td>
<td>Bleeding that is not actionable and does not cause the patient to seek unscheduled performance of studies, hospitalization, or treatment by a health care professional; may include episodes leading to self-discontinuation of medical therapy by the patient without consulting a health care professional</td>
</tr>
<tr>
<td>2</td>
<td>Any overt, actionable sign of hemorrhage (e.g., more bleeding than would be expected for a clinical circumstance, including bleeding fundus imaging) that does not fit the criteria for type 3, 4, or 5, but does meet at least one of the following criteria: 1) requiring nonsurgical medical intervention by a health care professional; 2) leading to hospitalization or increased level of care; or 3) prompting evaluation</td>
</tr>
<tr>
<td>3</td>
<td>Overt bleeding plus hemoglobin drop of 3 to &lt;5 g/dL (provided hemoglobin drop is related to bleed)</td>
</tr>
<tr>
<td></td>
<td>Any transfusion with overt bleeding</td>
</tr>
<tr>
<td>3a</td>
<td>Overt bleeding plus hemoglobin drop 25 g/dL (provided hemoglobin drop is related to bleed)</td>
</tr>
<tr>
<td></td>
<td>Cardiac tamponade</td>
</tr>
<tr>
<td></td>
<td>Bleeding requiring surgical intervention for control (excluding dental/urinary/hemorrhoid)</td>
</tr>
<tr>
<td></td>
<td>Bleeding requiring intravenous vasopressor agents</td>
</tr>
<tr>
<td>3b</td>
<td>Intracranial hemorrhage (does not include microbleeds or hemorrhagic transformation, does include intraspinal)</td>
</tr>
<tr>
<td></td>
<td>Subcategories confirmed by autopsy or imaging or lumbar puncture</td>
</tr>
<tr>
<td></td>
<td>Intraocular bleed compromising vision</td>
</tr>
<tr>
<td>4</td>
<td>Coronary artery bypass graft-related bleeding</td>
</tr>
<tr>
<td></td>
<td>Perioperative intracranial bleeding within 48 hours</td>
</tr>
<tr>
<td></td>
<td>Resuscitation after closure of cannula for the purpose of controlling bleeding</td>
</tr>
<tr>
<td></td>
<td>Transfusion of 25 U whole blood or packed red blood cells within a 4-hour period</td>
</tr>
<tr>
<td></td>
<td>Chest tube output 22 L within a 24-hour period</td>
</tr>
<tr>
<td>5</td>
<td>Fatal bleeding</td>
</tr>
<tr>
<td>5a</td>
<td>Probable fatal bleeding; no autopsy or imaging confirmation but clinically suspicious</td>
</tr>
<tr>
<td>5b</td>
<td>Definite fatal bleeding; overt bleeding or autopsy or imaging confirmation</td>
</tr>
</tbody>
</table>
**Background**

**Figure 12b.** Major-bleeding definitions (continues)

<table>
<thead>
<tr>
<th>Scores</th>
<th>Bleeding site</th>
<th>Decrease in hemoglobin</th>
<th>Packed red blood cell transfusion</th>
<th>Death from hemorrhage</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>TIMI*</td>
<td>Intracranial</td>
<td>Hb &gt; 5 g/dL</td>
<td>-</td>
<td>-</td>
<td>Hemodynamic instability</td>
</tr>
<tr>
<td>GUSTO</td>
<td>Intracranial</td>
<td>-</td>
<td>Need for transfusion associated with hemodynamic instability</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ACUITY</td>
<td>Intracranial or intracocular</td>
<td>Hb ≥ 3 g/dL and known source of bleeding, Hb ≥ 4 g/dL and unknown source</td>
<td>Need for transfusion</td>
<td>-</td>
<td>Hematoma &gt; 5 cm in diameter or need for on-site intervention</td>
</tr>
<tr>
<td>CURE</td>
<td>Symptomatic intracranial or intracranial with visual deficit</td>
<td>Hb ≥ 5 g/dL</td>
<td>≥ 2 U</td>
<td>Fatal hemorrhage</td>
<td>Hemodynamic instability or need for on-site intervention</td>
</tr>
<tr>
<td>OASIS-5</td>
<td>Symptomatic intracranial, intracranial or retroperitoneal hemorrhage</td>
<td>Hb ≥ 3 g/dL associated with frank hemorrhage</td>
<td>≥ 2 U</td>
<td>Fatal hemorrhage</td>
<td>-</td>
</tr>
<tr>
<td>OASIS-7</td>
<td>Symptomatic intracranial</td>
<td>Hb ≥ 5 g/dL</td>
<td>≥ 2 U</td>
<td>Fatal hemorrhage</td>
<td>Bleeding related to myocardial revascularization surgery</td>
</tr>
<tr>
<td>PLATO*</td>
<td>Intracranial and cardiac tamponade</td>
<td>Hb ≥ 5 g/dL</td>
<td>≥ 4 U</td>
<td>Fatal hemorrhage</td>
<td>Hemodynamic instability</td>
</tr>
</tbody>
</table>
Regardless of the bleeding definition used, advanced age, female gender, lower body weight, use of invasive procedures and renal insufficiency have consistently been found to be strong independent predictors of bleeding complications in patients with ACS (figure 13).

**Figure 13.** Predictors of bleeding in acute coronary syndrome (Moscucci et al, Predictors of major bleeding in acute coronary syndromes. EHJ (2003) 24, 1815–1823. Reprinted with permission).

![Graph showing predictors of bleeding](image)

Women had a 43% higher risk for developing a major in-hospital bleeding compared to men in the GRACE registry. 91 Smaller body and vessel size, reduced creatinine clearance (for a given weight and serum creatinine), higher prevalence of comorbidities and higher risk of drug overdosing were main contributors to this observation. 92 In women, excess dosing of antithrombotic agents may account for up to 25% of the bleeding risk.93 Apart from female gender, older age and renal insufficiency were strong predictors of excess dosing of antithrombotic agents. A rapport from the CRUSADE registry of more than 140 000 ACS patients showed that 42% of patients received at least one excess dose of antithrombotic agent during their hospitalisation and had significantly higher risk of bleeding and prolonged length of hospital stay.92 Careful adjustment of doses of pharmacological agents cleared by kidneys, based on the estimated renal function, is of crucial importance in order to reduce the risk for bleeding.

**Prevention of bleeding complications and improvement of outcomes**

Despite the strong association between bleeding and adverse outcome in patients with ACS the causal relationship between bleeding complications and adverse outcome remains uncertain.94 Bleeding complications may be a surrogate marker of comorbidities and identify patients at high risk for adverse events given the overlapping
Background

between predictors of bleeding and predictors of ischemic complications. However, consequences of bleeding including hemodynamic instability, anemia, blood transfusion and early discontinuation of antiplatelet and anticoagulation therapies may have a direct negative impact on outcomes (figure 14). 86, 95, 96

Figure 14. Hypothetical mechanisms linking bleeding and mortality (Steg et al; Bleeding in ACS and PCI. European Heart Journal (2011) 32, 1854–1864. Reprinted with permission)

Interventional studies have provided some evidence about a causal relationship between bleeding and adverse outcome. In the OASIS-5 trial including 20,078 patients with NSTEMI, treatment with fondaparinux compared to enoxaparin, significantly reduced the incidence of 9-day major bleeding (2.2% vs 4.1%, p<0.01). At 30 days, the number of deaths was significantly lower among patients assigned to fondaparinux (295 vs. 352, p=0.02). 97 In the HORIZON-AMI trial enrolling 3,602 patients with STEMI and comparing bivalirudin vs UFH+GPI, treatment with bivalirudin was associated with a significant reduction in major bleeding at 30 days (4.9% vs 8.3%, p<0.01) and mortality at 30 days (2.1% vs. 3.1%, p=0.047). In the WOEST trial of 573 patients undergoing PCI with an indication for oral anticoagulation, the bleeding rate as well as the all-cause mortality rate was significantly lower in patients who received double therapy compared to patients who received triple therapy. 98 Finally, in the MATRIX study, a reduction of major bleeding by radial access was associated with lower all-cause mortality compared to femoral access. 99

Prevention of bleeding complications in STEMI patients undergoing PCI should always take into consideration: 1) choice of antithrombotic strategy 2) kidney function and 3) access site. An antithrombotic strategy that reduces the risk of bleeding while maintaining anti-ischemic efficacy should be preferred especially in a subgroup of patients with high risk of bleeding such as elderly, women and patients with renal insufficiency. Careful attention when dosing antiplatelet and anticoagulation agents that are cleared by kidneys is warranted. Finally, reducing access site bleeding can be achieved by using the radial instead of femoral approach.
Gender differences in patients with ACS

Coronary artery disease is the leading cause of mortality for women and afflicts 6.6 million women annually in the US. In Sweden, 26,600 patients were diagnosed with MI in 2015. Despite a significant improvement in cardiovascular disease (CVD) mortality in both genders, the annual CVD mortality rate has remained higher for women than for men the last 30 years. Within 1 and 5 years of a first MI, regardless of age, the mortality is significantly higher in women compared to men (women vs men: 26% vs 19% and 47% vs 36%, respectively). Furthermore, data suggest a significant interaction between age and gender whereby younger women are at particularly higher risk of mortality after MI compared to men.

Women are older when they present with their first MI and have more often comorbidities such as DM, hypertension and CKD, compared to men. The older age onset of IHD in women is mainly attributed to the protective role of circulating estrogen on the endothelium. However the exact mechanism by which estrogen protects against atherosclerosis is incompletely understood. Traditional risk factors for IHD are similar in both genders. In the INTERHEART study, 9 potentially modifiable risk factors (smoking, hypertension, DM, waist-to-hip ratio, dietary patterns, physical activity, alcohol consumption, plasma apolipoproteins, and psychosocial factors) accounted for 96% of the population-attributable risk of MI in women. Clustering of risk factors in women at the time of the first MI may be explained by older age. However, a recent study in young women with MI showed that women fail to assess their personal risk for IHD and they reported limited access to preventive cardiac care before MI.

Pathophysiological mechanisms of MI may significantly differ between men and women. Plaque rupture remains the most common mechanism of MI but a recent study showed that only 56% of fatal MI in women was found to be due to a plaque rupture compared to 76% in men. Plaque rupture is particularly infrequent in premenopausal women whereas autopsy studies have shown an increased prevalence of plaque erosion compared to men and postmenopausal women. These findings may explain the higher incidence of non-obstructive coronary artery disease in women with MI. Additionally, unusual pathophysiological mechanisms of MI such as spontaneous coronary artery dissection as well as Takotsubo cardiomyopathy mimicking ACS are more common in women.

Gender differences in clinical presentation are evident in patients with ACS and may influence both time to presentation and outcomes. Despite the fact that chest pain remains the most common symptom in both genders, women more often have atypical chest pain, angina-equivalent such as dyspnoea or more general symptoms such as fatigue and indigestion. Women present later to treatment for AMI than men, a difference that has remained unchanged over the last two decades despite educational initiatives to increase awareness of the symptoms suggesting MI.

Reperfusion therapy in STEMI patients from a gender perspective

Fibrinolytic therapy is still an important reperfusion strategy when timely reperfusion with PCI is not available. Fibrinolytic therapy, especially when administrated early, significantly improves outcomes, regardless of gender and age. However, the short- and long-term mortality in STEMI patients treated with fibrinolysis is about twice as high in women than in men. The incidence of complications such as shock, heart
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failure (HF), re-infarction, stroke and bleeding is significantly higher in women. Furthermore, female gender is an independent predictor of intracranial bleeding with fibrinolytic therapy. Moreover, relative contraindications to fibrinolytic therapy such as advance age, uncontrolled hypertension and small body weight are more often present in women than in men and contributed to the lower rate of reperfusion therapy provided in women with STEMI in the fibrinolytic era.

Because women have higher risk for complications with fibrinolytic therapy they derived a greater benefit from PPCI. Use of PPCI as reperfusion strategy reduces the risk for intracranial bleeding and is an independent predictor of survival in women with STEMI. In the GUSTO II-B angioplasty sub study, PPCI compared to fibrinolytic therapy prevented 56 deaths in women and 49 in men per 1 000 treated. Despite the improvement in outcomes in women in the era of PPCI, controversy still remains as to why short and long term mortality after STEMI is reported to be higher in women than men. It remains unclear if gender is an independent predictor of outcome due to differences in the biology of the disease in men and women with STEMI or if this is a confounded observation due to baseline differences in cardiovascular risk profile and/or health care utilisation between genders.

Pre-specified gender analysis of RCTs have shown that the observed unadjusted higher short- and long-term mortality in women compared to men are attenuated and no longer significant after adjustment for age and other baseline characteristics. In addition to higher age and comorbidities in women, disparities in the management between genders may further contribute to the higher risk for adverse outcomes in women compared to men. Women are less likely to receive primary reperfusion therapy despite the higher survival advantage in women than in men and less likely to get newer evidence based-therapies such as thienopyridines and angiotensin converting enzyme inhibitors (ACEI). Also, women are more likely to have a prehospital missed diagnosis of STEMI and a higher risk for inter hospital transfer to a PCI-capable facility and hence are at risk for reperfusion delay. Ambulance service may give lower priority for transporting women than men with possible STEMI diagnosis, resulting in potentially longer ischemic time. While the reasons for these disparities are unclear, they may significantly contribute to increase mortality in women, presented with STEMI.

Gender differences in the effectiveness and safety of antiplatelet therapy

Treatment with DAPT (aspirin and an ADP receptor blocker), improves outcomes in patients with an ACS. A meta-analysis of all the most important randomised trials on clopidogrel, with focus on gender differences, showed that clopidogrel significantly reduced the risk for adverse cardiovascular outcomes by 14% with similar efficacy in both genders. Clopidogrel significantly increases the risk of bleeding in both genders. However, limited data exist in terms of the efficacy and safety of clopidogrel in men and women with STEMI treated with PPCI. In the PLATO trial, ticagrelor significantly reduced the combined ischemic endpoints compared to clopidogrel. In a pre-specified gender analysis, women showed similar absolute and relative reduction of the primary endpoint within the ticagrelor arm and similar effects were also seen in terms of major bleedings. Notably, the PLATO trial included STEMI patients planned for PPCI as well as patients with NSTEMI intended for either invasive or medical treatment.
Chronic kidney disease in patients with ACS

It is assumed that more than 20% of patients with ACS have at least moderate CKD and almost 40% have some renal impairment.\textsuperscript{133, 134} Based on estimated glomerular filtration rate (eGFR), previous studies have shown a powerful relationship between the severity of CKD and poor outcomes in ACS and STEMI patients.\textsuperscript{135-141} Multiple possible mechanisms for the cardio-renal syndrome, the strong association between CKD and increased risk for death and cardiovascular disease, exist. A high prevalence of known risk factors for cardiovascular disease and death such as DM, HT and heart failure is observed in patients with CKD.\textsuperscript{134} CKD is also associated with increased levels of inflammatory factors, abnormal apolipoprotein levels, enhanced coagulability, anemia, left ventricular hypertrophy and increased arterial calcification, factors that may contribute to the higher risk for cardiovascular disease.\textsuperscript{142, 143} Furthermore, there is an association between the risk of bleedings and eGFR.\textsuperscript{144} CKD is one of strongest predictors of bleeding events\textsuperscript{92} and overdosing of antithrombotic agents significantly contributes to these results.\textsuperscript{93} A rapport from the CRUSADE registry of more than 140 000 ACS patients showed that 42% of patients received at least one excess dose of antithrombotic agent during their hospitalisation and had significantly higher risk of bleeding.\textsuperscript{92} Another important contributor to the worse prognosis of CKD patients is under treatment. In Gulf Registry of Acute Coronary Events prospective registry, including 6 518 consecutive patients with an ACS, patients with CKD were less likely to receive antiplatelet agents, ACEI, beta blockers and statins and were less likely to undergo invasive management.\textsuperscript{145} Concerns for further worsening of renal function and/or therapy-related toxic effects in patients with CKD are possible explanations for this observation. However, the benefit of revascularisation may well exceed the risk associated with the invasive management. A report from the SWEDEHEART register including 23 262 NSTEMI patients showed that the adjusted risk for death at 1 year was 36% lower in patients with CKD who underwent early invasive management with PCI compared with those who did not. The benefit from early invasive strategy was not uniform across the five CKD stages and declined with lower renal function.\textsuperscript{146}

Measurements and estimations of glomerular filtration rate

The best overall index of renal function is considered to be the glomerular filtration rate (GFR) and the gold standard method for its assessment is the measurement of renal inulin clearance or other methods using radiolabeled isotopes or nonradioactive contrast agents such as iohexol.\textsuperscript{147, 148} Unfortunately, these methods cannot be used routinely in daily practice because of their complexity and cost. Therefore, formulas to estimate GFR, based on creatinine, have been developed. The Cockcroft-Gault (CG), and the Modification of Diet in Renal Disease (MDRD)\textsuperscript{149, 150} are the most widely used. However, their performance significantly varies in populations not similar to the one from which the equations were derived.\textsuperscript{151} During the last years, new equations such as the Chronic Kidney Disease-Epidemiology Collaboration (CKD-EPI)\textsuperscript{152} based on creatinine has emerged and challenged the older formulas due to its higher accuracy. Nevertheless, all creatinine based equations share a main limitation, the unpredictable production and tubular secretion of creatinine in various individuals and populations. Cystatin C is a proteinase inhibitor, eliminated mainly via glomerular filtration, without tubular reabsorption and degradation and is a more reliable predictor of GFR than creatinine.\textsuperscript{153} The relative Grubb cystatin C equation (rG-CystC)\textsuperscript{154} has shown a high
Background

accuracy, comparable to MDRD, using the cystatin C concentration, and has probably overcome many limitations of the creatinine based estimates in different populations. However, measurements of cystatin C have suffered from a lack of universal standardisation. Furthermore, it remains unclear if inflammation, myocardial necrosis and atherosclerosis affect cystatin C levels in patients with MI.\textsuperscript{155,156}

As significant disagreements in CKD classification between formulas have been proven in MI populations and overestimation of GFR by formulas may have led to overdosing of antithrombotic drugs and contributed to the observed higher bleeding rate.\textsuperscript{151} Despite the crucial role of renal function in the management of STEMI patients, GFR estimates have not been validated against mGFR in that population during the acute phase. Therefore, it is still unclear if their prognostic impact only depends on their accuracy to estimate GFR or the coefficients that are included in the formulas.
AIMS

The aims of the research program on which this thesis is based were to

- compare the efficacy and safety of the two most commonly used antithrombotic strategies, bivalirudin vs UFH with or without GPI in both groups, in STEMI patients treated with PPCI in a large real world population

- provide pharmacodynamic data of two novel ways of ticagrelor administration, crushed and chewed tablets, in comparison with administration of the standard integral tablets

- study the association between gender and risk of short term mortality and bleeding in STEMI patients planned for PPCI and treated with ticagrelor

- validate the performance of the most commonly used creatinine and cystatin C based equations for the estimation of GFR against measured GFR during the index hospitalisation of a STEMI population.
MATERIAL AND METHODS

Paper I
Source data
In the study for paper I we used data from The Swedish Web-system for enhancement and development of evidence-based care in heart disease evaluated according to recommended therapies (SWEDEHEART). Details about the register have been previously published. The SWEDEHEART register includes the former separate registers RIKS-HIA, SCAAR, SEPHIA and the Swedish Thoracic Surgery Registry. SWEDHEART registers all patients admitted to a coronary care unit (CCU) and all patients undergoing coronary catheterisation in Sweden. More than 100 variables, including patient demographics, past medical history, medical treatment before admission, medical treatment and interventions during CCU care, in-hospital outcomes and complications, discharge diagnosis and discharge medication are collected. Source data have continuously been validated by comparison of the register information with the hospitals’ patient records by an external monitor with over 95% agreement.

Data from the SWEDEHEART register was merged with the National Patient Registry (NPR) to obtain information on previous medical history and readmission for a new MI and the Swedish population register to obtain information on mortality, by using each patient’s unique personal identification number. NPR provides all discharge diagnoses for patients admitted to a hospital in Sweden. Peri-procedural characteristics and in-hospital complications including bleedings were obtained from the SWEDEHEART register. Mortality and stent thrombosis data were available for all patients from the National Board of Health and Welfare’s Cause of Death Register and the SWEDEHEART register, respectively. Data on readmission due to a new MI were collected from the NPR register.

Study population
From January 1st 2008 to October 10th 2014 we selected patients using the following inclusion criteria: 1) patients with STEMI presenting within 12 hours after symptom onset, 2) treated with PPCI 3) received UFH or bivalirudin as adjunctive anticoagulant. Inclusion dates were chosen to coincide with the presentation of the HORIZONS AMI trial. Only the first time an individual appeared in the register with STEMI was included for analysis. The included population was 23 800 patients. Based on the adjunctive antithrombotic regimen patients were coded into two treatment groups: (i) UFH with or without GPI (will be referred as UFH group) and (ii) bivalirudin with or without GPI or UFH (will be referred as bivalirudin group). Information about death and stent thrombosis were collected up to November 14th 2014 (all patients had at least 30 days of follow up) whereas information about readmission for a new MI were available up to December 31st 2013.

Outcome definition
The primary efficacy outcome of the study was all-cause mortality during 30 days after the index procedure. Secondary efficacy outcomes included 1-year all-cause mortality, 30-day and 1-year definite stent thrombosis and re-infarction at 30-day and 1 year after discharge. Primary safety outcome was the rate of non-CABG major in-hospital
Material and Methods

bleeding and secondary outcome was the composite of non-CABG major or minor in-hospital bleedings.

Definite stent thrombosis (ST) was defined according to the Academic Research Consortium definition. Standardised clinical and biochemical criteria for the diagnosis of acute MI and STEMI has been used in the register in accordance with the European guidelines and diagnoses have been coded according to the International Classification of Disease at the treating physician’s discretion. In-hospital major bleeding was defined as any fatal or cerebral bleeding, required surgical intervention or blood transfusion or leading to a decrease in hemoglobin > 5.0 g/dL. Minor in-hospital bleeding included any bleeding that led to hemoglobin drop > 2.0 g/dL, led to early discontinuation of antithrombotic treatment or any pseudo-aneurysm at the access site that required treatment other than manual compression.

Paper II  
Study design and population
This was a single center, open-label, randomised, investigator initiated, pharmacodynamic study. Patients > 18 years of age, with stable angina pectoris, scheduled for outpatient coronary angiography, were randomly assigned, at least 90 minutes before the intervention, in a 3:1:1 fashion (according to a computer generated randomisation list) to one of the following treatment modalities: A) Integral ticagrelor tablets, 180 mg LD B) Crushed ticagrelor tablets, 180 mg LD or C) Chewed ticagrelor tablets, 180 mg LD. The allocation sequence was concealed from the researcher enrolling and assessing participants in sequentially numbered, sealed envelopes.

Exclusion criteria were: pregnancy or lactation, known allergy to the study medication, chronic therapy with ticagrelor, prasugrel, clopidogrel or ticlopidine, treatment with warfarin or new oral anticoagulants (NOAC) within 4 days before admission, active bleeding, bleeding diathesis or coagulopathy, history of gastrointestinal or genitourinary bleeding in the last 2 months, history of intracranial bleeding, major surgery in the last 4 weeks, known relevant hematological deviation (severe anemia, severe thrombocytopenia), known severe liver disease or severe renal failure, increased risk of bradycardia or inability to chew tablets.

Administration of the different ticagrelor formulations.
All three groups received two tablets of ticagrelor (180 mg) and 150 mL of water. In the first group (A), two integral ticagrelor tablets were administered as an oral dose, followed by 150 mL of water. In the second group (B), two ticagrelor tablets (180 mg) were placed in a point-of-care (POC) crushing device and crushed. The total content of the crushed tablets was transferred to a dosing cup, 50 mL of water was added and the suspension was mixed before drinking. Afterwards, 100 mL of water was administered. In the third group (C), the patient was instructed to chew two tablets of ticagrelor for at least 10-15 seconds followed by oral administration of 150 mL of water.

Blood sampling for platelet aggregation measurements
Platelet aggregation assessment was performed at three time-points: before administration of ticagrelor (baseline, sample 1) 20 ± 5 minutes (sample 2) and 60 ± 10 minutes (sample 3) after administration of ticagrelor. In all cases, the blood samples were drawn from a recently inserted venous catheter for repeated sampling or by direct venipuncture. The first 2-3 mL of blood was discarded to avoid platelet aggregation and then blood was collected in 3.2 % citrated tubes. Platelet aggregation was measured with the VerifyNow P2Y12 (Accumetrics Inc, San Diego, CA) POC test. The test has
Material and Methods

been described in detail earlier.\textsuperscript{160} Briefly, VerifyNow is a turbidimetric test which measures agonist-induced aggregation as an increase in light transmittance. The system contains a lyophilised preparation of human fibrinogen-coated beads, which causes a change in light transmittance by agonist-induced platelet aggregation. Platelet reactivity (PR) results are reported in arbitrary P2Y12 reaction units (PRU). The percent inhibition of platelet reactivity (IPR) was defined as: \([\text{PRU baseline} – \text{PRU sample 1 or 2}]/\text{PRU baseline}\) \times 100. Based on a recently published consensus document, high residual platelet reactivity (HRPR) was defined as PR > 208 PRU (non-responders).\textsuperscript{161} Patients with PR values \(\leq 208\) were considered as responders to the drug.

Outcome
We report residual platelet reactivity, percent IPR and proportion of patients with HRPR at baseline, 20 and 60 minutes. Safety outcomes include TIMI major, minor or minimal bleeding within 24 hours after randomization.

Paper III
The ATLANTIC study was an international, randomised, double-blind, placebo-controlled study with 30 days follow-up. The design of the study, inclusion and exclusion criteria, outcome definitions and principal results have previously been described.\textsuperscript{74,162} Briefly, 1,862 patients presenting with STEMI, less than 6 hours after symptom onset, were included and randomised to pre-hospital (in the ambulance) versus in-hospital (in the catheterisation laboratory) treatment with a loading dose of ticagrelor (180mg) in addition to aspirin and standard clinical care. All patients were to subsequently receive ticagrelor 90 mg twice daily for 30 days, after which it was recommended that ticagrelor should be continued for up to 12 months. Pre-hospital administration of a GP IIb/IIIa inhibitor was left to the physician’s discretion. Periprocedural use of parenteral anticoagulants was also left to the physician’s discretion, according to the local practice. The proportion of patients who did not have \(\geq70\%\) resolution of ST segment elevation before PCI and / or did not meet the criteria for TIMI flow 3 in the infarct related artery (IRA) at initial angiography was the primary outcome of the ATLANTIC trial. Secondary outcomes (clinical efficacy outcomes) included the composite of death, MI, stent thrombosis, stroke, or urgent revascularisation at 30 days and definite stent thrombosis at 30 days. Safety outcomes included major and minor bleeding over the 30-day treatment period. Bleeding risk was evaluated using the TIMI (The Thrombolysis In Myocardial Infarction), BARC (Bleeding Academic Research Consortium) and PLATO (Study of Platelet Inhibition and Patient Outcomes) bleeding definitions.\textsuperscript{162} The main objective of our analysis was to study the association between gender and primary and secondary outcomes of the main study with a special focus on the clinical efficacy and safety outcomes. The interaction of gender subgroups with randomised treatment effects was also investigated.

Paper IV
Design and Study Population
This was a single center, prospective observational study including patients with STEMI treated with primary PCI (PPCI). Patients with known advanced renal failure (on dialysis), cardiogenic shock at arrival or known allergy to iodine were excluded. Between November 2011 and February 2013, forty patients were successfully included.
Material and Methods

GFR, plasma Creatinine and Cystatin C measurements
Blood samples were obtained via a direct venous puncture at the time of randomisation (before coronary angiography) and before discharge (between day 4 and 7 after admission). Plasma creatinine and cystatin C were analysed at the central laboratory of Östergötland County Concil (at Linköping University Hospital and at Norrköping Hospital).

Furthermore, from patient records we retrospectively collected high sensitive Troponin T that was routinely measured six to eight hours after admission as a marker of infarct size.

Determination of GFR
GFR was determined by measurement of the plasma clearance of iohexol, a non-radioactive radiographic contrast medium (Omnipaque 300 mg I/ml; Nycomed Amersham AB, Sweden) before discharge, soon after blood samples for creatinine and Cystatin C measurements were obtained. Four millilitres of iohexol were injected intravenously in an antecubital vein and clearance was calculated from the remaining iohexol concentration in two plasma samples. The sampling time-point was determined from eGFR, using MDRD-IDMS. If eGFR was > 40 mL/min/1.73 m² sampling was performed at 3 and 4 hours after injection. For eGFR < 40 mL/min/1.73 m² samples were drawn 6 and 8 hours after injection. Plasma iohexol concentration was determined by high-performance liquid chromatography (HPLC). The total coefficient of variation (CV) for the analysis method, during a three months period was 4.0 % for a control sample with an assigned value of 31 mg/L and 3.7% for a control sample with an assigned value of 62 mg/L (n=56). For a person weighing 70 kg and sampling at 4 hours, these iohexol concentrations corresponded to GFR values of approximately 100 mL/min and 60 mL/min, respectively, without correction for body surface area (BSA). GFR was expressed in relative values: mL/min/1.73 m² using the DuBois-DuBois formula for calculation of BSA.

Determination of plasma creatinine
Plasma creatinine was analysed by a kinetic alkaline picrate colometric method, a modification of the original method described by Jaffe using Advia 1650 and 1800 instruments (Siemens Healthcare Diagnostics). Precision measured during a three months period on four instruments showed a total CV of 4.3% at a creatinine level of 86 µmol/L and of 3.2% at a creatinine level of 380 µmol/L (reference values used: 60 - 105 for adult males and 45 - 90 for adult females). Calibration is traceable to a primary reference material (SRM 967) with values assigned by isotope dilution mass spectrometry (IDMS) and a zero-point calibrator was used.

Measurement of cystatin C
Plasma cystatin C was measured by an automated particle-enhanced immunoturbidimetric method on Advia 1650 and 1800 analysis system (Siemens Healthcare Diagnostics) with reagents obtained from DakoCytomation and according to the procedure recommended by the reagent producer. Precision measured during a three months period on two instruments showed a total CV of 4.2 % at a cystatin C concentration of 1.2 mg/L and 3.2% at 4.6 mg/L (reference values used, 0.55 - 1.15 mg/L for persons 1-50 years of age and 0.63 - 1.44 mg/L for persons > 50 years). All samples were analysed within one day after collection.
**Material and Methods**

**Definition and staging of Chronic Kidney Disease**
According to the National Kidney Foundation Kidney/Disease Outcome Quality Initiative (NKF K/DOQI) CKD is defined as kidney damage persisting for more than 3 months. Patients with reduced kidney function should be staged into five CKD stages based on GFR. Patients in stage 1 (GFR $\geq 90$ mL/min/1.73 $m^2$, normal kidney function) and stage 2 (GFR 60-89 mL/min/1.73 $m^2$, mild CKD) must have signs of kidney damage such as albuminuria or pathological imaging in order to be staged into CKD. Patients in stage 3 (GFR 30-59 mL/min/1.73 $m^2$) and stage 4 (GFR 15-29 mL/min/1.73 $m^2$) have moderate or severe CKD, respectively. Dialysis or GFR <15 mL/min/1.73 $m^2$ is defined as end-stage renal failure (stage 5). In this article, from now on CKD is defined as CKD stage 3-5, i.e. GFR less than 60 mL/min/1.73 $m^2$.

**Objectives**
The objective of this study was to validate the performance of the most commonly used equations for estimation of GFR against mGFR during the index hospitalisation in a STEMI population. We used the Cockcroft-Gault (CG), the abbreviated Modification of Diet in Renal Disease (MDRD-IDMS), the Chronic Kidney Disease-Epidemiology Collaboration (CKD-EPI) based on creatinine and the relative Grubb cystatin C (rG-CystC) equations to estimate GFR (figure 15).

**Figure 15.** Equations for estimation of Glomerular Filtration Rate (eGFR). The Cockcroft-Gault (CG), the abbreviated Modification of Diet in Renal Disease (MDRD-IDMS), the Chronic Kidney Disease-Epidemiology Collaboration (CKD-EPI) based on creatinine and the relative Grubb cystatin C (rG-CystC) equations

<table>
<thead>
<tr>
<th>Equation</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG</td>
<td>$\text{GFR} = \frac{140 - \text{age}}{\text{weight} \times \text{creatinine}}$</td>
</tr>
<tr>
<td>MDRD-IDMS</td>
<td>$175 \times \left(\frac{\text{creatinine}}{88.4}\right)^{-1.104} \times \text{age}^{-0.203} \times 0.742$ (if women)</td>
</tr>
<tr>
<td>CKD – EPI</td>
<td>-</td>
</tr>
<tr>
<td>Women:</td>
<td>-</td>
</tr>
<tr>
<td>If creatinine $\leq 62$: $144 \times \left(\frac{\text{creatinine}}{88.4}\right)^{-1.209} \times 0.993$</td>
<td></td>
</tr>
<tr>
<td>If creatinine $&gt; 62$: $144 \times \left(\frac{\text{creatinine}}{88.4}\right)^{-1.209} \times 0.993$</td>
<td></td>
</tr>
<tr>
<td>Men:</td>
<td>-</td>
</tr>
<tr>
<td>If creatinine $\leq 101$: $141 \times \left(\frac{\text{creatinine}}{1.0 \times \text{BMI}}\right)^{-1.094} \times 0.993$</td>
<td></td>
</tr>
<tr>
<td>If creatinine $&gt; 101$: $144 \times \left(\frac{\text{creatinine}}{1.0 \times \text{BMI}}\right)^{-1.094} \times 0.993$</td>
<td></td>
</tr>
<tr>
<td>Relative Grubb cystatin C equation</td>
<td>$84.89 \times \text{Cystatin C}^{-1.640}$</td>
</tr>
</tbody>
</table>

Weight in kg, age in years, plasma creatinine in $\mu$mol/L, cystatin C in $mg/L$. 

*34*
SAMPLE SIZE CALCULATION

Sample size calculation was not performed in paper I and paper III that were retrospective analyses of prospectively collected data. The study for paper IV was a methodological, observational study and sample size calculation was not performed. In the study for paper II, previous studies showed that around 50% of patients had HRPR 30 minutes after LD ticagrelor (integral tablets). A recent study has shown that administration of crushed ticagrelor tablets resulted in a mean plasma concentration of ticagrelor that was four to five times higher at 30 minutes compared with plasma concentration after administration of integral tablets. We assumed that chewed ticagrelor tablets would have at least as fast uptake as crushed ones. Given that the antiplatelet effect of ticagrelor is linearly related to the blood concentration of ticagrelor, we also assumed that 20% of patients with the novel ways of ticagrelor administration (crushed and chewed) would have HRPR 20 minutes after administration of LD. A sample of 100 patients (60 patients in the integral, 20 patients in the crushed and 20 patients in the chewed group) would give an 80% power to detect statistically significant differences in the HRPR rates.
STATISTICAL ANALYSIS

In paper I and III, continuous variables were presented by their mean and standard deviation (SD) or median and 25th -75th percentiles as appropriate. Categorical variables were presented as counts and percentages. Baseline and peri-procedural characteristics were compared according to treatment or gender by Chi-square tests for categorical variables and Student’s t-test or Mann Whitney U test for continuous variables, depending on if the variable of interest was normally distributed or not. In paper II, the Kolmogorov-Smirnov test was used to test for normality of the distribution of PR values. Baseline characteristics were compared according to randomised treatment by Fisher’s exact test for categorical variables and Kruskal-Wallis test for continuous variables. Friedman’s test was used for within group comparisons of PR over time. PR and IPR of the three groups of patients were compared using Kruskal-Wallis test. Pairwise comparisons of the groups were performed with Mann Whitney U test. Percentage of HRPR in the three groups was compared using Chi-squared test. Pairwise comparisons were also performed using the same test. A p-value <0.05 was considered to indicate statistical significance. Due to the relatively small number of hypotheses being tested under the pairwise comparison, the likelihood of type I error was estimated as low and adjusted p values were not used. A p-value <0.05 was considered to indicate statistical significance.

Paper I

Given the observational nature of our study, methods for risk-adjusted outcomes, such as multivariable analyses, propensity score adjustments and propensity score matched analyses were used. In order to study the association between treatment and efficacy outcomes, hazard ratios (HR) with 95% confidence intervals (CI) were derived from Cox proportional hazard models. In the unadjusted (crude) model, treatment was the only explanatory variable. In the first adjusted model (Model 1), we used a backward stepwise selection algorithm with a significant cut-off level of 0.05 for variable inclusion. Treatment was forced into the model. The selection of the other variables possibly associated with the efficacy outcomes was based on results from previous studies as well as clinical experience: year of the index procedure, gender, age, smoking status, history of HTN, dyslipidemia, DM, HF, MI, previous PCI, CABG, stroke, history of atrial fibrillation (AF), cancer within 3 years of admission, COPD, history of gastrointestinal bleeding, treatment with warfarin, time from symptom onset to diagnostic ECG, BMI, baseline hemoglobin, eGFR according to the MDRD formula, cardiogenic shock at arrival, aspirin and ADP receptor blockers before or during PCI, access site, MVD, IRA, PCI with stent, PCI with DES, thrombectomy and successful revascularisation.

To assess the association between treatment and safety outcome logistic regression models were constructed, including the same variables as above using a backward stepwise selection algorithm with a significance level of less than 0.05 for inclusion. Odds ratios (OR) with 95% CI were presented.

We calculated an individual propensity score, reflecting the individual probability for a patient to be treated with bivalirudin based on available covariates within a multiple logistic regression model. All covariates included in the multivariable analysis were also included in the propensity score model. As a linear relationship between propensity
Statistical analysis

score and outcomes cannot be assumed, we divided subjects into five equal-size groups using the quintiles of the estimated propensity score and performed a multivariable analysis of outcomes including treatment and the propensity score as a categorical variable according to the method described by Austin (Model 2). Furthermore, based on the individual propensity score we matched patients in the UFH group with patients in the bivalirudin group using 1:1 nearest neighbor matching without replacement and caliper width of 0.1. We evaluated the success of matching by using standardized difference between the two groups. In the propensity matched population we used multivariable Cox proportional hazard models to assess efficacy outcomes and multivariable logistic regression models to assess bleeding complications. Treatment and the four covariates that significantly differed between the two groups (year of the index procedure, thrombectomy, PCI with DES, and ADP receptor blockers before or during PCI), were included in the multivariable model (Model 3).

Paper II
A forward stepwise binary logistic regression analysis was used to identify independent predictors of HRPR at 20 minutes after administration of ticagrelor. Known and potential predictors of HRPR were included in the model, in accordance with previous studies. Variables included in the model were age, gender, DM, BMI, smoking status, eGFR by the MDRD formula, platelet count, treatment with beta blockers or diuretics, PR at baseline and randomization group as a dichotomous variable (integral tablets versus crushed or chewed tablets). Odds ratios with 95% CI were presented for the significant predictors.

Paper III
In order to study the association between gender and the clinical efficacy and safety outcomes, independent of randomised treatment as well as the possible interactions between gender and randomised treatment with respect to the outcomes, hazard ratios with 95% CI were derived from Cox proportional-hazards models. In the unadjusted (crude) model, gender was the only explanatory variable whereas in the adjusted multivariable model, gender was forcibly included and the other variables were chosen by using a forward stepwise selection algorithm with a significant cut-off level of 0.05 for inclusion. Adjustment variables included age, weight, prior MI, prior PCI, history of DM, HTN, non-hemorrhagic stroke, gastrointestinal bleeding, time from symptom onset to pre-PCI ECG, admission Killip class (as a dichotomous variable 1 / >1), baseline hemoglobin, eGFR according to the MDRD formula, use of GP IIIb/IIa inhibitor and location of MI. To evaluate the importance of early mortality, a landmark analysis of the clinical efficacy outcomes was also performed from 48 hours after randomisation (arbitrarily defined) to the end of the study. In order to explore the importance of bleeding on the clinical efficacy outcomes, a separate analysis was also performed by censoring the patients who reported a PLATO major bleeding at the time of the onset of a bleeding event.
A comparable statistical process was used to compare gender differences in the effect of randomised treatment by constructing logistic regression models. Odds ratios with 95% CI were presented. The above process for Cox regression and logistic regression was also used to explore any interaction between gender and treatment.

Paper IV
The percentage of CKD patients obtained with the four formulas and mGFR was compared with the McNemar test. Agreement between GFR estimates and between
mGFR and eGFR regarding CKD classification to discriminate GFR greater and less than 60mL/min1.73m² was assessed by Cohen’s kappa statistics. A kappa value of 0.20 or less was considered slight agreement; 0.21 – 0.40, fair agreement; 0.41 – 0.60, moderate agreement; 0.61 – 0.80 substantial agreement; and 0.81 – 1.00, almost perfect agreement.¹⁷⁰

Furthermore, we constructed Bland-Altman plots according to the original article by Bland and Altman.¹⁷¹ Because iohexol clearance was considered as the gold standard method, differences between eGFR and mGFR were plotted.

We assessed the diagnostic performance of the eGFR equations compared to mGFR with respect to correlation, bias, precision and accuracy.¹⁷² Correlation between eGFR and mGFR was obtained from Pearson correlation and reported as correlation coefficient (R). Bias was defined as the median percentage error between eGFR and mGFR, positive values indicating an overestimation of mGFR. A bias of less than 10% was considered as clinically acceptable.¹⁷³ Precision was assessed as the interquartile range (IQR) expressed in mL/min/1.73 m² of the difference eGFR – mGFR. Accuracy within 30% (P30) was the percentage of estimates within 30% of mGFR. A P30 of more than 75% was accepted sufficient for clinical decisions.¹⁷⁴ Differences in accuracy between 0 – 2% were regarded as equivalent accuracy, between 3 – 4% as slightly higher accuracy and ≥ 5% as significant higher accuracy. The 95% of P30 was calculated according to the formula:

\[
95\% CI = P30 \pm 1.96 \times \left( P30 \times \frac{(1-P30)}{\text{number of measurements}} \right)^{0.5}.
\]

We used the Cockcroft-Gault (CG), the abbreviated Modification of Diet in Renal Disease (MDRD-IDMS), the Chronic Kidney Disease-Epidemiology Collaboration (CKD-EPI) based on creatinine and the relative Grubb cystatin C (rG-CystC) equations to estimate GFR. P-values < 0.05 were considered to indicate statistical significance.

Missing values

**Paper I**

For the variables included in the multivariable analyses 28% of the patients had at least one missing value and 1.5% of the total values were missing. Multiple missing values imputation was performed by using SPSS and an automatic imputation method was used based on a scan of our data source.

**Paper III**

For most of the variables included in the multivariable models, only few patients had missing data (<10 patients). However, 7% of patients had missing values for creatinine, 5% for hemoglobin and 4% for Killip class. The number of patients with complete data for multivariable Cox proportional-hazard models of the primary outcome was 1613 (88%). To investigate the influence of missing values, multivariable analysis was performed before and after simple missing values imputation as well as with only gender and age as explanatory variables (because these variables had no missing values).
ETHICAL CONSIDERATIONS

All studies were approved by the Ethical review board in Linköping, conformed to the Declaration of Helsinki and followed the principles of good clinical practice. The study for paper I was a retrospective analysis of prospectively collected data in the SWEDHEART register. All patients were informed about the inclusion in the register and had the right to decline participation, but informed consent for participation in our study was not judged to be possible. The merge of the registers was approved by the National Board of Health and Welfare. Analysis of data was performed with anonymised data only and the possible risk of revealing confidential medical records was minimised.

The study for paper II was a randomised clinical trial, approved by the Regulatory Authorities. Participants in this study were treated according to our standard clinical praxis. They received only a loading dose ticagrelor before catheterisation followed by maintenance clopidogrel dose if PCI was performed. The antiplatelet effect of clopidogrel administered the day before the intervention is equivalent to the effect of ticagrelor administered at arrival to the outpatient clinic. Therefore the risk for bleeding complication and ischemic complications (e.g. acute stent thrombosis or myocardial infarction) in our participants should not be higher compared to patients pre-treated with clopidogrel. We hypothesised that chewed or crushed ticagrelor administration would result in a faster inhibition of platelet aggregation at 20 minutes compared with the intact tablets. Nevertheless, ticagrelor was given at least 1.5 hour before the coronary angiography and no difference in the degree of platelet aggregation was expected at the time of angiography or angioplasty between the three different groups. Therefore patients randomised to one of the three different routes of ticagrelor administration were not expected to have any additional risk. The risk for allergic reaction to ticagrelor is very low and probably not higher compared to the risk for allergic reaction to clopidogrel. The risk for other common side-effects of ticagrelor such as dyspnea or bradycardia was assessed to be very low after administration of a single dose ticagrelor.

The study for paper III was a pre-specified analysis of the ATLANTIC trial, a randomised double blind clinical trial, with central randomisation, 100% local monitoring of the medical records in the centers, CRF cross-checking, declaration of all adverse events, mandatory pharmacovigilance declarations when AE were related to the drug, adjudication of all the events declared by the investigators by an independent clinical endpoint committee international randomised control trial. Our analysis could be performed without any additional risk for the patients.

The study for paper IV was an observational, methodological study. This study could be performed safe and without any significant risk for the patients. Considering blood samples and iohexol clearance measurement the discomfort and risks were considered limited to mild temporal discomfort and a very small risk of local complications due to venous puncture.
RESULTS

Paper I

Baseline and peri-procedural characteristics
Of the total population of 23,800 patients, 8,783 (36.9%) were included in the UFH group and 15,017 (63.1%) in the bivalirudin group. The UFH group had more men, higher prevalence of dyslipidemia, previous MI and PCI whereas patients in the bivalirudin group were older and more often had a history of cancer. The rate of cardiogenic shock at arrival was significantly higher in the UFH group (5.1% vs 3.6%, p<0.01). Angiographic findings including culprit vessel were similar in the two groups while PCI success rate was higher in the bivalirudin group. Stents, radial access and thrombectomy were significantly more often used in the bivalirudin arm. Almost 80% of the bivalirudin treated patients received at least one dose of UFH before/during PPCI. Concomitant GPI administration was 68.5% in the UFH arm compared to 3.5% in the bivalirudin arm (p<0.01).

Mortality
The primary efficacy outcome, 30-day all-cause mortality, occurred in 5.3% in the UFH group and in 5.5% in the bivalirudin group, respectively (crude HR 0.97; 95% CI 0.86 - 1.08). After multivariable adjustment (model 1), there was no significant difference (HR 0.94; 95% CI 0.82 - 1.07). Similarly with short-term mortality, 1-year all-cause mortality rate did not significantly differ between groups (HR 0.93; 95% CI 0.84 - 1.03 in the multivariable model 1). Propensity score adjusted models (model 2 and 3) showed similar results (figure 16).

Stent thrombosis
The rate of definite stent thrombosis within 30 days was 0.6% in the UFH group and 0.5% in the bivalirudin group (crude HR 1.35; 95% CI 0.95 - 1.91). After multivariable adjustment, no significant difference was detected.
At one year, the incidence of stent thrombosis was significantly higher in the UFH group 1.1% vs 0.7%, (crude HR 1.56; 95% CI 1.19 - 2.04). However, after multivariable adjustment, no significant difference remained, regardless which risk-adjusted model was applied (figure 17).

Myocardial infarction
The incidence of re-infarction, within 30 days after discharge, was 2.9% in the UFH group and 2.1% in the bivalirudin group, HR 1.33; 95% CI 1.18 - 1.59. However, after adjustment, the difference was no longer statistically significant. The adjusted incidence of re-infarction within 1 year after the index procedure did not significantly differ between groups (figure 18).
## Results

### 30 day all-cause mortality

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Crude</th>
<th>Multivariable model</th>
<th>Propensity score adjusted model</th>
<th>Propensity matched population</th>
</tr>
</thead>
<tbody>
<tr>
<td>UFH</td>
<td>467 [5.3]</td>
<td>826 [5.5]</td>
<td>0.94 [0.82 - 1.07]</td>
<td>0.91 [0.80 - 1.02]</td>
</tr>
<tr>
<td>Bivalirudin</td>
<td>427 [5.8]</td>
<td>0.88 [0.76 - 1.01]</td>
<td>0.87 [0.75 - 1.02]</td>
<td></td>
</tr>
</tbody>
</table>

### 1 year all-cause mortality

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Crude</th>
<th>Multivariable model</th>
<th>Propensity score adjusted model</th>
<th>Propensity matched population</th>
</tr>
</thead>
<tbody>
<tr>
<td>UFH</td>
<td>728 [8.3]</td>
<td>1335 [8.9]</td>
<td>0.93 [0.85 - 1.02]</td>
<td>0.92 [0.83 - 1.01]</td>
</tr>
<tr>
<td>Bivalirudin</td>
<td>648 [9.2]</td>
<td>0.89 [0.80 - 1.00]</td>
<td>0.95 [0.87 - 1.04]</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 16.** Association between treatment and all-cause mortality. Crude hazard ratio (HR) and p-value for UFH versus bivalirudin (with or without glycoproteins IIb/IIIa inhibitors in both groups) was calculated from Cox proportional hazard model with only treatment as explanatory variable. Additionally, three different risk adjusted models (multivariable model, propensity score adjusted model and propensity matching) were developed to calculate adjusted HR and p-value.

### 30 day stent thrombosis

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Crude</th>
<th>Multivariable model</th>
<th>Propensity score adjusted model</th>
<th>Propensity matched population</th>
</tr>
</thead>
<tbody>
<tr>
<td>UFH</td>
<td>56 [0.6]</td>
<td>71 [0.5]</td>
<td>0.97 [0.67 - 1.39]</td>
<td>0.99 [0.67 - 1.45]</td>
</tr>
<tr>
<td>Bivalirudin</td>
<td>41 [0.6]</td>
<td>49 [0.7]</td>
<td>0.74 [0.49 - 1.13]</td>
<td>0.96 [0.63 - 1.45]</td>
</tr>
</tbody>
</table>

### 1 year stent thrombosis

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Crude</th>
<th>Multivariable model</th>
<th>Propensity score adjusted model</th>
<th>Propensity matched population</th>
</tr>
</thead>
<tbody>
<tr>
<td>UFH</td>
<td>100 [1.1]</td>
<td>110 [0.7]</td>
<td>1.56 [1.19 - 2.04]</td>
<td>1.07 [0.69 - 1.64]</td>
</tr>
<tr>
<td>Bivalirudin</td>
<td>68 [1.0]</td>
<td>69 [1.0]</td>
<td>0.92 [0.66 - 1.30]</td>
<td>0.96 [0.63 - 1.45]</td>
</tr>
</tbody>
</table>

**Figure 17.** Association between treatment and definite stent thrombosis. Crude hazard ratio (HR) and p-value for UFH versus bivalirudin (with or without glycoproteins IIb/IIIa inhibitors in both groups) was calculated from Cox proportional hazard model with only treatment as explanatory variable. Additionally, three different risk adjusted models (multivariable model, propensity score adjusted model and propensity matching) were developed to calculate adjusted HR and p-value.
Results

Figure 18. Association between treatment and readmission for myocardial infarction. Crude hazard ratio (HR) and p-value for UFH versus bivalirudin (with or without glycoproteins IIb/IIIa inhibitors in both groups) was calculated from Cox proportional hazard model with only treatment as explanatory variable. Additionally, three different risk adjusted models (multivariable model, propensity score adjusted model and propensity matching) were developed to calculate adjusted HR and p-value.

Bleeding

The incidence of in-hospital major bleeding was 1.8% in the UFH group compared to 1.1% in the bivalirudin group (HR 1.65; 95% CI 1.33 - 2.05). After adjustment, the risk for major bleeding remained significantly higher in the UFH group, compared to the bivalirudin group (47% to 65% higher risk depending on the risk-adjusted model used). The composite of major or minor in-hospital bleeding was significantly higher in the UFH group (3.1%) compared to the bivalirudin group (2.4%) (Crude HR 1.35; 95% CI 1.15 -1.58). The risk remained significantly higher after adjustment, regardless of the risk-adjusted model used (figure 19).
Results

Figure 19. Association between treatment and readmission for myocardial infarction. Crude hazard ratio (HR) and p-value for UFH versus bivalirudin (with or without glycoproteins IIb/IIIa inhibitors in both groups) was calculated from Cox proportional hazard model with only treatment as explanatory variable. Additionally, three different risk adjusted models (multivariable model, propensity score adjusted model and propensity matching) were developed to calculate adjusted HR and p-value.

Paper II

Baseline characteristics
There were no significant differences in baseline characteristics and medication between the groups.

Residual Platelet reactivity
There was no difference in PRU values at baseline between groups. At 20 and 60 minutes after the LD, chewed ticagrelor resulted in significantly lower PRU values compared to other two treatment modalities. Crushed ticagrelor achieved significantly lower PRU values compared to integral tablets 20 minutes after the LD whereas no difference was observed at 60 minutes (figure 20).

High Residual Platelet Reactivity
The percentage of patients with HRPR at different time points in the integral, crushed and chewed ticagrelor groups are presented in Figure 4. HRPR rates differed significantly between the three groups 20 and 60 minutes after the LD. No patient in the chewed ticagrelor group had HRPR 20 minutes after the LD. At the same time point, 68.3% of patients in the integral ticagrelor group and 30% of patients in the crushed ticagrelor groups had HRPR. After 60 minutes, none of the patients in the crushed or the chewed ticagrelor groups had HRPR compared to 20% of patients in the integral ticagrelor group, a significantly higher rate than the two other groups (figure 21).
Results

In a multivariate analysis including potential predictors of HRPR, integral ticagrelor administration (vs. crushed/chewed tablets combined) was the most powerful predictor of HRPR (OR for HRPR: 12.63; 95% CI: 4.22 - 37.76). The other significant predictors of HRPR had a modest effect; PRU value at baseline (OR: 1.01; 95% CI: 1.00 – 1.02) and BMI (OR: 1.15; 95% CI: 1.00 – 1.32).

Figure 20. Mean values (standard deviation) of P2Y12 platelet reactivity unit (PRU) for integral vs crushed vs chewed ticagrelor: 274 (54), 279 (43), 271 (50) at baseline, 227 (100), 142 (90), 81 (56) 20 minutes after loading dose and 96 (106), 59 (46), 28 (41) 60 minutes after loading dose of 180 mg ticagrelor. No significant reduction in PRU was observed in the integral ticagrelor group within 20 min (p = 0.52). PRU was significantly reduced between 20 and 60 min in the integral ticagrelor group (p < 0.01) and between all the time points in the crushed and chewed ticagrelor group, (p values not shown). Comparisons by using Friedman’s test. Reprinted with permission.

Patients with High Residual Platelet Reactivity (%)

Figure 21. High Residual platelet reactivity (defined as > 208 platelet reactivity units) at baseline and 20 and 60 minutes after ticagrelor administration.*P-values for comparison of the three groups by Chi-squared test. Pairwise comparison between groups by chi-squared. Reprinted with permission.
Paper III

Demographic and clinical characteristics stratified by gender

The study population consisted of 369 (20%) women. They were older (mean age 69 vs. 59 years, p<0.01), had lower BMI, higher TIMI risk score, more often had a history of HTN, COPD or stroke. Men had more frequent prior PCI.

Time delays

Women had significantly longer delay times, both from symptom onset to prehospital ECG (median 88 vs 70 minutes, p<0.01).

Procedural characteristics, angiographic findings, and medication during the index event

Coronary angiography was more often performed by femoral access in women (39.7% vs 30.4%, p<0.01). The genders did not differ according to IRA, with the left anterior descending artery involved in 38% of women and 39% of men. PPCI, with or without stent, was performed significantly more often in men (88.5% vs 83.7%, p<0.01) whereas the use of DES was similar. Thromboaspiration, GP IIb/IIIa inhibitors and intravenous anticoagulants were used less often in women

Association between gender and outcomes independent of randomised treatment

Female gender was associated with significantly higher risk for the composite outcome of death, MI, stroke, urgent revascularisation or definite acute stent thrombosis compared to male gender (6.8% vs 3.9%, crude HR 1.79, 95% CI 1.12 – 2.86). This difference was mainly driven by a 3-fold higher risk for all-cause mortality in women compared to men (5.7% vs 1.9%, HR 3.13, 95% CI 1.78 – 5.51). However, in the multivariable model, the gender effect was lower and not significantly associated with the composite primary outcome (HR 1.34, 95% CI 0.77 – 2.32) or with all-cause mortality (HR 1.98, 95% CI 0.97 – 4.04) (Figure 22).

Figure 22. Association between gender and clinical outcomes independent of randomised treatment.
Results

Women had significantly higher risk for major bleeding complications compared to men, irrespective of the bleeding definition used. However, after adjustment for baseline and clinical characteristics, female gender was no longer an independent predictor of risk for bleeding at 30 days (figure 22). Female gender was associated with lower risk for TIMI flow < 3 in the IRA at initial angiography (OR 0.64, 95% CI 0.46-0.91). The risk of incomplete ST-segment elevation resolution (<70%) before PCI did not differ significantly between the genders (adjusted OR 1.15, 95% CI 0.75-1.76). The risk of abnormal TIMI flow in the IRA and incomplete ST segment elevation resolution after PCI was similar in both genders (figure 23).

Association between gender and efficacy and safety of randomised treatment

Pre-hospital administration of ticagrelor compared to in-hospital administration resulted in a non-significant difference in the primary, secondary and safety outcomes. No significant interactions between randomised treatment and gender in terms of these outcomes were found in the unadjusted or adjusted (data not shown).

![Figure 23](image)

Figure 23. Association between gender and efficacy outcomes independent of randomised treatment

Paper IV

The median creatinine on arrival was 86 µmol/L and the median cystatin C was 1.1 mg/L. Cystatin C increased by 19% during hospitalisation whereas a 6% increase in creatinine value was observed (median values).

CKD prevalence

On arrival, the CG showed a tendency to overestimate the CKD prevalence compared to other formulas but no statistical significant differences could be detected. The lowest prevalence of CKD was obtained when the CKD-EPI and the rG-CystC formulas were used. At discharge, the rG-CystC significantly overestimated the prevalence of CKD.
compared to mGFR whereas the CG showed a similar tendency. The CKD prevalence according to the CKD-EPI and the MDRD-IDMS formulas were comparable to that obtained by mGFR. The rG-CystC formula significantly overestimated the prevalence of CKD compared to the CKD-EPI (p=0.02) and the MDRD (p=0.07) (Table 1).

Table 1. Prevalence of moderate-severe CKD on arrival and at discharge

<table>
<thead>
<tr>
<th></th>
<th>CKD, % (n)</th>
<th>p-value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>On arrival</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CG</td>
<td>35 (14)</td>
<td></td>
</tr>
<tr>
<td>MDRD-IDMS</td>
<td>27.5 (11)</td>
<td></td>
</tr>
<tr>
<td>CKD-EPI</td>
<td>25 (10)</td>
<td></td>
</tr>
<tr>
<td>rG-CystC</td>
<td>25 (10)</td>
<td></td>
</tr>
<tr>
<td>At discharge</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CG</td>
<td>42.5 (17)</td>
<td>0.3</td>
</tr>
<tr>
<td>MDRD-IDMS</td>
<td>32.5 (13)</td>
<td>1.0</td>
</tr>
<tr>
<td>CKD-EPI</td>
<td>30 (12)</td>
<td>1.0</td>
</tr>
<tr>
<td>rG-CystC</td>
<td>47.5 (19)</td>
<td>0.03</td>
</tr>
<tr>
<td>Iohexol</td>
<td>32.5 (13)</td>
<td></td>
</tr>
</tbody>
</table>

†p values for comparison of percentage of moderate-severe Chronic Kidney disease (CKD) patients obtained with the four formulas compared to mGFR by using McNemar test. CG (Cockcroft-Gault), MDRD-IDMS (Modification of Diet in Renal Disease - Isotope Dilution Mass Spectrometry), rG-CystC (relative Grubb cystatin C), CKD-EPI (Chronic Kidney Disease-Epidemiology Collaboration). n number.

Level of agreement between eGFR and mGFR to discriminate GFR <60 mL/min/1.73 m² is illustrated in Table 2. At discharge, the CKD-EPI had an almost perfect agreement with mGFR according to the Cohen’s kappa value, followed by the MDRD-IDMS. The rG-CystC formula had a substantial agreement but lower than the MDRD-IDMS whereas the CG achieved the lowest agreement among the formulas tested (moderate agreement). When agreement between different GFR estimates, both on arrival and at discharge, was examined, the creatinine-based formulas had an essentially unchanged agreement to each other whereas rG-CystC showed an almost perfect agreement with MDRD-IDMS and CKD-EPI on arrival but a substantially lower agreement at discharge (Table 2).

Table 2. Agreement between different estimates and mGFR to discriminate GFR greater and less than 60 mL/min/1.73 m²

<table>
<thead>
<tr>
<th></th>
<th>CG</th>
<th>MDRD-IDMS</th>
<th>CKD-EPI</th>
<th>rG-CystC</th>
<th>Iohexol</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG</td>
<td>+++ / +++</td>
<td>0.71 / 0.68</td>
<td>0.65 / 0.74</td>
<td>0.38 / 0.60</td>
<td>+++ / 0.58</td>
</tr>
<tr>
<td>MDRD-IDMS</td>
<td>0.71 / 0.68</td>
<td>+++ / +++</td>
<td>0.94 / 0.94</td>
<td>0.81 / 0.59</td>
<td>+++ / 0.77</td>
</tr>
<tr>
<td>CKD-EPI</td>
<td>0.65 / 0.74</td>
<td>0.94 / 0.94</td>
<td>+++ / +++</td>
<td>0.87 / 0.64</td>
<td>+++ / 0.83</td>
</tr>
<tr>
<td>rG-CystC</td>
<td>0.58 / 0.60</td>
<td>0.81 / 0.59</td>
<td>0.87 / 0.64</td>
<td>+++ / +++</td>
<td>+++ / 0.70</td>
</tr>
</tbody>
</table>

Cohen’s Kappa value for comparison at arrival / discharge. +++ comparison was not performed. CG (Cockcroft-Gault), MDRD-IDMS (Modification of Diet in Renal Disease - Isotope Dilution Mass Spectrometry), rG-CystC (relative Grubb cystatin C), CKD-EPI (Chronic Kidney Disease-Epidemiology Collaboration).

Correlation, bias, precision and accuracy
The MDRD-IDMS, the CKD-EPI and the rG-CystC estimates had better correlation to
mGFR, compared to the CG. The rG-CystC estimates yielded the best correlation with mGFR. All creatinine-based estimates showed a low bias. The rG-CystC formula had a marked bias of -17.8%. The rG-CystC formula had the highest precision, followed by the CKD-EPI and the MDRD-IDMS that yielded an almost equal value, whereas the CG showed the lowest precision between the formulas tested. The CKD-EPI and the MDRD-IDMS formulas had the highest accuracy. The rG-CystC formula had a slightly lower accuracy compared with the CKD-EPI and the MDRD-IDMS. The CG formula showed a considerably lower accuracy compared to the other equations (Table 3). We found a moderate correlation between the cystatin C rise during hospitalisation (absolute values) and troponin values 6-8 h after admission (R=0.51, p=0.01).

**Table 3.** Correlation, bias, precision and accuracy (P30) of prediction equations to estimate relative m-GFR (mL/min/1.73 m²)

<table>
<thead>
<tr>
<th>At discharge</th>
<th>Correlation (R)</th>
<th>Bias, median error (%)</th>
<th>Precision (IQR), mL/min/1.73 m²</th>
<th>P30 (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG</td>
<td>0.73</td>
<td>-1.2 (-1.3)</td>
<td>22.5</td>
<td>75.0% (62 – 88%)</td>
</tr>
<tr>
<td>MDRD-IDMS</td>
<td>0.78</td>
<td>-0.8 (-1.3)</td>
<td>17.9</td>
<td>82.5% (70.5 – 94.5%)</td>
</tr>
<tr>
<td>CKD-EPI</td>
<td>0.81</td>
<td>0.9 (1.5)</td>
<td>17.1</td>
<td>82.5% (70.5 – 94.5%)</td>
</tr>
<tr>
<td>rG-CystC</td>
<td>0.89</td>
<td>-12.2 (-17.8)</td>
<td>14.8</td>
<td>80.0% (68 – 92%)</td>
</tr>
</tbody>
</table>

Bias was defined as the median percentage error between eGFR and mGFR, positive values indicate an overestimation of mGFR. Precision was assessed as the interquartile range (IQR) expressed in mL/min/1.73 m² of the difference eGFR – mGFR. Accuracy within 30% (P30) was the percentage of estimates within 30% of mGFR. Correlation between eGFR and mGFR was reported as correlation coefficients (R). CG (Cockcroft-Gault), MDRD-IDMS (Modification of Diet in Renal Disease - Isotope Dilution Mass Spectrometry), rG-CystC (relative Grubb cystatin C), CKD-EPI (Chronic Kidney Disease-Epidemiology Collaboration).
DISCUSSION

Despite recent advantages in the management of STEMI patients, including implementation of PPCI as the reperfusion therapy of choice, short and long term mortality after the index event remains high (figure 24).

![Figure 24. 30-day and 1-year mortality after PCI in STEMI patients in Sweden, 2007-2015 (Swedeheart annual report 2016)](image)

Therefore, actions to further improve outcomes are warranted. In this direction, judicious choice of antithrombotic drugs in order to obtain a balance between the risk for ischemic and bleeding complications is crucial. Novel ways of orally administrated antiplatelet agents may improve their pharmacodynamic properties and reduce the risk for acute stent thrombosis. The effectiveness and safety of new treatment strategies should be tested in both genders. Furthermore, identification of gender as an independent predictor of adverse events may lead to target therapeutic interventions that will improve outcomes. Finally, accurate estimation of renal function in STEMI patients during the initial hospitalisation is important to avoid overdosing of antithrombotic agents and contrast media as well as making decisions about use of other life-saving therapies and additional risk stratification.

Our results showed that bivalirudin monotherapy with bailout use of GPI significantly reduced the risk for major in-hospital bleeding with no difference for the risk of short and long term mortality compared to UFH with or without GPI. Novel ways of ticagrelor administration resulted in a faster and stronger platelet inhibition compared to standard ticagrelor administration that may have a clinical implication in STEMI patients, especially those treated with bivalirudin. Prehospital administration of ticagrelor showed similar efficacy and safety in both genders. Women had a significantly higher risk for early mortality and bleeding complication compared to men.
but this difference was significantly attenuated after adjustment for age and baseline characteristics. Finally, the CKD-EPI followed by the MDRD-IDMS formula more precisely estimated renal function in STEMI patients during the acute phase compared to other formulas whereas further research is needed to clarify the role of cystatin C.

**Bivalirudin versus UFH, with or without GPI (Paper I)**

In this large real-world study of STEMI patients treated with PPCI, we found no significant difference in 30-day or one-year mortality, myocardial infarction or stent thrombosis in patients treated with bivalirudin±GPI compared with UFH±GPI. Bleeding complication rates were significantly higher with UFH±GPI. Our study showed no difference in short or long-term mortality between the two treatment groups. The mortality benefit associated with bivalirudin in the HORIZONS-AMI trial was mainly attributed to the reduced risk for bleeding complications, but the mortality benefit persisted even when patients with a major bleeding were excluded. Despite a significant reduction in the risk of major bleeding in the BRIGHT and EUROMAX trial, no difference in mortality was observed in accordance with our results. The recent HEAT-PCI showed no significant difference in 30-day mortality and bleeding complications between the two treatment strategies.

In the EUROMAX trial, UFH was combined with GPI in a similar proportion of cases as in our trial (69%) whereas in the BRIGHT trial and the HEAT-PCI trial, GPI was used as bailout treatment in both bivalirudin and UFH group. Recently, the MATRIX study became the first RCT after HORIZONS-AMI suggesting a mortality benefit with bivalirudin over UFH±GPI. However, there was no difference in the primary outcome, hence the observed difference in mortality may have been a play of chance. Reasons for the observed differences between these studies are incompletely understood but may, at least partially be explained by differences in UFH dose, bivalirudin infusion time, use of modern P2Y12-inhibitors, access site and rate of GPI use between groups. Notably, none of the earlier studies was powered to detect differences in mortality. Consistently with our results, a recent meta-analysis, including the five most recent RCTs with more than 10 000 patients, showed no difference in short term mortality between bivalirudin±GPI and UFH±GPI. In concordance with the BRIGHT trial, lack of difference between the two treatment strategies persisted at one-year follow-up.

In our study, the mortality rates were substantially higher than in RCTs, but similar to observational STEMI trials, emphasising the importance of post-marketing studies in real-world high-risk populations, which include patients that are usually not included in RCTs. In our analysis we used three different risk-adjustment models to compensate for the observational nature of our data and the findings strongly concurred.

Bleeding complications have been associated with worse outcome, but the mechanisms are not clarified. In our study Bivalirudin ± GPI reduced the unadjusted risk of non-CABG major in-hospital bleeding by 65%, a difference that remained after adjustment. An abundance of evidence from observational and randomised trials support our findings, demonstrating a safety benefit with bivalirudin over UFH, with various degrees of GPI administration. Bivalirudin’s predictable biological activity, short half-life and the absence of thrombocytopenia are plausible explanations for the reduced bleeding risk. More frequent use of GPIs with UFH in our study, may obviously have contributed to the increased bleeding rate. Anyhow, GPIs may have still have a role in selected patients, but the optimal rate remains unclear.

In contrast to previous studies we did not observe higher rate of ST associated with bivalirudin, after adjustment for baseline differences. The rate of early ST in the
Discussion

UFH±GPI group is consistent previously reported, but the rate in the bivalirudin±GPI group was substantially lower in our study. Lower rate of ST associated with bivalirudin in our data, at least partially, be explained by: 1. Initial prehospital treatment with UFH is common practice in Sweden (79.5% in our study) irrespective of later treatment strategy, which has been shown to lower ST rate. Early implementation and high rate of third generation P2Y12-inhibitors. 3. Widespread use of bivalirudin infusion. In the BRIGHT trial, with a high dose post-PCI bivalirudin infusion in all patients treated with bivalirudin, the risk of early stent thrombosis was 0.4% in bivalirudin compared to 0.6% in UFH, which is in accordance with our results. Similar trends were observed in a subgroup analysis of the EUROMAX trial in patients receiving a post-PCI bivalirudin infusion. In contrast to the HORIZONS-AMI trial, we found a significantly higher rate of reinfarction in the UFH group. However, after adjustment, the difference was not statistically significant. In accordance with our results, recent studies and meta-analyses have not confirmed a lower reinfarction rate with bivalirudin compared to UFH±GPI, despite the superior safety profile of bivalirudin. Given the current conflicting evidence, our study provides important information regarding the efficacy and safety (short and long-term) of the most commonly used antithrombotic strategies during PPCI in a large real-world population with STEMI.

Novel ways of orally administrated ticagrelor - The IPAAD-Tica study (Paper II)

Our study shows that a LD of crushed or chewed ticagrelor tablets achieved a more rapid and more effective platelet inhibition compared to LD of standard integral tablets. In addition, and to the best of our knowledge, for the first time, we show that administration of chewed ticagrelor tablets is feasible and may provide a faster and stronger platelet inhibition than administration of either crushed or integral tablets. Within 20 minutes after chewed ticagrelor LD, no patient had HRPR and a very low residual PR was observed (median PR 84 PRU). As the degree of platelet inhibition by ticagrelor is linearly related to the plasma concentration of the drug, our data indicate that chewed ticagrelor achieved the most rapid and the highest rate of drug absorption. Furthermore, the faster and stronger platelet inhibition with chewed ticagrelor compared to crushed ticagrelor implies that other mechanisms than the mechanical “fractioning” of the tablets may have contributed to the improved absorption of the chewed ticagrelor. Initiation of enzymatic metabolic degradation of the tablet in the mouth due to the prolonged contact of the drug with the saliva as well as enhanced oral transmucosal absorption of the drug may be explanations for our results. In this study we confirm earlier knowledge regarding pharmacodynamic properties of integral and crushed ticagrelor LD. In a previous study, patients with stable angina were randomised to ticagrelor versus clopidogrel LD. Within 60 minutes, 80% inhibition of PR was observed in the ticagrelor group in accordance with our results in the integral ticagrelor group. A recent study compared crushed versus integral ticagrelor tablets in STEMI patients. Crushed ticagrelor provided earlier inhibition of platelet aggregation than integral tablets. At 60 minutes, 63% in the integral ticagrelor group and 35% in the crushed ticagrelor group had HRPR. In our study, compared to integral ticagrelor, 50% lower rate of HRPR was observed with crushed ticagrelor at 20 minutes. Lower baseline PRU values and earlier onset of drug action in our stable population may explain the difference between the two studies.
Discussion

Our findings may be of clinical importance in STEMI patients. According to an earlier trial, more than half of the STEMI patients treated with LD of integral ticagrelor still have HRPR up to 4 hours after administration of the drug. Given the fact that suboptimal platelet inhibition is an important predictor of ischemic complications, such as stent thrombosis, there is a time window after PPCI, in which patients are at increased risk for ischemic complications. In the ATLANTIC trial, despite a short median time between pre-hospital and in-hospital administration of ticagrelor, prehospital administration significantly reduced the risk of stent thrombosis, suggesting that fast and strong platelet inhibition at the time of PCI is clinically important. A significantly faster and stronger platelet inhibition, by overcoming the delayed intestinal absorption of orally administrated drugs, with chewed ticagrelor administration may improve clinical outcomes compared to integral tablets. A recent study in STEMI patients confirmed the superior pharmacodynamic properties of crushed ticagrelor compared to integral tablets. Crushed tablet preparation in the prehospital setting, such as in the ambulance, may be cumbersome whereas chewed ticagrelor administration is more comfortable and according to our data, may provide a more effective platelet inhibition compared to crushed ticagrelor administration. Theoretically, a stronger platelet inhibition at the time of PCI may increase the risk of bleeding. In the ATLANTIC trial, prehospital administration of ticagrelor appeared to be safe, compared to in hospital administration. In our study, the risk of bleeding was not increased with crushed or chewed ticagrelor administration.

Association between gender and risk for short term mortality and bleeding in STEMI patients treated with primary PCI and novel antiplatelet therapy (Paper III).

In the present analysis, unadjusted data showed a significantly higher risk of the composite outcome of death, MI, stroke, urgent revascularisation or definite acute stent thrombosis in women, mainly driven by a three times higher risk for short-term mortality. After multivariable adjustment female gender was not an independent predictor of worse outcome. Undoubtedly, older age and clustering of comorbidities in women could explain a large part of the observed difference in short term clinical outcomes in our study. However, the adjusted risk for death, while not statistically significant, was still considerably higher in women, generating the hypothesis that gender could be an independent predictor of early mortality if the population of the study was larger. Any possible contribution of gender, as independent risk factor, on adverse outcome may depend on the population studied. The age gap between genders in our population was substantially higher compared to previous studies, with a mean age difference of 10 years. In spite of appropriate statistical methods that were used for adjustment, adjustment for such age-difference is awkward. Additionally, the age difference may indicate a selection bias. As identification and randomisation took place in the ambulance, young women with suspected STEMI were possibly not included in the study as alternative reasons for ST-segment elevation on prehospital ECG may have been considered more plausible in this group. These factors may explain the observed trend toward increased adjusted risk for early mortality in women.

Previous observational studies in STEMI cohorts, without focus on PPCI treated patients have shown a higher risk for early mortality in women. Significant differences in the rate of reperfusion therapy between genders and hidden confounders such as frailty in elderly women with STEMI may have influenced their results. Recent
Discussion

Observational studies including patients treated with PPCI\textsuperscript{122, 192, 193} and pre-specified gender analysis of RCTs\textsuperscript{126, 127, 132, 189, 194} have shown that the impact of gender on mortality could mainly be explained by differences in age and comorbidities between genders, in accordance with our results. On the contrary, another observational study and a meta-analysis have reported higher multivariable adjusted risk of early mortality in women with STEMI treated with PPCI\textsuperscript{7, 121}. Differences in the covariates included in the multivariable analysis between studies may explain the conflicting results. Previous studies have clearly demonstrated the importance of body surface area and the eGFR on prognosis\textsuperscript{195, 196}. In a meta-analysis, Berger et al showed that female gender was an independent predictor of early mortality in STEMI patients. However, 30-day mortality was not statistically significant different between genders after additional adjustment for angiographic disease severity\textsuperscript{197}.

In the present study, women had a 2-3 times higher unadjusted risk for non-CABG related bleedings, depending on the definition used. After adjustment for baseline characteristics, no significant difference remained. Female gender has previously been associated with higher risk for bleeding complications in patients with ACS\textsuperscript{5, 179, 198, 199}. Known predictors of bleedings like advanced age, diabetes, hypertension, renal insufficiency and anemia, are usually more often encountered in women with STEMI\textsuperscript{86}. Additionally, smaller body and vessel size, higher use of femoral access and overdosing of antithrombotic medication in women may explain the higher observed risk for bleeding. Procedural-related improvement such as increased use of radial access or smaller femoral sheaths and careful dose adjustment of antithrombotic medication, have resulted in a significant decline in the risk of bleeding/vascular complication during cardiovascular interventions the last years and have probably contributed to our results\textsuperscript{200}.

In terms of ST-segment elevation resolution and TIMI flow 3 rates before and after PCI, no significant difference between genders was observed apart from a lower risk of abnormal coronary flow in IRA at initial angiography in women. The higher rate of TIMI flow 3 at initial angiography may be explained by the higher rate of normal coronary arteries as well as less obstructive coronary artery disease in women with STEMI, which is in concordance with previous data\textsuperscript{201}.

The analysis of the effect of gender on outcomes dependent on the randomised treatment showed no statistically significant interactions. Pre-hospital administration of ticagrelor did not improve pre- or post-PCI coronary reperfusion as assessed by TIMI flow in IRA and resolution of ST elevation pre-PCI. Clinical efficacy outcomes were not significantly improved either. However, prehospital administration of ticagrelor was safe in both genders with similar rate of major and minor bleedings.

GFR estimations in STEMI patients during the initial hospitalisation.

In our study, CKD-EPI followed by the MDRD-IDMS formula showed a good overall performance to estimate GFR. Validation of the most widely used creatinine based GFR estimates, in the acute phase after a STEMI, showed largely confirmatory result. The similar overall accuracy of the MDRD-IDMS and the CKD-EPI equations was consistent with the original CKD-EPI external validation cohort, where P30 was 84% for the CKD-EPI formula and 81% for the MDRD-IDMS formula\textsuperscript{152}. The low bias for the MDRD-IDMS and the CKD-EPI formulas was comparable with the results in previous studies\textsuperscript{202}. The CKD-EPI showed the best ability to discriminate patients with CKD and that can be explained by the
population studied. The CKD-EPI performs better in patients with GFR≥60 ml/min/1.73 m^2 and reduces the rate of false-positive diagnosis of CKD (eGFR<60 ml/min/1.73 m^2). The original MDRD formula was developed by studying 1628 patients with non-diabetic CKD using the Jaffe method of creatinine measurement but was re-expressed for use with the standardised serum creatinine assay. The best accuracy is obtained in patients with CKD whereas the formula has a tendency to underestimate the mGFR in patients without CKD. As in previous studies, the CG formula had a tendency to overestimate the prevalence of CKD compared to other GFR estimates. The CG formula has many limitations. First, it was derived from a small population, predominantly men and an arbitrary correction for women was proposed. Second, it calculates creatinine clearance that may significantly differ from GFR. Third, the formula has not been re-expressed for use with the standardised serum creatinine assay. However, the CG has extensively been used and previous studies have shown that the eGFR based on CG is a better predictor of adverse outcomes than that based on the MDRD-IDMS and probably the CKD-EPI equations in patients with myocardial infarction. According to our results, the predictive ability of the CG formula should be attributed to the coefficients included in the formula and not to the better estimation of GFR, as other authors have previously suggested. Validation of the rG-CystC formula in our STEMI population showed inconsistent results with previous studies. In the development and validation population, the rG-CystC equation showed a very good performance, at least as good as the MDRD-IDMS. In the absence of a universal calibrator for cystatin C, the choice of the cystatin C measurement method is of crucial importance and can importantly affect the performance of a cystatin C-based equation that is not derived by using the same calibrator and method, resulting in significant methodological error in GFR estimations. In our study, we calculated eGFR by using a rG-CystC formula that was recommended by the manufacturer. Therefore an important methodological error in the cystatin C measurements was considered improbable. It should be noticed that the performance of the rG-CystC equation altered during the study period. At arrival, CKD-EPI and rG-CystC showed an almost perfect agreement, classified almost the same patients as having CKD but at discharge their agreement was substantially lower and they showed an important discrepancy in CKD classification that reached statistical significance. During hospitalisation, cystatin C increased by 19%, disproportional to creatinine rise. Theoretically, that could be attributed to the early detection of renal injury by cystatin C and not by creatinine. Nevertheless, that can be ruled out as the rG-CystC considerably overestimated the CKD prevalence compared to mGFR and the CKD-EPI showed an almost perfect agreement with mGFR at discharge.

Our findings suggest that other pathophysiological events were probably involved in the rise of cystatin C and the observed bias of the rG-CystC, e.g. plaque rapture, inflammation and myocardial necrosis. Cystatin C has been shown to be an independent predictor of mortality and MI in patients with stable angina, ACS and STEMI and previous studies have suggested that cystatin C is not only a marker of renal function but is also correlated with atherosclerosis, inflammation, plaque vulnerability and rupture. Cystatin C, independently of the estimated renal function, has been shown to be associated with increased risk of developing cardiovascular disease. It is an endogenous inhibitor of cathepsins that are cysteine proteases secreted by all nucleated cells and played an important role in the extracellular matrix degradation. An imbalance between cystatin C and cathepsins may trigger a pathophysiological cascade involving...
leading to atherosclerosis and collagen degradation in the cap of the atherosclerotic plaque, increasing the risk of rupture. Levels of cathepsins are elevated in STEMI patients at arrival. Additionally, cathepsins are probably involved in healing process after STEMI and cathepsin-mediated turnover of collagen is increased during the first days following the myocardial infarction. The observed increase of cystatin C in our population may represent a response to the highly activated cathepsins, trying to counterbalance the effect of those enzymes. This hypothesis is supported by experimental studies showing that cystatin C is produced by cardiomyocytes as a response to myocardial ischemia and elevated cystatin C results in inhibition of cathepsins. We examined a possible relationship between cystatin C increase and troponin level in our population. We found a significant but moderate correlation between cystatin C rise and troponin and a weaker correlation between creatinine rise and troponin. This partially reflects the cardio-renal syndrome but could also indicate a relationship between the infarct size and the cystatin C elevation. Apart from an elevated production of cystatin C during the acute phase of myocardial infarction, an altered glomerular endothelial permeability due to infarct-related inflammation may impair the filtration rate of cystatin C that is a more than 100 times larger molecule than creatinine, leading to an accumulation of cystatin C in the blood.
CONCLUSIONS – CLINICAL IMPLICATIONS

In STEMI patients treated with PPCI, bivalirudin was the optimal antithrombotic therapy compared to UFH with or without GPI. Treatment with bivalirudin and low rates of concomitant GPI administration was associated with a significant reduction in the risk of non-CABG major and major or minor in-hospital bleeding compared to UFH and high rates of GPI administration. This safety benefit with bivalirudin was not associated with a significant reduction in mortality. Furthermore, it is still a matter of debate if bivalirudin increases the risk for early stent thrombosis compared to UFH and provisional GPI administration. Taking into account the substantially higher financial cost with bivalirudin compared to UFH, physicians may consider individualised selection of antithrombotic drug during PPCI guided by the estimated risk of acute stent thrombosis and bleeding based on risk scores or clinical experience. In patients with high risk of bleeding, treatment with bivalirudin should always be preferred. In patients at increased risk of stent thrombosis but low risk of bleeding UFH with bailout GPI administration remains an option.

Novel ways of orally administrated ticagrelor were feasible and provided a faster and a more effective platelet inhibition compared to standard ticagrelor tablets. Our findings may have an important clinical implication in STEMI patient in whom insufficient platelet inhibition at the time of PPCI has been associated with an increased risk of stent thrombosis. In bivalirudin treated patients, early administration of chewed ticagrelor tablets may mitigate the risk for early stent thrombosis and further improve outcomes.

Women with STEMI treated with PPCI and ticagrelor had a significantly higher unadjusted risk of early mortality and bleeding complications compared to men. However, these differences were significantly attenuated after adjustment for baseline characteristics and age. Scores for bleeding risk estimation incorporate female gender together with baseline and procedural characteristics such as advanced age, anemia and reduced renal failure that are more often presented in women than in men with STEMI. In women with high risk of bleeding, bivalirudin use and cautious antithrombotic dose-adjustment based on estimated renal function are some of the actions warranted in order to reduce the bleeding risk and improve outcomes.

According to our results MDRD and CKD-EPI should be the formulas used for estimation of GFR in the acute phase of STEMI. On the other hand, CG should be abandoned as it overestimates the prevalence of CKD. Finally cystatin C based formulas should be used with cations in the acute phase of STEMI until more studies confirm or reject our findings. Due to the crucial role of renal function on the acute management, risk stratification, avoidance of overdosing of drug cleared by the kidneys and early initiation of other life-savings therapies, accurate estimation of GFR by formulas may have a significant influence on outcomes.
FUTURE DIRECTIONS

Bivalirudin is superior to UFH and provisional GPI in the reduction of bleeding. However, UFH monotherapy at the lower recommended dose with bailout GPI administration appears as a promising alternative. It remains unclear if bivalirudin monotherapy has a superior safety profile compared to UFH monotherapy. Furthermore, it is still unclear if premedication with a single dose UFH, prolonged post-PCI infusion of bivalirudin and early administration of ticagrelor may mitigate the risk of early stent thrombosis in bivalirudin treated patients. Results from the VALIDATE-SWEDEHEART trial, a register-based RCT, randomised 7000 ACS patients to bivalirudin or UFH monotherapy, are awaited to provide further evidence in this area.

The superior pharmacodynamic properties of chewed ticagrelor compared to crushed ticagrelor should be confirmed in a new study. The efficacy and safety of chewed or crushed ticagrelor compared to integral ticagrelor in STEMI patients should be tested in a RCT.

The association between gender and the risk of early mortality and bleeding in STEMI patients treated with PPCI, novel antiplatelet therapy and bivalirudin or UFH monotherapy with bailout-only GPI administration should be evaluated in a large study. A pre-specified gender analysis of the VALIDATE-SWEDEHEART is planned.

Our findings suggested that inflammation and myocardial necrosis were involved in the observed rise of cystatin C during the acute phase in STEMI patients. Further research is needed to confirm our finding and provide pathophysiologic explanations for this observation.
ACKNOWLEDGEMENTS

This thesis could not have been completed without the great support of so many people during these past four years. I would like to express my sincere gratitude to all of you who have helped, supported and inspired me during these years. In particular, I wish to express my gratitude to:

**All patients** who participated in our studies.

**Linköping University** and “**Institution of Medicine and Health**” for giving me the opportunity to perform my PhD training.

Professor **Eva Swahn** - my main supervisor. Thank you for “dragging” me into clinical research, I do not really know if I had been engaged without you. For sharing with me your deep knowledge about research. I am grateful for your support all these years and your generous attitude. For your inspiring enthusiasm that you succeed to share with your colleagues. For always being available and providing rapid feedback. For giving me the opportunity to choose my own scientific paths. For good advice, not just about scientific questions but also about life. For opening your home and creating a welcoming atmosphere for me. For offering me your friendship.

**Joakim Alfrdsson**, my co-supervisor. Thank you for your great contribution to my thesis. For constructive, valid and valuable remarks regarding my projects. For keeping me on tracks many times by supporting or discouraging new ideas and there have been many. For your vast knowledge about registry-based research and statistic that you have generously shared with me. Thank you for your patience and your time. It was a pleasure and a privilege to have you and Sofia as fellow passengers during this exciting trip.

**Sofia Sederholm-Lawesson**, my co-supervisor. Thank you for your deep involvement in my thesis. For your important contribution in the design, performance, analysis and presentation of my projects. For introducing me to SPSS and to mystics “syntaxes”. For your tremendous generosity, kindness and enthusiasm. For your linguistic and editorial revision of all my articles. For your efforts to promote research in our department. It has been a pleasure to travel with you to international congresses presenting our results.

**Magnus Janzon**, head of the department of Cardiology at University Hospital in Linköping. Thank you for your kind support, encouragement and for giving me the opportunity to participate in international congresses as well as to take time off for my research. “Research at the centre” has been the department’s moto and Magnus has an important contribution to that.

**Elisabeth Logander** your skills makes you the best research nurse ever. Thank you for successfully guiding me in the labyrinth of different applications and advising me about Good Clinical Praxis. Your advices will follow me in the future.
Acknowledgements

Maria Eriksson, research nurse, for your help with IPAAD-Tica study and editorial revision of my abstracts.

All my co-authors, in all papers for their invaluable remarks and recommendations.

My colleague Anders Björkholm for teaching me fundamentals in coronary angiography and PCI.

My colleague Tim Tödt for pushing me to break my limits.

My friend and colleague Manolis Charitakis, who recently defended his thesis, for your friendship, support and for our endless discussions about statistics and new research projects.

My friend and colleague Giorgios Panayi for always taking time to listen.

My good friend Andreas Bousios for your friendship and help to organise the party.

All my past and present colleagues in the Cardiology department and catheterisation laboratory at Linköping for their friendship, support and involvement in my projects.

All nurses in the catheterisation laboratory at Linköping and Norrköping for participating in my projects and for pleasant times at work and outside the hospital.

All nurses in the Seldinge outpatient clinic at Linkoping University Hospital for their participation in the data collection of the IPAAD-Tica study.

Richard Cairns from Worldwide Clinical Trials UK who performed the statistical analysis of the ATLANTIC gender analysis and Liz Anfield, Prime Medica Ltd, Knutsford, Cheshire, for editorial support of the ATLANTIC gender analysis.

My parents, for the principles and the unconditional love you gave me.

To my beloved family. My wife Despina for your love and endurance and for showing me what is truly meaningful in life. My wonderful daughters, Silia and Anastasia for the joy and happiness you give me every day.
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Papers

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