Biomarkers of Renal Function in Acute Coronary Syndromes

AXEL ÅKERBLOM
Abstract

The thesis aimed to investigate cystatin C and creatinine-based estimates of glomerular filtration rate (eGFR), both at admission and during follow-up, on the combined endpoint of cardiovascular death and myocardial infarction in patients with acute coronary syndrome (ACS). We also evaluated two cystatin C assays and assessed genetic determinants of cystatin C concentrations.

We used the PLATElet inhibition and Patient Outcomes study, where all types of ACS patients (n=18624) were randomized to ticagrelor or clopidogrel treatment. Multivariable Cox regression models, including clinical variables and biomarkers (troponin and NT-proBNP), and c-statistics were calculated.

Cystatin C and the creatinine-based CKD-EPI equation exhibited similar significant prognostic impact on the combined endpoint, with Area Under Curves (AUC) 0.6923 and 0.6941, respectively. Follow-up samples of renal biomarkers did not improve risk prediction.

Patients randomized to ticagrelor treatment were associated with a non-sustained larger increase in renal markers at discharge, but neither the change nor the difference between the randomized groups affected cardiovascular risk.

Two different cystatin C assays exhibited good correlation 0.86 (95% confidence interval 0.85-0.86), however moderate level of agreement. Risk prediction with a combination of creatinine and cystatin C did not outperform the creatinine-based CKD-EPI equation, AUC: 0.6913 and 0.6924, respectively (n=13050).

The genetic polymorphism rs6048952 independently affected the cystatin C concentration with mean levels of 0.85mg/L, 0.80mg/L and 0.73mg/L for the A/A, A/G, and G/G genotypes, respectively.

The genetic polymorphism did not affect outcome overall, however in the non-ST-elevation ACS subgroup a signal that genetic polymorphism may be associated with cardiovascular death was observed (p=0.002).

In conclusion: cystatin C or eGFR, irrespective of equation or assay, are important cardiovascular risk factors in ACS patients. Nonetheless, the incremental value of adding any renal variable, to a multivariable risk model, is small. Further research on the impact of cystatin C genetic polymorphism is warranted.

Keywords: cystatin C, glomerular filtration rate, GFR, creatinine, acute coronary syndrome, ACS, kidney, renal, mortality, death, myocardial infarction

Axel Åkerblom, Uppsala University, Department of Medical Sciences, Cardiology, Akademiska sjukhuset, SE-751 85 Uppsala, Sweden.

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ISSN 1651-6206
urn:nbn:se:uu:diva-197852 (http://urn.kb.se/resolve?urn=nbn:se:uu:diva-197852)
Sometimes you’re ahead,  
sometimes you’re behind.  
The race is long,  
and in the end,  
it’s only with yourself.

Mary Schmich

To my Family
List of Papers


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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Adenine</td>
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<td>Å</td>
<td>Ångström</td>
</tr>
<tr>
<td>ACS</td>
<td>Acute Coronary Syndrome</td>
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<td>AKI</td>
<td>Acute Kidney Injury</td>
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<tr>
<td>ANCOVA</td>
<td>Analysis of Covariates</td>
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<tr>
<td>BMI</td>
<td>Body Mass Index</td>
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<td>BSA</td>
<td>Body Surface Area</td>
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<td>C</td>
<td>Cytosine</td>
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<td>CCr</td>
<td>Creatinine Clearance</td>
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<td>CRP</td>
<td>C-Reactive Protein</td>
</tr>
<tr>
<td>CABG</td>
<td>Coronary Artery Bypass Grafting</td>
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<tr>
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<td>Coronary Artery Disease</td>
</tr>
<tr>
<td>CG</td>
<td>Cockcroft Gault</td>
</tr>
<tr>
<td>CIN</td>
<td>Contrast Induced Nephropathy</td>
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<tr>
<td>CKD</td>
<td>Chronic Kidney Disease</td>
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<td>Chronic Kidney Disease – Epidemiology Collaboration</td>
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<td>DNA</td>
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<td>DTPA</td>
<td>Diethylenetriaminepentaacetate</td>
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<td>ECG</td>
<td>Electrocardiogram</td>
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<tr>
<td>eGFR</td>
<td>Estimated Glomerular Filtration Rate</td>
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<tr>
<td>G</td>
<td>Guanine</td>
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<tr>
<td>GFR</td>
<td>Glomerular Filtration Rate</td>
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<tr>
<td>GWA</td>
<td>Genome-Wide Association</td>
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<td>GRACE</td>
<td>Global Registry of Acute Coronary Events</td>
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<td>IABP</td>
<td>Intra-Aortic Balloon Pump</td>
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<tr>
<td>IDI</td>
<td>Integrated Discrimination Improvement</td>
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<tr>
<td>IDMS</td>
<td>Isotope Dilution Mass Spectrometry</td>
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<tr>
<td>ISIS</td>
<td>International Study of Infarct Survival</td>
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<tr>
<td>LBBB</td>
<td>Left Bundle Branch Block</td>
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<tr>
<td>MDRD</td>
<td>Modification in Diet and Renal Disease</td>
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<td>mGFR</td>
<td>Measured Glomerular Filtration Rate</td>
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<tr>
<td>MI</td>
<td>Myocardial Infarction</td>
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<tr>
<td>mRNA</td>
<td>Messenger Ribonucleic Acid</td>
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<tr>
<td>NKF</td>
<td>National Kidney Foundation</td>
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</table>
NKDEP  National Kidney Disease Education Program
NH-Stroke  Non Hemorrhagic Stroke
NSTEMI  Non ST-Elevation Myocardial Infarction
NSTE-ACS  Non ST-Elevation Acute Coronary Syndrome
NT-proBNP  N-terminal -pro B-type Natriuretic Peptide
PAD  Peripheral Artery Disease
PCI  Percutaneous Coronary Intervention
PLATO  A Study of PLATelet inhibition and Patient Outcomes
RNA  Ribo Nucleic Acid
SCr  Serum Creatinine
SNP  Single Nucleotide Polymorphism
STE-ACS  ST-Elevation Acute Coronary Syndrome
STEMI  ST-Elevation Myocardial Infarction
T  Thymine
TIMI  Thrombolysis in Myocardial Infarction
TnI  Troponin I
TnT  Troponin T
WHO  World Health Organization
Introduction

Atherosclerotic cardiovascular disease (CVD) is a chronic disorder developing insidiously throughout life and usually progressing to an advanced stage by the time symptoms occur. It remains the major cause of premature death in Europe, even though CVD mortality has fallen considerably over recent decades in many European countries.

Preambles in the European Society of Cardiology’s - Guidelines on Cardiovascular Disease Prevention [1]

Cardiovascular (CV) disease is the major cause of mortality in the world [1-6]. Although mortality rates have improved considerably in recent years, partly due to advances in treatment strategies and even more importantly to increased awareness and improved treatment of risk factors for coronary artery disease, the occurrence of cardiovascular disease and the recurrence rate remain high [1, 2, 4-10]. CV disease is strongly connected to lifestyle, especially the use of tobacco, unhealthy diet habits, physical inactivity and psychosocial stress [1, 4, 7-12]. Reduced kidney function is a major risk factor with both increased risk for recurrent cardiovascular morbidity and mortality [1, 13-17]. Furthermore kidney disease is of great importance, not only as a marker of risk but also, as an important piece of information in choosing treatment strategies as well as dosing adjuvant drugs [18, 19]. In patients presenting with ACS, an accurate method for estimating kidney function, a reliable risk prediction and an in depth knowledge of renal function and markers of glomerular filtration is of great importance.

The aim of my thesis is to investigate the relationship between biomarkers reflecting kidney dysfunction in patients with ACS and their relationship to CV death and myocardial infarction (MI). Furthermore, the thesis was aimed at improving CV risk stratification based upon the combination of biochemical and genetic data in this high risk population.
Background

Acute Coronary Syndromes

The acute coronary syndrome (ACS) is the acute phase of coronary artery disease encompassing the clinical manifestations of unstable angina, acute myocardial infarction, and sudden coronary arrest [18-21]. Despite having a partly similar pathophysiologic background, stable coronary artery disease is not classified as ACS and will not be discussed any further in this presentation [22].

The term ACS is applied to patients with an underlying suspicion of myocardial ischemia and is defined by three parameters; cardiac symptoms, ECG appearance and biomarkers reflecting myocardial damage [18, 19]. The presence of cardiac symptoms and either abnormal ECG appearance or a dynamic rise in cardiac biomarkers is necessary for ACS diagnosis [18, 19, 23]. ACS is subdivided into three groups: ST-elevation MI (STEMI), non-ST elevation MI (NSTEMI) and unstable angina (UA). The ECG appearance distinguishes between the ST-elevation ACS (STEMI) and the non-ST-elevation ACS (NSTEMI or UA) while a typical rise in biomarkers reflecting myocardial necrosis (> 99% percentile, with a rise and fall pattern preferably in troponin) is absent in patients with UA [18, 19, 23]. For ST-elevation ACS, cardiac symptoms and ST-elevation on ECG is sufficient for diagnosis, irrespective of biomarker results [18].

Typical cardiac ischemic symptoms have been widely described in the literature but are exhibiting large individual differences [19, 24]. However classical symptoms include: retrosternal pain or a strangling sensation often accompanied with shortness of breath and chest discomfort [9, 19, 24, 25]. Radiating pain towards the left shoulder, neck/jaw or back is common as well as nausea, a light-headed feeling or syncope [9]. Patients with ST-elevation ACS often present with acute onset and severe symptoms while the clinical presentation in non-ST-elevation ACS can often be vaguer [15, 16]. In one study examining patients with unstable angina, the total number of patients with atypical symptoms (not substernal chest pain) exceeded 50% [24].
The non-ST-elevation ACS cases have a better short term and in-hospital prognosis than STE-ACS. However they are accompanied by a worse long term prognosis [19, 26, 27]. Patients with NSTEMI have underlying disease more often and are generally older at presentation [19, 26-28]. Although mortality rates have improved considerably in recent years, partly due to advances in treatment strategies and even more importantly to increased awareness and improved treatment of risk factors for coronary artery disease, approximately 1 in 3 patients still die before arriving at the hospital [1, 3, 4, 7, 8, 21, 25]. However, in patients who survive until arrival at the hospital the 30-day mortality rate in Sweden is approximately 2-15% depending on underlying risk profile and age [26].

Pathology of ACS

During a person’s life span, starting in the second decade, the formation of atherosclerotic plaques in the coronary arteries commences insidiously [1, 5, 6, 29]. The growth of the plaques is often quiescent for long periods of time but may slowly become symptomatic, especially during stress or strenuous physical exercise, causing the previously mentioned stable angina pectoris.
The progression of the coronary plaques is thought to be influenced by inflammation as inflammatory mediators have been found in the plaque [5, 6]. A general increase in CRP is also independently associated with cardiovascular events, further reinforcing this hypothesis [30, 31].

A rupture or an erosion of an atherosclerotic plaque is a potentially dangerous situation where the highly thrombogenic necrotic core of the plaque is exposed to the blood [6]. This exposure triggers a cascade of events in the coagulation system which subsequently leads to thrombus formation [18-20]. The thrombus consists of red blood cells, fibrin and inflammatory cells [20, 21]. The extent of the thrombus may be subclinical and without any symptoms, but it may likewise lead to partial or complete occlusion of the coronary artery and hence ACS [21, 30]. In the ACS patient, coronary spasm and endothelial dysfunction will aggravate the situation with less blood passing the affected coronary segment [18, 32]. The complex interplay between these processes and the exact mechanism of ACS is not completely understood and is under debate. For example the initiation and the actual process leading to the plaque rupture is not known and it is important to mention that 40% of patients with myocardial infarction have a normal hsCRP and an actual plaque rupture can be established in only 70% of patients [6, 30]. Furthermore, up to one in four patients with ACS have no clearly demonstrable coronary artery stenosis and as many as three quarters of plaque ruptures are thought to be present in mild to moderate coronary artery stenosis lesions [18, 30, 32, 33]. Although hyperlipidemia and inflammation are thought to be vital parts of the plaque progression and instability this suggests that a universal cause and presentation of ACS is unlikely to be found [30].

Figure 2. Disease progression from native coronary arteries to the formation of an atherosclerotic plaque and finally at the far right, a plaque rupture with thrombus formation. Figure reprinted from information sheet by Astra Zeneca.

Patients with ST-elevation ACS generally have a complete occlusion of the vessel and consequently transmural ischemia (the entire myocardium in the affected segment is ischemic) [15, 16]. The non-ST-elevation ACS corresponds to subendocardial ischemia (the inner part of the myocardium) and is often the result of a subtotal occlusion [15, 16]. However in a substantial part of non-ST-elevation ACS a complete occlusion of the culprit vessel is
present, but complete transmural ischemia does not occur, often because of collateral vessels supporting the affected area with oxygen [34, 35], spontaneous reperfusion before the diagnostic ECG is taken or occlusion of a vessel supporting a small area not detectable by ECG [19]. The ST-elevation ACS is associated with rupture of the plaque instead of erosion to a higher degree compared to the non-ST-elevation ACS [36, 37].

Risk Stratification in ACS.

Risk stratification in ACS patients is endorsed by international guidelines and provides important information on treatment strategies and on future prognosis [19]. Several clinical factors have been observed to correlate with adverse prognosis.

Obviously age is of importance, as the chance of long-term survival decreases with older age, but the risk of other comorbidities is also significant. Some of the most important clinical risk factors are hence easily obtainable: age, gender, previous coronary artery disease or previous revascularization. These factors are often included in risk scores or risk prediction models [38-40]. Other risk factors for the development of atherosclerosis are current diseases such as hypertension, diabetes, kidney disease, and hyperlipidemia [7, 10]. Modifiable risk factors also include sedentary lifestyle, diet and smoking [1, 7, 10, 12].

There are several different risk scores today but the most commonly used are the TIMI and the GRACE risk scores, comprising a combination of both clinical variables and biomarkers [38-40]. The GRACE risk score is more complicated but has superior prognostic capabilities in retrospective evaluations, and is therefore recommended in international guidelines [19, 41]. Biomarkers are used as an integrated part of risk stratification. The heart-specific marker troponin (see below) is mainly used, and exhibits great diagnostic and prognostic capability [19, 23, 42]. However there are a larger number of biomarkers associated with cardiovascular outcomes, and several markers indicating organ dysfunction are typically related to outcome. These include plasma glucose (diabetes), CRP (inflammation), white blood cells (inflammation/stress) [31, 43]. Furthermore, clinical variables such as heart rate and systolic blood pressure and obviously abnormal lung findings (rales) as well as Killip class are associated with adverse outcome [40, 43]. The extent of abnormalities on ECG or the coronary angiography findings are also associated with future risk [44]. In general, both biomarkers and clinical variables exhibit a weaker association with predicting myocardial infarction but a more robust relationship with cardiovascular death [40].

This thesis focuses on kidney function markers, which have also proven to be important in risk stratification. There are several reasons to this but one must emphasize that the majority of kidney disease is due to diabetes or hypertension, and hence both kidney and cardiovascular disease share the same
comorbidities. Secondly, decreased kidney function triggers several biological processes, like inflammation, increased coagulation and altered metabolism, which also play adverse roles in cardiovascular system. Finally chronic kidney disease is a symptom of a general weakness of the cardiovascular system. Patients with decreased renal function also have an increased risk of bleedings and are thus difficult to treat. If they experience ACS they are often not given aggressive pharmacological treatment as well as may not be recommended coronary interventions [45]. It is likely that this will cause an increased event rate in a group of patients already at high risk.

Treatment of ACS

The treatment of ACS has gradually improved and encompasses both pharmacological and invasive treatments as well as monitoring in specific coronary care units. The overall goal with the treatment of ACS is to relieve symptoms, maximize myocardial salvage and prevent recurrent myocardial damage, recurrent MI or death.

Time is a key factor and several studies have shown that any delay in opening an occluded vessel will decrease the chance of myocardial salvage and increase the risk of cardiovascular morbidity and mortality [46]. The first study to demonstrate that a pharmaceutical drug could reduce mortality in patients with myocardial infarction was the ISIS-2 study in 1988, where aspirin and streptokinase (a thrombolytic) were evaluated [47]. Ever since the treatment of myocardial infarction or ACS has included antithrombotic and antiplatelet drugs (e.g. aspirin, clopidogrel, ticagrelor) with a recommended addition of antiarrhythmic drugs (e.g. beta blockers) and/or myocardial protective drugs (like ACE inhibitors) ) [18, 19, 48, 49].

Several drugs affecting the symptoms (like oxygen or nitrous oxide) are generally also recommended, although the scientific base is not as solid [18]. Several drugs however have proven to reduce morbidity and mortality [18, 19, 48, 49].

The introduction of revascularization therapies, thrombolitics and especially PCI, has proven to be an important step in preventing recurrent cardiovascular disease [18, 19, 32, 48-50].

The current treatment of ACS can be summarized in the following paragraphs:

- Relief of symptoms.
  Beta blockers, nitro and when applicable, diuretics and oxygen therapy are associated with symptom relief [18, 19, 48, 49].

- Reperfusion therapy
In STEMI patients the recommended treatment is PCI when available within 60-90 minutes [18]. Thrombolytic treatment is the second line of treatment in STE-ACS and is not recommended in either non-ST-elevation ACS or in combination with PCI [18, 19, 51].

- Obtaining adequate cardiovascular stability. Although not scientifically proven, a very intuitive approach is the use of restriction or the administration of intravenous fluids and diuretics. The use of medical therapy (inotropes) or mechanical circulatory support (ventricular assist devices or IABP) in patients were hemodynamic stability is difficult to obtain should be considered [18].

- Antithrombotic therapy to prevent re-infarction or stent thrombosis. This is obtained both with short acting antithrombotic treatment and long term antiplatelet therapy [18, 19, 48, 49].

- Cholesterol lowering therapy, preferably statins is also endorsed by international guidelines, and is one of the most important features of secondary prevention. [1, 10, 18, 19, 48, 49]

- Antiarrhythmic therapy with beta blocker therapy has proven effective in preventing arrhythmia in STEMI. However this is at the cost of increased risk for cardiovascular shock, especially in patients presenting with hypotension or congestive heart failure. Nonetheless, beta blocker therapy is indicated as it effectively reduces long term CV mortality and morbidity [18, 19, 32, 48, 52].

Kidneys and Renal Function

The kidneys (Latin: ren, Greek: nefros) serve several essential roles in humans, not only filtering the blood and excreting waste products but also playing a crucial role in regulatory functions such as maintaining blood pressure, water balance, electrolyte levels and acid–base balance [53]. The kidneys also play a major role in degradation of proteins as well as producing hormones affecting red blood cells production and mineral turnover [53, 54]. Renal impairment has traditionally been measured by either screening the creatinine concentration or by applying creatinine-based equations that estimate the GFR [55]. The glomerular filtration rate is considered to be the best overall method to evaluate renal function [53, 55-58] and is the most convenient and widespread kidney function estimate. There are, however several drawbacks to using creatinine or creatinine-based methods for this purpose and a complete evaluation of the kidney function cannot be obtained by a single creatinine value.
Renal Physiology

Approximately 20-25% of the overall cardiac output at rest will be diverted to the kidneys, where only a fraction of the blood is used for oxygenation and nutrition to the nephrons [59]. Instead, the majority of the blood will pass the kidneys relatively unaffected as only approximately 25% of the blood flow actually undergoes ultrafiltration [54, 59]. The functional unit in the kidney is the nephron, and humans are born with approximately 1 million nephrons, although the exact number is debated [54, 58-60]. The central part of the nephron is the glomerulus, which is a hank of capillaries in which the actual filtration occurs. Blood is transported to the glomeruli by the afferent arteriole and transported from the glomeruli by the efferent arteriole [59]. A series of tubules collects the filtrate and the epithelial cells in the tubule thereafter passively or actively reuptake electrolytes, water and proteins (with or without degradation) [59]. Large proteins are not filtered while smaller proteins with no electrical charge are generally freely filtered if their diameter is smaller than 20 Å [59] However, the vast majority are reabsorbed in the proximal tubule (>99%) [59]. The nephrons are located in the lateral part of the kidneys (cortex) while the tubules are present in both the cortex and the inner part of the kidney (medulla) [54].

*Figure 3. Morphology of the glomeruli and the Bowman’s capsule*
The renal clearance or the filtering capacity greatly exceeds the needs in healthy young adults and a measurable decrease in kidney function is not detectable until a majority of the glomeruli are non-functioning, as there is no linear relationship between nephron loss and decreased GFR [54, 59]. Loss of nephrons is most often a slow process not easily recognized because compensatory hyperfiltration by other nephrons occurs. An apparent change in, for instance, creatinine is not observed until more than 50% of the nephrons have been lost [61].

The dominating etiologies of kidney disease in adults are either hypertension or diabetes (or both) and thereby the incidence is highly affected by increasing age [55, 56]. There are however several other reasons for kidney disease and this very heterogeneous disease group is often due to infection, inflammation or structural defects that may, alone or in combination, rapidly deteriorate kidney function and may cause irreversible damage [54].

**GFR – Glomerular filtration rate**

The GFR is the most recognized measure of kidney function and is defined as the volume of primary urine filtered from plasma through the glomeruli (in both kidneys). It encompasses roughly 180L/day in a healthy individual [58]. In normal circumstances approximately 99% of this primary urine is, however, reabsorbed and consequently only a fraction of the primary urine will be voided.

GFR is dependent on the net filtration pressure over the glomeruli membrane and is hence dependent on both afferent and efferent arterioles as well as systemic blood pressure. The number of glomeruli and thereby the total filterable area also affect the GFR.

The GFR is usually adjusted for body size and presented in mL/min/1.73m² (relative GFR). However, sometimes the absolute GFR (the actual filtration rate in the individual) in mL/min is specified.

The GFR is an important variable for several reasons such as estimating kidney function, kidney disease progression, drug dosing and general hemodynamics. However it is very important to emphasize that the GFR does not provide a complete evaluation of the kidney function, rather an estimation of the filtering capacity of the kidneys. Furthermore and importantly, the GFR does not provide a lead to the cause or the extent of an impaired kidney function. This is especially evident as creatinine (an indirect marker of the GFR) in an acute strain to the kidney will not rise in concentration until after more than 24 hours and will not provide a casual explanation to the change in kidney function [62].

The “true” GFR, which would be defined as the actual simultaneous filtering in all nephrons in the kidneys, is a hypothetical concept and cannot be measured. This is of course because it is impossible to simultaneously measure the approximately one million nephrons that filter the plasma but also
because the primary urine changes its composition and volume as it is conducted from the tubuli to the urinary bladder. The amount of filtered plasma can, however, be estimated if the plasma contains a marker, where plasma concentration is known and where the marker is freely filterable and neither excreted nor reabsorbed by the tubule. The marker will then end up in the final urine, and the eliminated amount of marker in urine per a specific time unit equals the plasma volume that was filtered through the glomeruli during the same time period if the plasma concentration is known [53].

In simple terms, the glomerular filtration rate can thereby be described as the passive filtration rate for molecules to enter the tubule or in other words: GFR equals the volume of plasma that is completely cleared of a molecule by the nephrons within a unit of time. Normal values are approximately 130 ml per minute per 1.73 m² in young men and 120 ml per minute per 1.73 m² in young women [53, 55, 56]. The actual rate is influence by age, gender, body size, hydration and several other factors including drugs and disease. The mean GFR also decreases with age, as the largest filtration rate in observed in early adulthood with progressively decreased GFR during middle age [56]. However, the rate of the decline is a subject for debate: from the fourth decade it is thought to be in the range of 10 mL/min per decade [53]. Despite these drawbacks GFR is generally accepted as the best overall measure of kidney function [55-58].

**Measured GFR**

The infusion of an exogenous marker which not is present in the plasma and is freely filterable provides the classic setup for measured GFR (mGFR) methods. These methods provide an accurate measurement and hence an evaluation of kidney function, yet are comparatively sparsely used. This is because they are costly, cumbersome and time consuming, as well as having large intra-individual differences. The traditional gold standard method is renal clearance of inulin. However, renal clearance of $^{51}$Cr-EDTA (isotope labeled EDTA) and iohexol (contrast agent) as well as $^{125}$I-iothalamate (isotope labeled contrast agent) are considered to provide accurate measuring methods for GFR evaluation [53]. Plasma clearance of $^{51}$Cr-EDTA, $^{125}$I-iothalamate and iohexol are also considered accurate methods for measuring the GFR [53]. Although these methods provide accurate measurements, there is still an intra- and inter-individual variation when comparing these methods, indicating variation between methods [53]. The estimated equations later discussed in this thesis (for instance creatinine or cystatin C derived equations) are always calibrated against a gold standard model and hence even less likely to reflect the true GFR.
Clearance

The total plasma clearance (plasma clearance) is the plasma volume that is completely cleared of a substance per time unit. Total clearance thereby reflects the elimination of a substance in general and not only by the kidneys.

Renal plasma clearance (renal clearance) however is the volume of plasma which is specifically cleared by the kidneys, in contrast to other elimination ways (e.g., liver, bile, respiratory etc.). Measuring renal plasma clearance is thereby only possible if the elimination by the kidneys can be observed as a measurable marker/substance in the urine. If there are no other elimination pathway and the marker is stable and neither secreted nor absorbed, the renal plasma clearance (renal clearance) merely equals the GFR [53]. However, it is important to emphasize that the clearance is dependent on which marker is used as several markers are either not freely filterable or exhibit tubular secretion or absorption leaving the plasma clearance to either over- or under-estimate the GFR. Creatinine clearance is known to overestimate GFR as creatinine is partly secreted by the tubule [59, 63]. It is strongly dissuaded in clinical practice but it is still used in clinical practice as a misinterpreted GFR estimate [53].

The product of urine concentration of a substance (U) and urine volume (V) equals the mass of a substance excreted during the time that urine has been collected. This mass equals the mass of the substance filtered at the glomerulus if nothing is added or removed in the nephron. If this mass is divided by the plasma concentration (P), the volume of plasma which must have been filtered within the aforementioned period of time (t) is ascertained and hence the renal clearance is obtained.

$$\text{Clearance (mL/min)} = \frac{U \text{ (mg/mL)} \times V \text{ (mL)}}{P \text{ (mg/mL)} \times t \text{ (min)}}$$

The clearance however is only accurate if all the following circumstances are fulfilled:

- The marker must be freely filterable through the glomeruli
- The marker must not be secreted or absorbed
- The marker must be present at a constant concentration in the blood
- The marker must not affect the GFR

Furthermore the ideal GFR marker would be stable and non-toxic as well as easily and accurately measurable in urine and plasma. A high rate of protein binding or a large electric imbalance would also affect the filterable capacity of the candidate marker.
Traditionally renal clearance of inulin has been the marker of choice, but this method is both a cumbersome and a time consuming which means that it is not widely accepted as a routine measurement in clinical practice. These properties have also sparked the evolution of other markers and today several alternative markers are used in clinical practice. The overwhelmingly dominant methods for evaluating the kidney function in clinical practice however are estimates by endogenous creatinine or cystatin C [53].

Chronic Kidney Disease and CKD-Classification

Chronic kidney disease is a term encompassing disorders affecting the structure and function of the kidneys often with decreased GFR [64]. The CKD is a broad group of heterogeneous diseases where the prognosis and disease progression will be greatly affected by the underlying pathology. The formal definition of chronic kidney disease is either the presence of kidney damage (e.g. albuminuria) or an estimated glomerular filtration rate (eGFR) less than 60 mL/min per 1.73 m² [55, 65, 66]. The renal impairment should be present for >3 months before being considered as Chronic Kidney Disease [55, 65].

Albumin, the most common protein in human plasma, has a molecule weight of approximately 67kDa. Albumin is negatively charged and its diameter is around 36Å, making filtration impossible in the majority of the observed pores in the glomeruli [59]. The presence of albumin in the urine is thereby a sensitive marker for glomerular damage and is considered clinically significant if the urine albumin to creatinine ratio is > 30 mg/g (< 3 g/mol) [66, 67].

The CKD classification stratifies patients into five stages (CKD 1-5) where CKD 1 is kidney disease without decreased glomerular filtration rate while CKD 5 corresponds to kidney failure with a GFR of less than 15 mL/min/1.73m² or the need for treatment with dialysis or transplantation [55, 65, 66].

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
<th>GFR (mL/min/1.73m²)</th>
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<tbody>
<tr>
<td>1</td>
<td>Kidney damage with normal or increased GFR</td>
<td>≥90</td>
</tr>
<tr>
<td>2</td>
<td>Kidney damage with mildly decreased GFR</td>
<td>60-89</td>
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<tr>
<td>3</td>
<td>Moderate kidney disease</td>
<td>30-59</td>
</tr>
<tr>
<td>4</td>
<td>Severe kidney disease</td>
<td>15-29</td>
</tr>
<tr>
<td>5</td>
<td>Kidney Failure</td>
<td>&lt;15 or dialysis</td>
</tr>
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Risk factors for chronic kidney disease (CKD) include an age of more than 60 years, hypertension, diabetes, chronic nephritis (renal inflammation) and cardiovascular disease [53, 56, 58]. The prevalence of CKD is increasing and currently approximately 12%-15% of the population in the United
States is classified as having CKD [55, 58, 68, 69]. The incidence of patients in the clinically important CKD Stage 3 or worse is about 10-12% of the total population [55, 68]. In patients > 70 years old, approximately 40% are thought to have decreased kidney function [55].

When the GFR falls beneath 15 mL/min per 1.73 m² uremia occurs and toxic symptoms are often present. This situation is classified as kidney failure and long term survival is only possible if kidney transplantation or dialysis is started [53]. Globally, diabetes is the leading cause of kidney failure accounting for approximately 40% of the incidence of CKD Stage 5 in the USA, while the figure for Sweden is approximately 25% [70]. From an epidemiological and cardiovascular viewpoint, patients with a GFR of less than 60 mL/min/1.73m² are considered to have impaired renal function [18, 19, 55]. In a general ACS population, approximately 30-40% of the patients have a decreased glomerular filtration rate (< 60 mL/min/1.73m²) [18, 71].

### Renal Impairment and Cardiovascular Risk

Renal impairment, most commonly referred to as a decrease in the GFR, is associated with increased risk of cardiovascular events [1, 13-19, 71-74]. The pathophysiological effect of renal disease is multifactorial and contributing factors to the increased cardiovascular risk include: increased inflammatory response, endothelial dysfunction, platelet activation, hypertension and a risk of less aggressive medical and pharmaceutical treatment due to the impaired renal function [45, 75, 76]. Furthermore, it is believed that renal impairment has an independent adverse effect on specific drug efficacy, as in clopidogrel for instance [76]. Increased cardiovascular risk can be observed even at the level of 90 mL/min/m² [14].

Patients with renal dysfunction also have an increased risk of developing acute kidney injury (AKI). The use of contrast media during diagnostic and interventional vascular procedures (e.g. coronary angiography) represents the most common cause of acute kidney injury in hospitalized patients [77]. Previous investigations have postulated that approximately 15% of patients experience contrast-induced nephropathy (CIN), defined as an increase in serum creatinine of > 25% within 5 days [78]. However, the risk for CIN requiring dialysis is however small <1% [78]. To prevent CIN the first line of prevention is maintaining an adequate fluid output and hence saline infusion is recommended prior to the investigation [77].
Biomarkers

Biomarkers are molecules that are measurable and quantifiable in the blood and their presence or dynamic changes may signal disease or cellular dysfunction [79]. Their concentrations may change in several clinical situations, including ACS, and they are useful in diagnosing and following the course of the disease [19, 23, 42].

Over recent decades, a large number of biomarkers have played an important role in diagnosing ACS [18, 19]. The role of biomarkers is constantly evolving and the use of biomarkers has helped us understand the pathophysiology of the disease as well as increased the predictive ability to prevent adverse events.

The ideal biomarker will help to diagnose disease, aid clinical decision making or help predict risk accurately with a high sensitivity and/or specificity. Furthermore, it should be easily accessible, rapidly provide affordable and meaningful results that provide incremental value in combination with clinical risk factors and existing biomarkers [79].

Creatinine

Creatinine is a metabolite from the degradation of creatine phosphate in skeletal muscle and is considered non-toxic with no known biological function [54, 80]. Creatinine concentration is heavily dependent on extent of muscle mass but also on diet. However, although the dietary component may be very diverse, the production of creatinine is fairly constant [80]. The low molecular mass of 113Da makes it freely filterable by the glomerulus, and it has been used as a marker of kidney function for decades [80]. Creatinine is entirely eliminated by the kidneys: but not only filtered by the glomeruli, it is also secreted by proximal tubular cells and subsequently the creatinine clearance exceeds the GFR [53, 56, 63, 80]. Tubular secretion of creatinine varies among and within individuals but approximately at least 10% of urinary creatinine is derived from tubular secretion and the secreted proportion of creatinine increases, and may constitute of up to 60% of total creatinine clearance in patients with moderate or severely impaired kidney function [63, 80]. Some drugs, including trimethoprim and cimetidine, inhibit creatinine secretion, thereby reducing creatinine clearance and elevating the serum creatinine level, without actually affecting the GFR [63, 80]. With increasing age the muscle mass and the GFR decrease. A consequence of this is that both the production and the elimination of creatinine decrease in elderly individuals making creatinine as a marker of the GFR less reliable.

These properties are thought to be responsible for some of the variation seen in creatinine levels when evaluating patients representing different age, geographic and ethnic subgroups [81]. GFR estimating equations produced to overcome some of the differences have been presented, but they are still
prone to a significant variance among patients and over time [56]. In addition, a large GWAS study has reported that genetic polymorphism affects creatinine levels slightly, both by suspected changes in creatinine metabolism and in actual kidney function variation [82]. Nevertheless, creatinine as a single laboratory value is the most common method for a general evaluation of the kidney function, both internationally and in Sweden [53, 83-85].

Cystatin C

Cystatin C is a 13kDa (13,343 Da) cysteine proteinase inhibitor that was discovered in 1961 in human cerebrospinal fluid [86]. It was originally named human γ-trace but as a part of the cystatin superfamily it was later adopted as cystatin C. It has been observed in several body fluids, including urine, plasma, and ascitic fluid [87-89]. Cystatin C is secreted at a constant rate by most nucleated cells and consists of a single polypeptide chain consisting of 120 amino-acids [87-91]. It is positively charged, non-glycosylated and has outer borders of approximately 30 x 30 x 50Å [92]. Cystatin C encoding mRNA has been found in various tissues: for example, the heart, liver, lung, pancreas, intestine [89]. The complete amino acid sequence of human cystatin C was determined in 1981 by Grubb and Löfberg and the encoding gene is the CST3 gene located on the small arm of chromosome 20 (20p11.21) [87]. At present a total of 12 human cysteine protease inhibitors have been identified and they are all members of the human cystatin superfamily, sharing sequence homologies to cystatin C as well as clinical features. Cystatin A and B mainly exhibit intracellular inhibition of proteases, while the majority of the other members in the family, including cystatin C, predominantly exhibit extracellular inhibition of, for instance, cathepsin, legumain and papain [89, 92-95]. Because of its high prevalence in humans, it is thought to play an important role in inhibiting peptidases. An imbalance in cystatin C has been linked to arterial wall remodeling as well as to aortic disease [94, 96]. Cystatin C concentration and genetic variation has also been postulated to affect the progression and severity of coronary artery plaques [6, 88, 94-98].

As cystatin C is secreted at a constant rate, freely filtered by the glomerulus and then virtually fully absorbed and catabolized in the proximal tubules (to an extent of >99%), the serum concentration is considered to be an accurate marker of the glomerular filtration rate [87-89]. The cystatin C concentration is influenced to a small extent by circadian variation [61, 92, 99, 100]. Cystatin C is normally absent in the urine, and thereby it has been suggested that the presence of urinary cystatin C reflects tubular epithelial damage [101].

It has also been suggested that cystatin C is advantageous compared to creatinine in reflecting the glomerular filtration, as its non-GFR determinants are less affected by diet, race and muscle mass. Thus the eGFR derived by
cystatin C concentrations does not generally include anthropometric measures [53]. However, the non-GFR determinants of serum cystatin C are poorly understood [55]. Cystatin C concentrations are, for instance, independently of renal function, influenced by thyroid function with increasing levels of cystatin C in hyperthyroid patients and decreased levels in hypothyroid individuals [102]. Different genetic polymorphisms have also been suggested to affect cystatin C concentrations [82, 96, 103]. Furthermore, corticosteroid therapy increases cystatin C concentrations in vitro [104] and in vivo [99]. The in vitro results were shown to be due to a promoter-mediated increase in transcription of the cystatin C gene.

Furthermore, observations where cystatin C eGFR has been compared to creatinine-based eGFR, cystatin C has revealed possible interactions with age, gender, BMI, smoking status and CRP, something that could partly explain the large inter-individual variability with cystatin C [88, 105]. It is, however, important to emphasize that the latter comparison was based upon the mean of two 24h urinary creatinine clearance estimations and not a gold standard measurement, e.g. inulin clearance [105]. Recently, efforts have begun to harmonize cystatin C measurements via a global cystatin C calibrator.

Due to the challenges of assessing muscle mass in children, cystatin C is advantageous in a pediatric population [53, 92].

**Figure 4.** Cystatin C – complete amino acid sequence. Layout with permission from Anders Grubb.
**Cystatin C as a risk predictor**

Cystatin C has been shown to correlate well to cardiovascular risk [16, 72, 74]. This was not unexpected as cystatin C reflects the glomerular filtration rate, where decreased renal function is a clear risk marker for cardiovascular death, myocardial infarction [1, 13-17]. However, in several studies cystatin C has been shown to be a more sensitive marker, compared to creatinine, for cardiovascular risk in patients with normal or mildly impaired kidney function, for cardiovascular adverse events including CV death and MI, as well as severe atherosclerotic disease [16, 72, 74, 106-108]. Cystatin C exhibits a strong relationship to mortality in several specific populations, including patients with coronary artery disease [109, 110], myocardial infarction [72, 74], patients admitted to hospital for chest pain [74], patients without chronic kidney disease or individuals that are elderly but apparently healthy [16].

Cystatin C concentration is also related to the extent of cardiovascular disease, evaluated with coronary angiography, even after the adjustment of creatinine based eGFR [97, 111].

However, the clinical use of cystatin C, both as a marker of kidney function and as a risk predictor, has been sparse, partly due to a loss of standardized reference values, higher cost for the analysis, conflicting results and non-GFR determinants of unknown magnitude [55, 112].

**Estimates of the Glomerular Filtration Rate**

The most common way to evaluate kidney function in clinical practice is to evaluate the serum creatinine concentration as a single laboratory value or as a parameter in equations for estimating the GFR [53, 56]. During the past three decades the alternative renal marker cystatin C has caught increased interest. Creatinine concentration is generally adjusted for age, gender and estimated body size as surrogates for the non-GFR determinants of the renal marker [55, 113-115]. These estimates of the GFR are more accurate compared to a single laboratory creatinine value [53, 56]. The equations have turned more complex to provide greater accuracy and now include both cystatin C and creatinine as variables [53, 81].

Although estimates of GFR constantly improve, the innate difficulties of estimating the GFR have made some researchers believe it is a very difficult task to produce a single equation that will, with reasonable accuracy, estimate the GFR over the entire GFR spectrum and in all sub-populations [84].

One way of evaluating agreement between methods is applying the P30. This method specifies the amount of estimated GFR values that fall between ±30 percent of a measured GFR [53]. The term bias is also important, as it reflects a systematic error in one method constantly providing a different mean or median value compared to the other method [116].
Cockcroft Gault

In 1976 Canadian physicians Cockcroft and Gault published their equation for estimating creatinine clearance. It is, however, often used as a surrogate for the GFR even though not scientifically completely correct (see above). The equation was based upon 249 adult men (18-92 years old) with creatinine clearances ranging from approximately 30 to 130 ml per minute. The estimating equation is creatinine clearance = [(140 − age) × weight] / (72 × SCr) × 0.85 (if female). Creatinine clearance is expressed in milliliters per minute, age in years, weight in kilograms, and serum creatinine (SCr) in milligrams per deciliter [115].

The equation was a great improvement compared to evaluating a single creatinine value. However, the equation has major drawbacks that need to be addressed. First, the Cockcroft-Gault equation estimates creatinine clearance, and is hence not a marker of the GFR. Second the Cockcroft-Gault equation has a tendency to overestimate creatinine clearance because of the tubular secretion of creatinine, most importantly in patients with impaired kidney function (CKD Stage 3 or worse) [53, 56]. Third, the equation is not adjusted for body surface area (BSA) [56]. BSA adjustment adds important information and provides the relative GFR, which is of greater use in, for instance, risk prediction. Relative GFR has also been a standard with equations developed more recently.

Because of this, a direct comparison between the Cockcroft-Gault and other equations (giving the relative eGFR in mL/min/1.73m² as, for example, the CKD-EPI) is not easily done. Finally, the Cockcroft-Gault equation was based on creatinine concentrations derived by a non-compensated method that is not widespread today. Today most laboratories have calibrated their creatinine analyses to adhere to creatinine standardization by Isotope Dilution Mass Spectrometry (IDMS).

Given these issues, a widespread clinical use of the Cockcroft-Gault equation cannot be encouraged today [53]. However, it is still used in clinical practice as recommendations on dosing of drugs often have been decided based upon Cockcroft-Gault estimates, as these provides the absolute eGFR which in that specific setting may be of greater interest.

MDRD

The Modification in Diet and Renal Disease equation was presented in 1999 to overcome some of the difficulties with the Cockcroft-Gault equation [114]. The equation was derived by 1628 patients from the Modification of Diet in Renal Disease (MDRD) Study where 1070 were randomly selected as the test cohort while 558 patients were used as a validation cohort [114]. The study cohort included both men (60%) and women (40%) and 6% had diabetes. The MDRD study equation is an improvement over the Cockcroft-Gault equation but is on the other hand only reasonably accurate at GFRs of
more than 60 mL/min/1.73m² as the equation underestimates the GFR at higher ranges leading to misclassification and hence over diagnosing of CKD [117]. The increased bias and imprecision at high eGFR is partly due to that studied population was not representative for a general population as it exhibited a median mGFR of 40 mL/min/1.73m² measured with ¹²⁵I-iothalamate [114]. Thereby the study rather represented a population with decreased kidney function [114]. On average, GFR estimates of less than 90 ml per minute per 1.73 m² are lower than the directly measured values which may lead to a false positive diagnosis of chronic kidney disease in individuals who do not have the disease but have a mild reduction in GFR [56]. However, in individuals with measured GFR below 30 mL/min/m², the eGFR may be overestimated with the MDRD equation [53]. The MDRD equation has been presented in several variants and the original equation or variations of such are currently the most used GFR estimates reported by chemical laboratories and hospitals today [113].

Today, the use of the MDRD equation in countries that have converted to the IDMS calibration is only encouraged if the IDMS corrected MDRD equation is used to reduce inter-laboratory variation and to enable more accurate estimates of the glomerular filtration rate [118]. This was recommended by international federations including the NKDEP participating in the Creatinine Standardization Program [118].

**CKD-EPI**

The Chronic Kidney Disease – Epidemiology collaboration published the CKD-EPI equation in 2009 [113]. The equation was derived from a pooled analysis by 10 different clinical trials and investigations. A total of 8254 patients were analyzed, subdivided into a developing set (n=5504) and an internal validation cohort (n=2750) and subsequent external validation (n=3896 from 16 additional studies) [113]. The studies encompassed patients with both normal and impaired mGFR, with mean mGFR measured by ¹²⁵I-iothalamate in the development data set was approximately 68 mL/min/1.73m² [113]. This is important, as much of the current research is aimed at identification and treatment of patients with a mildly to moderately decreased glomerular filtration rate since these groups have been shown to have an increase in cardiovascular risk as well [15, 113]. Another important feature was the inclusion of several important subgroups such as diabetics and several different ethnicities, but the amount of elderly patients was low, illustrated with the mean age <50 years overall [113]. In the CKD-EPI equation, standardized creatinine values were used (to minimize the effect of pseudo-creatinitines), via the IDMS. The global spread of IDMS will allow different methodologies and assays to perform more uniformly in reporting the actual creatinine values.
The combined creatinine-cystatin C equation (by the CKD-EPI)
The Chronic Kidney Disease–Epidemiology collaboration published a combined equation in summer 2012 [81]. This new equation was thought to benefit from the strengths of both renal markers cystatin C and creatinine. The equation was an important step towards increased accuracy of the GFR estimate as factorized cystatin C values were believed to mimic an actual standardization of the cystatin C concentration [81]. The main message is that a combined creatinine-cystatin C equation is more accurate in predicting the true GFR than either kidney marker equation alone [81].

Important non-renal biomarkers
There are currently a multitude of biomarkers that are used both for guiding therapies and for risk stratification in current clinical practice. These biomarkers provide important information independently of other risk factors as they often mirrors a specific part of the disease process like P-glucose or HbA1C (diabetes), white blood cell count (stress and coagulation) or GDF-15 (inflammation) adding information to risk stratification. Three of the most important, which also appear in the papers are summarized below.

I. Troponin

Troponin is a part of the contractile element in the myocyte. As troponin is only present in the heart, an elevation in the blood truly reflects myocardial dysfunction or necrosis. However, an increased concentration of plasma troponin does not reveal the actual cause or the underlying condition, which does not necessarily have to be ACS. [23]. Nonetheless, one of the cornerstones for diagnosing myocardial infarction is an increase in the troponin level to more than the 99th percentile [18, 19, 23]. Even small increases in troponin correlate to adverse prognosis and increased risk of morbidity or mortality [119-122]. After a myocardial infarction, troponin levels peak at approximately 12 hours after the event. However, the concentration can remain elevated for up to 10 days after an myocardial infarction [79].

II. NT-proBNP

The polypeptide proBNP (pro- B-type Natriuretic Peptide) is synthesized both in atria as well as in the myocardium and released into the blood due to the stretching and dilation of the myocardium [42]. ProBNP is rapidly cleaved into BNP (B-type Natriuretic Peptide) and into NT-proBNP (N-terminal pro- B-type natriuretic peptide) [79]. BNP, a 32 amino acid polypeptide, exerts the biological functions with a diuretic effect on the kidneys, decreased thirst and acts as a vasorelaxant on the vascular bed [42]. NT-proBNP (the 76 amino acid N-terminal fragment of proBNP) on the other
hand is biologically inactive, but is more stable in plasma with a longer half-life and hence more reliable as a biomarker [79]. NT-proBNP has shown excellent prognostic capability for predicting CV death or MI in several populations including apparently healthy individuals, a general chest pain cohort and ACS populations [122-125] and has the same prognostic value as the complete GRACE score in predicting new cardiovascular events in patients surviving a myocardial infarction [79].

III. CRP

C-reactive protein is an acute phase protein which is predominantly produced in the liver. The biological role of CRP is uncertain, however, there has been much debate about whether CRP is causally linked to cardiovascular events or a surrogate marker of inflammation [126]. Irrespective of the cause, CRP responds fairly rapidly to stress or body injury with a large increase after a myocardial infarction, peaking at 2-4 days after initiation while the return to normal levels may take up to 12 weeks [79]. Recent research has shown elevated levels of CRP to correlate to cardiovascular risk, although not in the acute phase of myocardial infarction, rather prior to an event, possibly as a response to a general inflammation and plaque instability [14, 31, 122, 127]. CRP is particularly susceptible for confounding as it is also associated with smoking, hypertension, obesity, lack of physical activity and low socioeconomic factors. Nonetheless, the risk predictive capacity of CRP is about the strength as other conventional risk factor like hypertension [126].

Genetics

The human genome

The human genome consists of approximately 3 billion nucleotides, which encompasses the complete DNA (deoxyribonucleic acid) sequence [128] [129]. The genome is divided into 23 pairs of which twenty-two are considered autosomal, while the last two chromosomes are the gender-specific chromosomes X and Y [128]. The chromosomes are inherited from the individual’s mother and father and with the exception of monozygotic twins all human genomes are unique.

The chromosomes are long arrays of four versions of nucleotides: A (adenine), T (thymine), C (cytosine) and G (guanine). The nucleotides form two long chains where the strands combine vertically as base pairs A-T and C-G and vice versa. The complete human genome was sequenced in the global Human Genome Project which finished in 2003 [130].
Genes and genetic variation

A gene is the smallest functional unit of DNA encoding for a specific RNA and often a protein. A defined gene not only includes the actual genetic code for the protein but also a regulatory section responsible for the transcription of the gene [129]. However, the majority of DNA is comprised of “non-coding” sections and the reason for harboring this vast amount of genetic information remains unknown. Between two humans approximately 99.9% of the genome is identical while the remaining differences in genetic expression will constitute some of the observed diversity in personality, properties and disease susceptibility between individuals. Currently, the human genome is believed to carry more than 20,000 protein-coding genes [128, 129].

Genetic diversity may be due to large differences like a loss of chromosomes or structural differences (duplications, deletions, translocations or insertions) often with severe consequences. However the vast majority of the genetic variability is due to smaller chromosome abnormalities like single nucleotide polymorphisms (SNP), copy number variations and short tandem repeats [131].

If a variant is observed in more than 1% of the studied population, genetic polymorphism is present and the most common genetic polymorphism is the single nucleotide polymorphism [131]. The SNP is the smallest possible variation as it only differs one base pair in the genetic code and is responsible for much of the heritable phenotypic variation observed in the human population [131]. It has been estimated that approximately every 185-2000\text{th} nucleotide is different in two unrelated humans [131].

From gene to protein

The process from gene to protein starts with the transcription of the DNA, where gene information is copied to form single stranded RNA. This is followed by RNA splicing where the non-coding sections (introns) are cut out of the RNA while the coding parts (exons) are merged to form messenger RNA (mRNA). The final step is the translation when the transcribed mRNA is translated into a protein. In some genes the RNA transcript is functional without being translated to a protein.

Association studies

Genetic association studies evaluate the possible association between a genetic variable and a specified condition [132]. The common disease – common variant hypothesis is a cornerstone of genetic association studies and states that common variants in many genes will each lead to a small increase (or decrease) in the risk of a certain condition. The overall risk of a disease is determined by the combination of multiple variants and environmental exposures [133]. During the past decade, Genome-Wide Association Studies (GWAS) have discovered several genes that contribute to a small increase in
risk independently, and this has further fueled the hypothesis that most disease are polygenetic and several alleles collectively increase the risk of disease susceptibility [132]. In the early years of genetic exploration expectations were very high and it was anticipated that genetic information would yield major breakthroughs both regarding diagnosis and treatment in clinical practice [134]. Today the role of genetics is still strong but the expectations have been revised. One example of this is the genetic polymorphism that influences creatinine levels. Twenty different loci have been identified that affect creatinine levels in a large GWA study, but the information from these loci combined is only responsible for an expected variability in creatinine concentrations of 1.4% [82].
Aims

**General aims of the thesis**
The aim of the thesis is to evaluate biomarkers reflecting the glomerular filtration rate and their relationship to outcome in patients with acute coronary syndrome, in order improve cardiovascular risk stratification and the understanding of ACS.

**Objectives of the Thesis**

I. To investigate whether cystatin C and CKD-EPI are associated with cardiovascular death or recurrent MI in an ACS population in general and in patients with STEMI in particular.

II. To evaluate whether there is a prognostic difference when evaluating kidney function markers during follow up rather than at baseline in ACS patients.

III. To observe changes in the estimated glomerular filtration rate during the acute phase of ACS and to examine whether observed changes can predict adverse outcome.

IV. To assess possible differences between two cystatin C assays.

V. To evaluate the predictive capability of a combined creatinine-cystatin C equation.

VI. To study whether genetic polymorphism affects cystatin C concentration and whether this information is valuable in risk stratification either alone or in combination with biomarker levels.
The Main PLATO Trial

The PLATelet inhibition and Outcomes trial was a multicenter, randomized, double-blind trial, comparing two antiplatelet drugs in patients with ACS, conducted between 2006 and 2009 (NCT00391872) [28, 135]. The randomized comparison was between treatment with ticagrelor (180 mg loading dose, 90 mg twice daily thereafter) and clopidogrel (300 to 600 mg loading dose, 75 mg daily thereafter). The antiplatelet treatment was given in addition to optimal medical treatment including aspirin. Early revascularization treatment was encouraged thereby reflecting current guidelines.

In brief, eligible patients were individuals with onset of cardiac ischemic symptoms during the previous 24 hours, due to atherosclerosis, of ≥10 minutes’ duration at rest. The ACS patients also required further criteria as presented below [135].

i  ST-segment elevation ≥1 mm or new LBBB
   and planned for primary PCI

or

ii  non-ST-elevation ACS with 2 of the 3 prerequisites:
   1. ST-segment changes on ECG indicating ischemia
      and/or
   2. positive biomarker indicating myocardial necrosis
      and/or
   3. ≥1 risk criterion
      (a) age ≥60 years
      (b) previous MI or CABG
      (c) ≥50% stenosis in ≥2 coronary vessels
      (d) previous ischemic stroke, TIA, carotid stenosis
         (≥50%), or previous cerebral revascularization
      (e) diabetes mellitus,
      (f) peripheral artery disease
      (g) chronic renal dysfunction (eGFR <60 mL/min/m²)

Summary of inclusion criteria for the PLATO trial

Important exclusion criteria included need for dialysis, treatment with oral anticoagulants and stable coronary artery disease. The study population consisted of 18,624 patients, and thus was one of the largest prospective study cohorts of patients with acute coronary syndromes in the world. The details of the study have been published previously [28, 135].

In addition to the main PLATO study, investigator-initiated substudies were planned to evaluate the effect of ticagrelor compared to clopidogrel in specific, clinically important, subgroups. Predefined analyses include evalu-
ating the effect of medication in patients presenting with STEMI and NST-ACS separately as well as evaluation of patients with intent for coronary revascularization therapy. Specific emphasis was also placed on evaluating the treatment effect in specific subgroups such as patients with known kidney dysfunction or diabetes, as well as the influence of ECG changes or platelet reactivity on outcomes.

The main study and the substudies were suggested and designed by principal investigators from the Uppsala Clinical Research Center, Uppsala Sweden and the Duke Clinical Research Center, Durham, NC, USA [28, 135] in collaboration with sponsor representatives. In addition to the main study, biomarker and genetics substudies were conducted. The main study plus the biomarker and genetics programs were funded by AstraZeneca.

The Biomarker and Genetic Substudies
Within the PLATO trial we planned, designed and performed a biomarker and genetics program led by the principal investigators from Uppsala Clinical Research Center. The accumulation of the large biobank of blood samples, gathered at admission from around 16,000 patients and during follow up from around 4,000 patients was designed to provide information both on traditional biochemical parameters as well as on genetic information [135]. The purpose of the biobank was to gain insights into risk stratification, to gain an enhanced understanding of the pathophysiology of ACS and to refine predictive models of clinical outcomes. Biomarkers reflecting diverse disease processes were evaluated, including: myocardial dysfunction, metabolic changes, kidney function, inflammation and metabolism. Furthermore, within the trial we also accumulated genetic material from around 10,000 patients and this was evaluated by a Genome-Wide Association approach and thereby provided an unprecedented volume of genetic data in patients with ACS [135].
Methods

Blood Sampling and Storage

Venous blood samples were obtained via a direct venous puncture and anticoagulated in EDTA. After centrifugation, plasma samples were frozen and sent for central laboratory analysis. Two parallel laboratories were used, both the UCR laboratory (biomarker substudy tests) and the Quintiles laboratories (continuous safety analysis).

The UCR laboratory, located at Uppsala Clinical Research Center, Uppsala, Sweden, performed biomarker analyses of the biomarker sub study including both samples collected at randomization in all patients and repeated samples (including troponin I) in 4000 patients at discharge and after one and six months. The aim was to evaluate the usefulness of biomarkers as risk markers at follow up and also to evaluate the effects of the tested treatment on their biomarker levels during long-term treatment. The blood samples were stored in a biobank located in Uppsala.

Information on biomarkers analyzed by Quintiles Laboratories for the main PLATO trial was used for the original publication but also for safety monitoring. Analyses of troponin I, NT-proBNP, creatinine, glucose, HbA1c and lipids from all patients at randomization were performed continuously at the Quintiles Laboratories as part of the main study protocol. These assays (except for troponin I) were also followed over time (1, 3 and 6 months). These analyses were performed at five different Quintiles laboratories in the respective part of the world (Marietta, US; Livingston, UK; Sao Paulo, Brazil; Singapore; Irene, South Africa).

Biochemical Methods

Plasma cystatin C concentrations at baseline and during follow-up were analyzed with the Gentian assay using the Abbot Architect CI-8200. The analytical imprecision for cystatin C with the Gentian assay was 1.94% at the mean of 0.87 mg/L and 2.49% at 2.91 mg/L.

Serum creatinine was determined in a core lab using a rate blanked and compensated modified Jaffé method with the Roche BMD instrument. The method was standardized to isotope dilution mass spectroscopy with an analytical imprecision at 0.7% at the mean of 1.67 mg/dL [148 μmol/L] (intra-
assay) and 2.3% at 1.09 mg/dL [96 μmol/L] (inter-assay). The CKD-EPI equation was defined as $141 \times \min(\text{creatinine}/\kappa, 1) \times \max(\text{creatinine}/\kappa, 1) - 1.209 \times 0.993^{\text{Age}} \times 1.018 \times (\text{if female}) \times 1.159 \times (\text{if black})$, where $\kappa$ is 0.7 for women and 0.9 for men, $\alpha$ is $-0.329$ for women and $-0.411$ for men.

**Statistical Methods**

Throughout the papers in the thesis, the initial step for interpreting data was to produce demographic tables for each subgroup studied in order to evaluate the patient characteristics. In the first paper the quartiles of cystatin C concentration formed four strata. In the second paper, the patients were instead divided by randomized treatment (ticagrelor/clopidogrel) instead. In the fourth article patient characteristics was divided by genotype.

The following were used to test whether the treatment effect differed in relation to the biomarker: Cox proportional hazard regression with the treatment group (ticagrelor/clopidogrel), the baseline biomarker categorized in quartiles and the interaction treatment group-by-biomarker as independent variables. The Cochran-Armitage trend test was used to test for a linear trend in the event rate across quartiles of the biomarker and within the treatment group. Hazard ratios along with 95% confidence intervals were estimated for each quartile of the biomarker with the lowest quartile as the reference category using simple Cox proportional hazard regression including only the biomarker categorized in quartiles as an independent variable, as well as in a multiple Cox proportional hazard regression (including age, gender, diabetes, CHF, type of ACS, smoking status, hypertension, treatment group and previous MI, non-hemorrhagic stroke, PAD, CABG and PCI).

Kaplan-Meier curves were estimated for the time-to-event for each quartile of the biomarker and by treatment group.

C-statistics (based upon logistic regression models) was used in all the papers in the thesis as it provides a clinically useful and easily interpreted test of the discriminative value for a variable. This discriminatory test exhibits the probability that an individual with disease (or who will develop disease) has a higher biomarker concentration from a model designed to predict disease than an individual without disease (or who will not develop disease). This measurement is a cornerstone in risk stratification and is a well-established clinical tool for evaluating a biomarker. It is, however, sometimes considered to be a blunt measure as it is difficult to acquire statistically significant incremental values of yet another additional marker to an already multivariable analysis.

The c-statistic is often reported as the area under the receiver-operating characteristic curve (AUC). The size of the area indicates the probability, across the entire spectrum of concentrations for a biomarker, that a given individual with disease (or who develops disease) will have a higher bi-
omarker concentration of the test than an individual who does not have (or will not develop) disease. A perfect test has an AUC of 1.0, indicating optimal sensitivity and specificity. A test that is no better than chance has an AUC of 0.5 [116].

The IDI is also a measure for assessing the added predictive ability of a marker for a binary outcome. More specifically, the IDI can be viewed as the difference in the discrimination slopes between the new model (with a renal parameter) and the old model (without a renal parameter), where the discrimination slope is defined as the difference of mean predicted probabilities of event and non-events [136]. Although not interchangeable, the IDI and delta AUC provide similar information.

The evaluation of the change from baseline was analyzed separately at each visit with either a t-test or a non-parametric ANCOVA with the baseline value as a covariate, depending on the distribution of the parameter (Paper II).

Genetic Methods

Genetic analysis
A single venous blood sample (EDTA) was collected preferably at the time of enrolment. However, if this were not possible, the genetic samples could be drawn at any follow up visit. Participation in the genetic sub-study was voluntary and required an additional consent form at the time of enrollment in the genetic sub study.

Samples were transported to a central laboratory and were then kept frozen at all times and transported to the DNA extraction laboratory. The DNA samples were coded at the AstraZeneca genetics laboratories (Alderley Park, UK). The details of the genetic analysis and the two-step GWAS analysis are given in the fourth paper.

Linkage disequilibrium
LD is the correlation between two SNPs in a genome. A high $r^2$ value can be interpreted as the squared correlation between the two SNPs and can be interpreted as the likelihood of the two SNPs being inherited together. A low $r^2$ value reflects that the two SNPs are seldom in linkage disequilibrium and consequently rarely inherited together. An $r^2$ value of 0 indicates that the two SNPs are inherited completely randomly and subsequently are inherited totally independently of each other.

GWAS
Genome-Wide Association Studies provide a powerful tool for identifying common disease susceptibility variants, by relating SNPs from the entire
genome to biomarker concentrations, clinical outcomes or both. The GWAS studies provide the most effective method of evaluating complex diseases which originate from a combination of a familial predispositions with a large environmental contribution [133]. The current population was divided into sets: a discovery and a replication set where SNPs were evaluated for association to biomarker concentration. The primary endpoint was the SNP relationship to biomarker concentration although the secondary outcome was the SNP relationship to clinical outcome. The details of the current GWA study are given in Paper IV.

**Mendelian Randomization**

Mendelian randomization is a concept where the role of a specific biomarker is evaluated in three steps. First the association of a risk allele to biomarker concentration is established. Second, the role of a genetic variant in relation to outcome is assessed. The final step then evaluates the risk factor (e.g. cystatin C) and the relation to outcome. If a difference in outcome is observed, and no other obvious confounders are present, one can conclude that the biomarker is causally related to the outcome [137].
Results

The main PLATO trial

Timeframe and baseline characteristics
The PLATO trial enrolled 18,624 patients admitted to the hospital with acute coronary syndrome, with or without ST-segment elevation, from 862 centers in 43 countries. Patient enrollment took place between October 2006 and July 2008, while the follow-up period ended in February 2009 [28]. Consequently all patients were followed for at least 6 months and a maximum 12 months (median 9 months). Information on vital status was available for all patients except five. The results of the study were officially presented at the European Society of Cardiology congress in Barcelona on August 30th, 2009 [28].

The mean age of the randomized patients was 62 years and 72% were males. Mean body weight was 80 kg with a corresponding BMI of 27. Several patients had a history of coronary artery disease, including previous myocardial infarction (21%) and previous PCI (13%) or CABG (13%). Diabetes was present in 28% of the patients while smoking, hypertension and dyslipidemia were observed in 36%, 65% and 47% of the patients respectively. The two treatment groups were well balanced with regard to all baseline characteristics. At randomization the ECG finding was ST-segment elevation in 37% of patients while ST-segment depression and T-wave inversion were 51% and 32% respectively [28].

Primary results
Patients randomized to treatment with ticagrelor compared to clopidogrel were at a lower risk for the combined composite endpoint of cardiovascular death, myocardial infarction or stroke, 11.7% versus 9.8% respectively during one year of follow up (hazard ratio 0.84; 95% confidence interval [CI], 0.77 to 0.92; P<0.001). This absolute reduction of 1.9% (or relative reduction of 16%) was highly significant in the primary combined endpoint and significant differences were also observed in the predefined hierarchical testing of secondary end points: myocardial infarction alone (5.8% in the ticagrelor group vs. 6.9% in the clopidogrel group, P = 0.005) and death from vascular causes (4.0% vs. 5.1%, P = 0.001). The rate of all-cause mortality was also reduced with ticagrelor (4.5%, vs. 5.9% with clopidogrel;
P<0.001). There was no difference in the occurrence of stroke [28]. Importantly, no increase in total major bleeding was seen although a rise in total bleedings (major, minor or minimal) was observed [28].

The overall study results of ticagrelor treatment compared to clopidogrel treatment with regard to cardiovascular events were mirrored throughout several important subgroups in the trial, including, STEMI patients, in non-invasively managed patients and in diabetics [14, 138-141]. Importantly however, in these subgroups, like in most subgroups, a statistical significance was not achieved due to the lower number of patients but no interaction by subgroup was observed [14, 138-141].

In conclusion the PLATO trial showed ticagrelor to be superior to the current standard treatment with clopidogrel in patients with acute coronary syndrome with or without ST-segment elevation by significantly reducing the combined endpoint of death from vascular causes, myocardial infarction or stroke without increasing the rate of overall major bleeding [28].

The Biomarker Substudies of the PLATO Trial

Paper I – Biomarkers of Kidney Function as Risk Predictors

The first paper in the thesis covers cystatin C and creatinine based eGFR as risk markers in a current ACS population. Although cystatin C has proven to have incremental additive value in risk prediction in several populations [72, 74], its importance as a risk predictor in STEMI patients was unknown with the exception of a small study (n=71) [142].

We evaluated the predictive ability of both cystatin C and creatinine-based estimations eGFR in an ACS population consisting of both STE-ACS and NSTE-ACS. This was the first time the CKD-EPI was evaluated in a risk prediction analysis.

Plasma cystatin C and serum creatinine samples were collected at randomization, as per protocol within 24 hours of the onset of the cardiac symptoms in the entire population (n=16401). The estimated GFR was calculated and both the estimates as well as the laboratory values of cystatin C and creatinine were evaluated as predictors of the composite endpoint of cardiovascular death or myocardial infarction within one year. Creatinine as a laboratory marker, proven to be unpredictable for evaluating kidney function, was later excluded from the comparison. Two Cox proportional hazard models were used: one adjusting for clinical characteristics while the second included clinical characteristics and biomarkers NT-pro BNP, troponin I, and CRP.
The median cystatin C value was 0.83 mg/L. Increasing quartiles of cystatin C were strongly associated with poor outcome (6.9%, 7.1%, 9.5% respectively 16.2%). In the multivariable adjusted analysis the HR per standard deviation of cystatin C in the NSTE-ACS and STE-ACS populations was 1.12 (95% CI: 1.04-1.20) (n=8053) and 1.06 (95% CI: 0.97-1.17) (n=5278) respectively. There was no significant interaction for cystatin C by type of ACS (STE-ACS or NSTE-ACS). C-statistics with the area under curve (AUC) for the different eGFR and markers ranged from 0.6923 (cystatin C) to 0.6941 (CKD-EPI).

Figure 5. The Kaplan-Meier curves from the first article illustrate the increased risk of adverse cardiovascular events by quartiles of cystatin C.

To conclude, in Paper I we found that the cystatin C concentration contributes independently in predicting the risk of cardiovascular death or MI in a large ACS population and in NSTE-ACS separately. The incremental value of cystatin C was not statistically significant in STE-ACS patients. However, no interaction was found regarding sub-type of ACS. Thereby it is reasonable to suggest that cystatin C, as well as any renal marker studied in the paper, exhibits incremental value in risk prediction models to assess future cardiovascular events in ACS patients. However, cystatin C did not show the numerically highest incremental value in ACS patients. Rather, the CKD-EPI exhibited the numerically largest predictive value of all renal markers. Nevertheless, the additive predictive value of cystatin C or any creatinine-based eGFR measures in the unselected ACS patient is small when evaluated
in the context of a multivariable adjusted prediction model including both clinical variables and biomarkers.

**Paper II – Changes in Renal Biomarkers and Risk Prediction**

The second paper in the thesis evaluated serial cystatin C concentrations in ACS patients and whether ticagrelor treatment was associated with increased cystatin C concentrations. This was important because in the main PLATO trial an increase in creatinine and uric acid was observed in all patients but was more pronounced in patients randomized to ticagrelor compared to clopidogrel. It was also unclear whether the possible deterioration in estimated renal function would affect prognosis.

The second paper therefore aimed to evaluate a possible decrease in kidney function, evaluating the determinants of the change and assessing the possible effect on long term prognosis. We used a cohort consisting of patients with follow up blood samples (baseline cystatin C and creatinine values plus at least one additional sample). Baseline samples were collected within 24 hours of admission (baseline), at discharge from hospital, at 1 month and at 6 months of follow up.

The initial evaluation focused on the changes in biomarkers over time, in relation to randomized treatment, and was analyzed by covariance (ANCOVA) analysis. Approximately two thirds of the patients experienced an increase in renal marker concentrations in hospital and consequently decreased their glomerular filtration rate. Between the two treatment groups, a statistical difference at discharge was observed but there were no statistical differences between the geometric means of the two randomized groups at follow up.

**Table II.** Mean cystatin C and creatinine concentrations presented at different time points with Standard Deviation (SD)

<table>
<thead>
<tr>
<th>Cystatin C (mg/L)</th>
<th>n</th>
<th>Mean (SD)</th>
<th>Geometric Mean</th>
<th>n</th>
<th>Mean (SD)</th>
<th>Geometric Mean</th>
<th>Ratio of geometric means (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>2133</td>
<td>0.864 [0.33]</td>
<td>1.00 [0.37]</td>
<td>2162</td>
<td>0.861 [0.30]</td>
<td>0.99 [0.33]</td>
<td>1.03 [1.01-1.04]</td>
<td>0.0005</td>
</tr>
<tr>
<td>Discharge</td>
<td>2120</td>
<td>1.01 [0.42]</td>
<td>0.95</td>
<td>2166</td>
<td>0.98 [0.34]</td>
<td>0.93</td>
<td>1.01 [1.00-1.03]</td>
<td>0.155</td>
</tr>
<tr>
<td>1 m</td>
<td>1989</td>
<td>1.00 [0.37]</td>
<td>0.95</td>
<td>2014</td>
<td>0.98 [0.33]</td>
<td>0.94</td>
<td>1.01 [1.00-1.03]</td>
<td>0.155</td>
</tr>
<tr>
<td>6 m</td>
<td>933</td>
<td>1.00 [0.34]</td>
<td>0.96</td>
<td>993</td>
<td>0.99 [0.32]</td>
<td>0.95</td>
<td>1.01 [1.00-1.03]</td>
<td>0.1728</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Creatinine (μmol/L)</th>
<th>n</th>
<th>Mean (SD)</th>
<th>Geometric Mean</th>
<th>n</th>
<th>Mean (SD)</th>
<th>Geometric Mean</th>
<th>Ratio of Geometric Means (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>4210</td>
<td>85.8 [26.3]</td>
<td></td>
<td>4198</td>
<td>86.2 [27.2]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Discharge</td>
<td>N/A</td>
<td>N/A</td>
<td></td>
<td>N/A</td>
<td>N/A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 m</td>
<td>3938</td>
<td>92.8 [29.0]</td>
<td>89.5</td>
<td>3943</td>
<td>91.4 [28.5]</td>
<td>88.2</td>
<td>1.01 [1.00-1.02]</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>6 m</td>
<td>2822</td>
<td>92.9 [29.1]</td>
<td>89.5</td>
<td>2781</td>
<td>91.5 [27.1]</td>
<td>88.7</td>
<td>1.01 [1.00-1.02]</td>
<td>0.0385</td>
</tr>
</tbody>
</table>

Table 2. Serial cystatin C concentrations during follow up stratified by randomized treatment. Published with kind permission from the American Heart Journal.
The mean cystatin C concentrations in 2133 ticagrelor- and 2162 clopidogrel-treated patients were at baseline (0.86 mg/L and 0.86 mg/L), discharge (1.01 mg/L and 0.98 mg/L) (p<0.0005), 1 month (1.00 mg/L and 0.98 mg/L) (p=0.12) and 6 months (1.00 mg/L and 0.99 mg/L) (p=0.17) respectively (Table 2, Figure 6).

![Figure 6. Serial cystatin C concentrations during follow up stratified by randomized treatment. Published with kind permission from the American Heart Journal](image)

In a risk prediction analysis, c-statistics and the relative Integrated Discrimination Improvement (IDI) of the cystatin C concentrations regarding the primary outcome (cardiovascular death or myocardial infarction) were evaluated at the different time points by multivariable analysis including background characteristics and biomarkers: NT-proBNP and troponin I.

C-statistics (AUC) and the relative IDI of the primary outcome for the baseline cystatin C concentration were 0.687 and 5.2%, compared to 0.684 and 4.5% at discharge (n=4034) and 0.693 and 5.1% at one month (n=3096), respectively (Table 3).

As a separate analysis the determinants of the deterioration of cystatin C values were assessed and age, heart failure and type of ACS were major determinants of the cystatin C concentration (paper II).
Table 3. C-statistics (AUC) and relative IDI values for cystatin C and CKD-EPI, as predictors for the combined endpoint of cardiovascular death or MI from the individual time point until 12 months after the index event, adjusted for clinical risk variables and biomarkers

In conclusion we observed that the mean cystatin C concentrations in ACS patients increased overall. However the most important factor determining this was not ticagrelor treatment but baseline characteristics such as age, CHF or type of ACS. The initial greater increase in ticagrelor-treated patients was not sustained over time. Risk prediction did not improve with serial measurements of renal markers.

Paper III – Comparison of Two Assays and a New Equation

This paper compares two cystatin C assays in respect to correlation and level of agreement. This was important as by 2012 there were no standardized reference values for cystatin C and neither was there a reference method or standardized calibration method for cystatin C assays. Furthermore this article evaluates, for the first time, a new and more accurate combined creatinine-cystatin C equation for eGFR in a risk prediction setting [81].

By using two different methods of measuring cystatin C (by Gentian and Roche), we measured cystatin C in plasma and evaluated the results by Pearson’s correlation [143] and agreement with the method described by Bland–Altman [144]. The prognostic value by both methods in relation to CV death or MI during up to one year of follow up was evaluated by multivariable Cox regression analysis, c-statistics and Integrated Discrimination Improvement (IDI).

The agreement between the two assays was evaluated by plotting the difference against the mean of both methods in accordance to the Bland-Altman method [144]. This method investigates a possible relationship between the measurement error and the true value. With the true value unknown, the mean of the two measurements is its best estimate [144]. The level of agreement was visualized as the degree of the bias, estimated by the mean difference \(d\), the standard deviation (SD) of those differences, and the “limits of agreement”, \(d+2SD\) and \(d-2SD\) [144].
We observed a difference between the two assays as the mean cystatin C concentrations were 0.89 mg/L with an SD of 0.35 for Gentian compared to 1.02 mg/L with an SD of 0.37 for Roche (n=16279).

The calculated median eGFR derived by the Gentian and Roche assays alone were 104 mL/min/1.73m² and 94 mL/min/1.73m² respectively, while calculated eGFR by the combined creatinine-cystatin C equation by Gentian and Roche samples were 89.9 mL/min/1.73m² and 82.7 mL/min/1.73m² respectively. CKD-EPI was calculated for comparison and exhibited a median eGFR of 82.6 mL/min/1.73m² (n=13632).

The correlation was good, both overall: 0.86 (95% confidence interval 0.85-0.86) and in certain subgroups. The level of agreement evaluated by the Bland-Altman plot [144] as presented in Figure 1, demonstrated that 3.1% of observations fall outside the limits of agreement of +0.52 and -0.26 mg/L.

In the risk prediction analysis we evaluated both assays but also the combined equation (with both Gentian and Roche values) as well as the CKD-EPI as predictors of the composite endpoint of CV death and MI in a multivariable model (with adjustment for clinical risk factors and biomarkers) during one year of follow up (n=13050). The c-statistics for cystatin C by the two different methods was almost identical as illustrated in paper III.
In conclusion we found two clinically available cystatin C estimates that correlated well overall but the important level of agreement was only moderate. Risk prediction with cystatin C in combination with creatinine, irrespective of assay, adds important information on the composite endpoint of CV death and MI over one year of follow up. However the solely creatinine-based original CKD-EPI exhibited the highest predictive power.

The Genetic Substudy of the PLATO Trial

Paper IV – Genetic Polymorphism and cystatin C

This paper investigates a possible genetic contribution to the cystatin C concentration and whether this is related to clinical outcomes. A total of 9978 individuals (3892 in the discovery set and 5996 in the replication set) contributed to the analysis.

Figure 8 illustrates the genome-wide association of SNPs regarding cystatin C concentration with several significant hits in the CST region on chromosome 20.

The overall median cystatin C concentrations (interquartile intervals) were 0.83 (0.68-1.00) mg/L, corresponding to a calculated median GFR of 104 mL/min/1.73m². The rs6048952 genotypes had an additive effect on cystatin
C with levels of 0.85 mg/L, 0.80 mg/L and 0.73 mg/L for the A/A, A/G, and G/G genotype respectively (Figure 9).

![Figure 9](image)

Figure 9. Cystatin C concentration by genotype, adjusted by eGFR by CKD-EPI.

In a subsequent analysis, when adjusting for the top SNP, we found a locus significantly associated with the cystatin C concentration (rs16985615). This SNP has, to the best of our knowledge, not previously been published. Modeled as additive, the allelic effect, using the multivariable adjustments, was -0.045 mg/L per G allele for the rs6048952 and +0.027 mg/L per G allele for the independently associated SNP rs16985615.

We evaluated the addition of the kidney markers, individually, in a multivariable risk prediction model (with adjustment for clinical risk factors and biomarkers) as predictors of the composite endpoint of CV death or MI during one year of follow up (n=9978). The genotype-adjusted cystatin C was derived by cumulative adjustment for the rs6048952 and the rs16985615 polymorphism. The c-statistics with no kidney marker, by addition of CKD-EPI, by addition cystatin C and by genotype-adjusted cystatin C were: 0.6619, 0.6657, 0.6705 and 0.6703, respectively.

No significant SNP effects (rs6048952) could be observed for any of the clinical outcomes or combinations of outcomes in the entire ACS cohort.

The rs6408952 polymorphism was however also evaluated for CV death alone by multivariable adjustment (including cystatin C or CKD-EPI) stratified by type of ACS. In this subset we found a signal that a significant rela-
tion between genetic polymorphism and CV mortality in the non-ST-elevation ACS subgroup may be present.

Multivariable c-statistics regarding the combined endpoint of CV death or MI without cystatin C yielded an area under curve (AUC) of 0.6619. The AUC after the addition of cystatin C or the addition of genotype-adjusted cystatin C was 0.6705 and 0.6703, respectively (n=8623). The addition of CKD-EPI to the multivariable risk prediction model yielded an AUC of 0.6651.

In conclusion, genetic polymorphism in the cystatin C gene affects cystatin C concentrations. However, no relationship between this genetic polymorphism and outcome was observed in the entire ACS cohort. Nonetheless, we did observe a signal that genetic polymorphism, associated with lower cystatin C concentrations, irrespective of glomerular filtration rate, may be independently associated with lower risk of cardiovascular death in the clinically important non-ST-elevation ACS subgroup.
Discussion

This thesis focuses on biomarkers reflecting the glomerular filtration rate, which were investigated from several different aspects in a large contemporary ACS population.

**Kidney biomarkers as risk predictors**

In Paper I we found cystatin C concentrations to independently contribute to predicting the risk of cardiovascular death or recurrent myocardial infarction. This was statistically significant both overall and in the NSTE-ACS subgroup, but did not reach statistical significance in the STE-ACS subgroup. However, the point estimate in the STE-ACS subgroup was in line with the main results and no interaction by type of ACS was observed. Thus it is reasonable to suggest that cystatin C may have additional value as a risk predictor, even in the STE-ACS subgroup.

As creatinine-based equations are the predominantly used methods of estimating the GFR, we evaluated the most abundant GFR estimates and found the numerically strongest predictive value by the CKD-EPI equation, which was an independent risk variable in both STE-ACS and NSTE-ACS patients. This may be due to the better accuracy of the CKD-EPI equation in the current GFR range, as more than four out of five patients had an eGFR > 60 mL/min/1.73m². Further only approximately 1.5% of the patients in the PLATO study was classified as CKD stage 4 or worse.

Although the additive power of cystatin C or any GFR estimate to a multivariate analysis was significant, the magnitude of the additional value of any renal marker to overall risk prediction was low when clinical risk factors and biomarkers of myocardial infarction and left ventricular dysfunction were used in the prediction model, suggesting that kidney dysfunction covaries with other clinical characteristics. Kidney disease is often due to diabetes or hypertension, risk factors that co-vary with coronary and peripheral artery disease as well as with congestive heart failure. Our results, obtained by multivariable analysis including several co-varying factors, may be interpreted as decreased kidney function may be of subdued importance in this population, especially in the STEMI patients, in comparison to other known cardiovascular risk factors and conditions.
Changes in renal biomarkers and risk prediction

In the main PLATO trial both creatinine and uric acid concentrations rose in the majority of patients and the change was more pronounced in patients randomized to ticagrelor. Paper II addressed this issue as we chose to evaluate a third marker of kidney function (cystatin C) in serial analyses. In concordance with the creatinine and uric acid results, the mean cystatin C concentration increased in the majority of ACS patients with a non-sustained, more pronounced increase at discharge in patients randomized to ticagrelor treatment.

When the main PLATO trial was published, the increased kidney markers caused some concern and in an editorial published simultaneously with the main study, the use of ticagrelor in patients with decreased kidney function was not advised [145]. This increase in kidney function markers also led to a suggestion to check renal markers after one month of treatment, then as needed [146]. In our study we can show an evident rise in kidney biomarkers in general. As three different markers of kidney function (uric acid, creatinine and cystatin C) all show a similar pattern, we believe the general increase in kidney markers reflect an actual decline in GFR (as cystatin C is not secreted by the tubuli) in the majority of ACS patients, with a slightly greater, non-sustained, additional decrease in ticagrelor-treated patients.

The cause of the rise of kidney markers in general was determined by multivariable analysis and we found a multitude of clinical risk factors and medications to be of importance, for example age, type of ACS and congestive heart failure. Other factors included the initiation or presence of ACE inhibitors, as well as gender and whether an invasive approach was planned [147]. Compared to these clinical background variables, ticagrelor treatment affected the cystatin C concentration only modestly.

The more pronounced increase in cystatin C from baseline to discharge between the randomized groups was not sustained at 1 or 6 months. There were a predefined lower number of patients during the follow up, but the number of patients did not affect the statistical evaluation as the predefined number of patients was similar at discharge as at 1 month.

The effect of ticagrelor treatment was evaluated in respect to kidney function (GFR) in a substudy by James et al. [14]. The results clearly showed that ticagrelor treatment was more efficacious irrespective of eGFR and in fact, numerically more efficient in preventing the combined endpoint, the worse GFR [14]. We thereby believe the effect of ticagrelor on eGFR was subdued to the increased survival and the decreased risk of recurrent myocardial infarction proven by ticagrelor treatment in ACS patients. Further, the slight deterioration in renal function in the acute setting of ACS did not add any incremental prognostic information concerning cardiovascular death or myocardial infarction in this ACS cohort. We were unable to find any difference in risk prediction capabilities regarding baseline and follow up samples.
nor to find any importance of the change in biomarker concentration between baseline and follow up.

The actual cause of the non-sustained difference between the two treatments in renal markers remains unknown. Several theories have been presented and it has been postulated that it may be due to (1) adenosine-mediated arteriolar vasoconstriction, (2) possible changes in tubular excretion of uric acid or (3) an increase in plasma concentration of adenosine and thus an increased substrate for uric acid formation [146, 148]. The increased survival rate in patients with decreased kidney function, treated with ticagrelor, may also have provided a survival bias.

Comparison of two assays and a new equation

As decreased kidney function in several studies is associated with adverse cardiovascular events, it was somewhat unexpected to find that the cystatin C concentrations did not to a greater extent influence cardiovascular risk. Paper III investigated this issue as we evaluated the risk prediction capabilities, level of agreement and correlation in two commercially available cystatin C assays. Both assays were based on turbidimetric technology, but with different antibodies.

The striking result of this investigation was that although the two assays measure the exact same protein, their correlation was moderate and their level of agreement was not impressive. The level of agreement is of greater clinical relevance as it provides information about comparison of the actual measurements, not just the direction. As a consequence, the inter-individual difference between the two methods was not satisfactory for clinical use and there is an obvious risk of possible differences in staging patients into the clinically important CKD subgroups. This problem has been addressed and great efforts have been made to produce a clinically useful calibration for the cystatin C assays [149].

Risk prediction was similar between the two assays, despite this discrepancy in cystatin C concentrations. As the inconsistency between the methods did not affect risk prediction, one can hypothesize that the cystatin C method is a blunt measure of cardiovascular risk in a multivariable analysis.

We can also conclude from this study that risk prediction with cystatin C in combination with creatinine, irrespective of assay, adds important information on the composite endpoint of CV death and MI over one year of follow up. However, the solely creatinine-based original CKD-EPI exhibited the highest predictive power. In theory, the addition of another renal marker would improve accuracy and give better risk prediction, since the combined result would be a more precise marker of the actual GFR as any bias or any confounder linked to a specific marker would have less influence [150]. However, the CKD-EPI, which is derived from a large population based on gold standard equivalent $^{125}$I-iothalamate mGFR and which has been validated in several clinical populations with normal or near normal kidney func-
tion, may have an advantage with this setting, and hence exhibited the numerically largest predictive value.

**Genetic polymorphism and cystatin C**

Paper IV found that genetic polymorphism was important for the cystatin C concentration and hence a confounder when evaluating the cystatin C concentration. This has been reported previously but the extent of non-GFR determinants affecting the cystatin C concentrations in patients with no or mild chronic kidney disease has not been presented definitely. A previous evaluation where cystatin C genetic polymorphism was adjusted with MDRD has been published, but because this was in a population with mildly reduced or no GFR, the subsequent CKD-EPI equation would have provided a better GFR estimate. In our study the top SNPs influencing the cystatin C concentration were located, similar to previous studies, in the vicinity of the regulatory element of the CST3 gene. Due to the location with possible influence on a regulatory element, we believe that the genetic polymorphism is related to changed expression of the cystatin C gene. Alternative explanations would include increased degradation, altered glomerular filtration or analytical discrepancies but we believe that these explanations are less likely.

When we adjusted for the top SNP we found another locus that was independently related to the cystatin C concentration (rs16985615), which to the best of our knowledge has not been reported previously.

The genetic polymorphism did not independently influence the combined endpoint of CV death or MI in the entire study population although a trend towards decreased risk for patients with genetically low cystatin C was noted. Additionally, when we used the genetic information to adjust the measured cystatin C concentration, we did not observe incremental accuracy in risk prediction.

As cystatin C is known to be an important marker of cardiovascular death in the non-ST-elevation ACS subgroup [72, 74, 151] we chose to evaluate the influence of genetic polymorphism in this subgroup. In patients with non-ST-elevation ACS the genetic polymorphism, irrespective of glomerular filtration, added significant incremental prognostic value in predicting cardiovascular death. To our knowledge this information has not previously been published and this finding will thereby reinforce the hypothesis of a biological role for cystatin C in the development of cardiovascular disease.

The effect of genetic variability on cystatin C concentrations has a relatively larger impact on patients with normal or mildly reduced glomerular filtration rate (>60 mL/min/m²). It is therefore especially important to be aware of this genetic variability in these patients as this information may alter identification, classification and stratification of patients with mildly decreased kidney function. Consequently the genetic polymorphism is of less importance in CKD Stage 3 or worse as these groups are already known
to be at great cardiovascular risk and can already be easily identified with current methods.

The emphasis in the current study is that genetic polymorphism of the cystatin C gene is not an independent risk factor in ACS overall, but may independently predict cardiovascular death in non-ST-elevation ACS, a subgroup where cystatin C concentrations are known to have prognostic impact. The role of the genetic polymorphism was attenuated in a combined endpoint including MI as well as when STEMI patients were evaluated. This was expected since cystatin C exhibits a weaker association with MI and has not been proven to be independently predictive in STEMI patients [72, 151].

Nonetheless, the current study provides further support for evaluating cystatin C, and thus the genetic regulation of cystatin C, especially as a marker of cardiovascular death, in non-ST-elevation ACS populations. The link between genetic polymorphism in the cystatin C gene and outcome needs to be further evaluated as well as interpreted with caution.

Importantly though, the current study does not provide a causal relationship to the observation that low concentrations of cystatin C, due to genetic variation, are associated with low risk, irrespective of the actual cystatin C concentration. It has been proposed that cystatin C, an abundant proteinase inhibitor, lowers the oxidative stress and opposes cathepsins responsible for atherosclerotic plaque destabilization and hence protects against cardiovascular adverse events [94, 95]. In case-control studies, genetic polymorphism of the CST3 gene has been linked to severity of cardiovascular disease, yet no prospective trial has shown a causal or independent relation to cystatin C polymorphism [82, 97, 98, 103].

It was somewhat unexpected therefore that we observed the lowest risk in patients with genetically determined low plasma cystatin C concentrations. A possible explanation could be that an imbalance between the inhibitor cystatin C and the proteinases is of importance. Nonetheless, we believe the results presented in this thesis support the hypothesis of a biological role of cystatin C, even though we cannot provide a causal explanation to the possible advantage of low plasma cystatin C concentrations or the presence of the specific allele.

**General discussion**

There are several further issues that need to be addressed because they have possibly affected the results of our studies.

Regarding the risk prediction analyses, it is important to remember that cystatin C and biomarkers in general are better for predicting death (total mortality or cardiovascular mortality), rather than myocardial infarction [16, 72, 74, 106, 110]. In the main PLATO trial, the majority of the 1878 primary endpoint were recurrent myocardial infarction (1097) while 795 were deaths from vascular causes and 231 were stroke events. The effect of biomarker concentration on the primary endpoint was likely attenuated in our
study by the inclusion of myocardial infarctions. When biomarker concentration was related to MI alone, a borderline statistical relationship was observed. Further, in studies where mortality was the leading outcome a more robust relationship between kidney function biomarkers has been observed. These studies have included older patients [16, 106, 122], more severely ill patients with several co-morbidities and a longer follow up period [16, 72, 74, 106, 110].

Another important consideration is that the patients’ characteristics may have affected the distribution of outcome in the trial. All participants in the study population were recruited in the first 0-24 hours of an ACS event. They had hence survived the first critical phase of ACS, since it is considered that up to one in three patients with ACS die prior reaching a hospital. If a general population with no evident CAD were evaluated, it would be reasonable to assume that some of these individuals would develop ACS, but suffer from a malign arrhythmia and not make it to the hospital. This subgroup of patients, suffering from ACS with sudden death as their initial (and only) symptom, is not represented in the current study.

The eGFR is related to cardiovascular outcomes, but the effect of the eGFR is diminished when adding other clinical and biomarker variables. The NT-proBNP is one of the strongest predictors of mortality and the effect of cystatin C or eGFR is minimized when NT-proBNP is added. In clinical practice, individuals with congestive heart failure and hence increased NT-proBNP concentrations, often develop chronic kidney disease. Since we adjusted for NT-proBNP, we believe the risk for overestimating and inflating the prognostic capabilities of cystatin C is minimal. Other clinical studies, that not adjusted for NT-proBNP, may partly have reflected possible co-existing heart failure, which is accompanied by a high risk of cardiovascular death [16].

In patients with ACS, the majority of recurrent cardiovascular events occur within the first month, and a large part of these are in fact in-hospital events occurring within the first 24 – 48 hours of the index event. A substantial part of these events are iatrogenic or peri-procedural events. Typical examples include PCI or CABG procedures, which are performed to lower overall cardiovascular risk but which may cause a minor coronary vessel closure, with subsequent increase in troponin, and hence a myocardial infarction. From a clinical perspective, these events are not considered to have the same impact as a spontaneous re-infarction occurring after an initial episode of ACS [152]. In the PLATO study, where physicians were encouraged to perform revascularization therapy when applicable, peri-procedural myocardial infarctions did occur and due to the difference in pathophysiology between a spontaneous and a peri-procedural MI, the prognosis is likely different. It is therefore reasonable to expect that the common risk stratification tools and the biomarkers used to foresee spontaneous re-infarctions are not applicable in estimating the risk of peri-procedural MIs. Thus the pres-
ence of peri-procedural myocardial infarctions may have attenuated the results.

Although there is evidence that an episode of ACS may begin asymptomatically several days prior to clinical symptoms, it is not likely that this subclinical activation itself would change the kidney biomarker concentrations, since a rise in cystatin C is not observed until after 24 hours in, for instance, AKI patients [62]. The cystatin C or creatinine value at admission in ACS patients would thereby reflect a basal level of risk, rather than the actual hemodynamic and metabolic situation in the ACS patients. This basal risk will reflect long term prognosis (rather than short term outcome) and is likely influenced by previous illnesses and previous risk factors. This covariation will probably attenuate the actual power of the eGFR marker.

The main hypothesis of kidney disease and cardiovascular risk is that the worse kidney function there is the greater is the risk for cardiovascular adverse events. GFR is difficult to measure and all measurements and estimations rely on the gold standard, the renal clearance of inulin. The filtration rate of the biologically inactive and inert exogenous substance inulin is by tradition the gold standard and it cannot be ruled out that an actual decline in kidney function (functional, hormonal or declined filtration) can occur in patients that have an ordinary inulin filtration. One must hypothesize that decreased kidney function can present two different faces, quantitative (decreased GFR) and qualitative (decreased function e.g. proteinuria) and it is likely that they will both affect cardiovascular risk. This hypothesis is illustrated in early diabetics where a transient hyper-filtration precedes the subsequently decreased GFR, indicating a non-linear relationship between kidney disease and GFR [53]. This has also been taken into account in the CKD guidelines, where an individual can exhibit CKD without impaired glomerular filtration rate [65]. This observation will be more important in individuals with normal or mildly reduced kidney function, as in the PLATO population.

Limitations
There are inborn difficulties with a variable like GFR. Confounders like medical treatments and concomitant disease may alter both the actual GFR as well as creatinine and cystatin C concentrations. Further the actual GFR is neither constant nor unaffected by physiological differences or everyday variation. We cannot rule out the possibility that we may have encountered unknown confounders that may have affected our results, despite having used a randomized material in order to avoid them.

In comparison to a community-based ACS population, the PLATO study population is healthier, younger, has better kidney function, less comorbidities and consequently exhibits a lower risk. The population cannot therefore be regarded unconditionally as an appropriate sample of the average ACS population. Further, in a clinical study there are always issues and factors that cannot be adjusted for, that may affect the trial. Factors that may
lead to a biased patient enrollment include patient motivation, expected compliance with study drugs as well as actual patient location. These factors may have excluded certain subgroups of patients from the clinical trial, and as they are difficult to address, it cannot be ruled out that they may have influenced the study population and hence the results.
Conclusions

I Cystatin C concentrations contributed independently to predicting the risk of cardiovascular death or myocardial infarction in a large ACS cohort.

II The additive power of cystatin C or any GFR estimate to a multivariate analysis was significant, irrespective of time when the concentration was measured. However, the magnitude of the additional value to overall risk prediction was low when clinical risk factors and biomarkers were used in a prediction model.

III In ACS patients, renal function deteriorated in the majority as indicated by an increase in mean cystatin C and creatinine concentrations. This proposed change in GFR did not affect outcome.

IV Baseline cystatin C concentrations measured with two different assays yielded different median and mean concentrations. Although the correlation was good, the level of agreement between the two assays was only moderate.

V Risk prediction with a new equation combining both creatinine and cystatin C concentrations did not clinically improve risk prediction compared to using the CKD-EPI equation.

VI Genetic polymorphism influenced cystatin C concentrations. Genetic variation in the cystatin C gene was not related to outcome in an ACS population overall. However in the clinically important subgroup of non-ST-elevation ACS patients, genetic polymorphism was signaling a possible association with cardiovascular death.
Clinical Implications and Future Directions

This thesis reinforces the fact that CKD is an important risk factor in respect to cardiovascular outcomes. We were, however, not able to provide a definitive answer to which equation to use for risk prediction in this thesis.

Producing new and improved methods for estimating the GFR is important not only for pharmacological dosing and decision making in clinical practice, but also for risk prediction.

We were unable to show a better risk prediction with the new combined equation, although this equation likely reflects the GFR better than a single marker equation. The use of combined equations with two renal markers will make the estimating process more complex, but more accurate, and hence future GFR estimates will likely consist of two or more markers.

It is our belief that the use of mGFR, at least methods based on renal clearance, will be of less importance in the future as improvements in GFR estimating equations, with combinations of markers and possibly genetic information, will provide information accurate enough and hence be the method of choice when kidney function is discussed.

Another important question is whether a single equation can provide reasonable accuracy for several different subpopulations. Although this would be desirable, the innate difficulties with estimating the GFR may be more prominent when studying several different populations and these challenges might not be completely diminished by adding more markers. This said, the best GFR estimate may not necessarily be ideal for risk prediction as in our study we observed a signal that genetic polymorphism may influence clinical outcomes in the non-ST-elevation ACS population.

Thereby, the objective to find a single equation that can provide both accurate GFR estimates and risk predictions, in several diverse populations, may prove very difficult. The alternative will consequently be to produce several different equations which all have a stronghold in their population or for their separate purposes, respectively. Future research will provide the answer.
Acknowledgements

I wish to express my sincere gratitude and appreciation to everyone who has helped me to complete this thesis, with special thanks to:

**Stefan James** - My primary supervisor, for your enthusiasm, support and for being a clinical and research role model. Your never ending enthusiasm for improving my research and enhancing my knowledge has been invaluable.

**Lars Wallentin** - My co-supervisor, for your inspiration and for sharing your unprecedented expertise and knowledge in the field of cardiovascular research.

**Agneta Siegbahn, Richard C. Becker, Andrzej Budaj, Steen Husted, Robert F. Storey, Jay Horrow, Anders Himmelmann, P. Gabriel Steg, Hugo Katus, Evangelos Giannitsis and Marc J. Claeys** - My co-writers, who have given me great feedback and important comments. Thank you for your enthusiasm and encouragement!

**Anders Larsson** – Co-writer and renal expert, enthusiastic in giving and sharing the wonders of laboratory medicine and the world of chemistry.

**Nils Åsenblad, Niclas Eriksson, Maria Bertilsson and Åsa Johansson** - The main statisticians and geneticists who have organized the vast amount of information and helped us find our gold nuggets.

**Research group:** Bertil Lindahl, Lars Wallentin, Frank Flachskampf, Stefan James, Jonas Oldgren, Claes Held, Gerhard Wikström, Bertil Andrén, Bo Lagerqvist, Erik Björklund, Christina Christersson, Emil Hagström, Nina Johnston, Kai Eggers, Thomasz Baron, Christoph Varenhorst, Mohammad Kavianipour, Catrin Henriksson, Ziad Hijazi, Birgitta Jönelid, Dan Henrohn, Julia Aulin, Ola Vedin, Gorav Batra and Daniel Lindholm.

You have given me good advice and support as well as made both conference trips and everyday research an interesting and exciting part of my life.

**UCR lab, with Birgitta Högberg and Mats Flodin** - You made all the laboratory analyses and answered my basic questions. Thanks!
PLATO patients – Without your courage, we would know less about CV disease and other patients would not benefit from new effective drugs.

PLATO nurses – For good work in recruiting and taking care of all patients.

Department of Cardiology – for letting me work with what I am interested in as well as providing me with great clinical training and time for research.

My Colleagues at the Uppsala University Hospital in general and the Department of Cardiology in particular. Thank you for sharing all your knowledge and for your great support and friendship.

Emil Hagström – Good times at the clinic and at conferences. Thanks for hilarious conversations, inspiring road trips and great plans.

Johan Ärnlöv and Inga Soveri for providing me with key insights and invaluable advice regarding kidney function estimates and laboratory analyses.

Niclas Abrahamsson, Jens Ellingssen, Martin Sandelin, Martin Wohlin, Johan Zelano, Gabriel Westman, Kirtisiri Casey Chetty and Olov Norlén.
Colleagues at the Uppsala University Hospital who have given me memorable discussions, great times as well as nice dinners over recent years.

A special thanks to all my good friends outside work, no one mention, no one forgotten.

Ebba Bergman and Ulla Nässander Schikan who have provided great help with publications and project place presentations.

UCR personnel and CEC for providing an excellent environment for research and work. For pleasant lunches and great humor!

To my parents Kerstin and Bernt Åkerblom and my sister Elisabet Sjöstrom with family for all the support and all those great times you can only have with family! You have always been there for me and you provide the most important link to the past, present and future!
The Laurell family - for all the support, generosity and your interest in our well-being.

Finally, to the most important things in my life, my lovely wife Karin and our two wonderful daughters Ellen and Agnes.
Without your strength, compassion and love this would not have been possible. Your presence and the joy of being with you is worth everything!
Hjärt-kärlsjukdom i allmänhet och akut koronart syndrom (AKS) i synnerhet är den ledande orsaken till död och sjuklighet globalt. I Sverige och andra höginkomstländer har det skett stora förbättringar vad gäller omhändertagande, behandling och prognos men insjuknandet i AKS är fortsatt högt även om dödligheten minskat. Nya behandlingsstrategier och läkemedel har bidragit till minskad dödlighet, men den största förbättringen harrör sannolikt från en ökad medvetenhet om hjärt-kärl sjukdomar och bekämpningen av kända riskfaktorer.


Nedsatt njurfunktion är således ett stort kliniskt problem och trots den markant förhöjda risken för akut kranskärlssjukdom (AKS) kan patienter med njurfunktionsnedsättning i många fall gå miste om effektiv behandling då man bedömer att riskerna för exempelvis blödning eller ytterligare njurskada är för stora. Det är därmed av stor vikt att korrekt kunna bedöma njurfunktionen för att identifiera patienter som har nytta av behandling, samtidigt som riskerna inte blir för stora. En noggrann uppskattning av njurfunktionen är även avgörande för bedömningen av risken för nya hjärt-kärlhändelser eller komplikationer (riskprediktionen).

Mitt avhandlingsarbete rör biomarkörer (ämnen som kan påvisas i blodprov i olika koncentrationer) som riskmarkörer och jag har huvudsakligen studerat de två mest kända kroppseginna njurfunktionsmarkörerna; kreatinin och cystatin C. Dessa två ämnen speglar njurfunktionen genom att deras koncentration i blodet stiger kraftigt med nedsatt njurfunktion (nedssatt filtreringsförmåga) och har tydligt länkats till att kunna förutspå död eller ny hjärtinfarkt hos patienter med akut koronart syndrom (AKS). Det har under längre tid debatterats vilken av dessa markörer som skattar njurfunktionen bäst och samtidigt hur man matematiskt ska värdera att markörerna påverkas av en mängd olika variabler som inte har med njurfunktion att göra. I klinisk praxis används ofta kreatinin, och sätts då i relation till ålder, kön med flera variabler. Cystatin C är mindre benägen att påverkas av andra icke njurrelaterade faktorer, men samtidigt är cystatin C betydligt mindre välstuderad.

I avhandlingsarbetet har blodprover från fler än 16000 patienter från en stor global studie på AKS patienter studerats. Samtliga patienter lämnade blodprov när de insjuknade i AKS, och dessa analyserades med avseende på kreatinin och cystatin C nivåerna i blodet. Genom att länka patienternas nivåer i blodet, med hur det sedan gick för dem under uppföljningen (under ett år), har vi kunnat dra slutsatser om vilken information som kreatinin och framförallt cystatin C ger oss om prognosen för dessa patienter.

Vi undersökte flera olika njurfunktionsmått och ekvationer men gjorde även genetiska analyser samt undersökte ett helt nytt beräkningssätt av njurfunktionen. Vi studerade dessa njurfunktionsmått i olika subgrupper och vid olika tillfällen, allt för att försöka hitta den optimala tidpunkten, den noggrannaste metoden och den mest korrekta tolkningen av resultaten när vi undersökte relationen mellan njurfunktionsmarkörer och risken att återinsjukna i ny hjärtinfarkt eller avlida hos patienter med pågående AKS.
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69


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