BIOMARKERS IN THE ASSESSMENT OF PROGNOSIS AND ORGAN INJURY IN CARDIOGENIC SHOCK

TONI JÄNTTI

ACADEMIC DISSERTATION
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To my family

‘Every good idea sooner or later degenerates into hard work’

Calvin Trillin
ABSTRACT

Cardiogenic shock is the most severe form of acute heart failure. It is a syndrome in which an initial insult results in deterioration of cardiac output, leading to systemic hypoperfusion often with accompanying microcirculatory dysfunction and systemic inflammatory responses resulting in organ injury and high mortality. Despite modern advanced therapies, short-term mortality in cardiogenic shock remains high at approximately 40%. Mortality in cardiogenic shock is often caused by multi-organ failure, but the incidence, sequence and predisposing factors for organ injury and impairment in cardiogenic shock patients remains largely unstudied. Novel invasive therapies, such as mechanical circulatory support devices may be life-saving in certain situations but also carry a high risk of complications, increasing the need for risk assessment tools for selection of suitable patients while avoiding harm and futility.

The aim of this thesis was to investigate biomarkers associated with end-organ injury in cardiogenic shock, to assess the incidence of organ injury, and to assess the prognostic importance of the biomarkers in cardiogenic shock patients. The study population was 179 patients enrolled in the multinational, prospective, observational CardShock study with blood samples available.

Study I evaluated the prevalence and prognostic importance of several liver biomarkers and their early changes within the first 24 hours of study enrolment. We found that although elevated alanine aminotransferase (ALT) was frequent already at baseline, it was not independently associated with 90-day mortality, whereas an early increase in ALT by 20% or more within the first 24 hours was strongly and independently associated with higher 90-day mortality. Findings in study I suggest that early increases in ALT in patients in cardiogenic shock are related to organ hypoperfusion, which was supported by the association of ALT increase with lower cardiac index and other clinical markers of hypoperfusion.

Study II assessed albumin levels in cardiogenic shock patients and their association with 90-day mortality. Hypoalbuminemia was present in 75% of patients in the early phase of cardiogenic shock. Hypoalbuminemia was associated with pre-existing comorbidities, higher levels of C-reactive protein and lower levels of hemoglobin. Plasma albumin levels at baseline were independently associated with 90-day all-cause mortality, with mortality increasing across lower albumin quartiles in a linear fashion.

Study III investigated two biomarkers – plasma proenkephalin (P-PENK) and plasma neutrophil gelatinase-associated lipocalin (P-NGAL) – which have previously been shown to be associated with acute kidney injury (AKI). Levels of both P-PENK and P-NGAL differed between patients who did and did not develop...
AKI defined by an increase in creatinine within 48 hours ($\text{AKI}_{\text{crea48h}}$) as well as between 90-day survivors and nonsurvivors. High baseline levels of both P-PENK and P-NGAL were able to predict the occurrence of $\text{AKI}_{\text{crea48h}}$ with reasonable accuracy. High levels of P-PENK and P-NGAL at 24 hours were independently associated with increased 90-day all-cause mortality after adjustments for $\text{AKI}_{\text{crea48h}}$ and two risk scores validated for cardiogenic shock patient populations.

Study IV examined the associations of a novel biomarker, circulating miR-423-5p, with clinical findings, other biomarkers and outcome. Above median levels of circulating miR-423-5p were associated with markers of hypoperfusion, organ injury and dysfunction. A miR-423-5p level above median also predicted 90-day mortality independently of established risk factors in cardiogenic shock patients. The association of miR-423-5p with high-sensitivity troponin T, an established marker of cardiac injury, depended on the etiology of cardiogenic shock.

In conclusion, the studied biomarkers (ALT, albumin, P-PENK, P-NGAL and miR-423-5p) relate to organ injury, provide additional prognostic information compared with clinical risk scores and can be utilized in the risk assessment of patients in cardiogenic shock.
Sydänperäinen shokki on sydämen äkillisen vajaatoiminnan vaikea-asteisin ilmenemismuoto. Kyseessä on oireyhtymä, jossa tapahtumaketjun käynnistävä vaurio johtaa sydämen minuuttivirtauksen alenemaan, joka puolestaan yhdessä mikroverenkierron toimintahäiriöön ja elimistön tulehdusvasteen kanssa johtaa elimistön verenkiertojaukseen, elinvaurioihin ja korkeaan kuolleisuuteen. Sydänperäisen shokin hoitomuotojen kehittymisestä huolimatta kuolleisuus on pysynyt edelleen korkeana noin 40%:ssa. Uudet kajoavat hoitokeinot kuten mekaaniset verenkierron tukilaitteet saattavat tietyissä tilanteissa pelastaa ihmishenkiä, mutta niihin toisaalta liittyvät korkeat komplikaatioriskit ovat lisänneet tarvetta työkaluihin hoidosta hyötyvien potilaiden tunnistamiseksi häittojen ja hyödyttömiön hoitojen välttämiseksi.

Tämän väitöskirjan tavoitteena oli arvioida sydänperäiseen shokkiin liittyvien elinvaurioiden biomerkkiaineita ja niiden käyttökelpoisuutta sydänperäisen shokin ennusteen arviinnissa. Tutkimusaineistona oli 179 monikansalliseen, havainnoinaan ja etenevään CardShock -tutkimukseen osallistunutta potilasta, joilta oli verinäyte saatavilla biomerkkiaineiden analysoimiseksi.

Ensimmäisessä osatyössä arvioitiin useiden maksaan liittyvien elinvaurioiden biomerkkiaineiden poikkeavien löydösten yleisyyttä tässä aineistossa sekä aikaisten 24 tunnin sisällä hoidon alusta tapahtuvien muutosten ennusteellista merkittävyyttä. Tutkimuksessa havaitsimme, että vaikkakin alaniiniaminotransferaasi (ALT) oli usein koholla jo tutkimuksen alkuhetkellä, koholla olevat arvot eivät ennustaneet kuolleisuutta itsenäisesti muista tekijöistä riippumatta toisin kuin ensimmäisten 24 tunnin aikana tapahtuva ALT-arvojen nousu yli 20%, mikä oli itsenäinen ja merkittävä 90-päivän kuolleisuuden riskitekijä. Ensimmäisen osajulkaisun löydösten perusteella aikainen ALT-arvojen nousu näyttää yhdistyvän pääte-elinten verenkiertovajaukseen, mitä tukivat tutkimuksessa todetut ALT-nousun yhdistyminen matalampaan sydänindeksiin ja muihin verenkiertovajauksen kliinisiin ilmentyiin.


Kolmas osatyö selvitti kahden biomerkkiaineen, plasman proenkefaliinin (P-PENK) ja neutrofiilin gelatinaasiin assosioituvan lipokaliinin (P-NGAL)

ACKNOWLEDGEMENTS

This thesis was carried out at the Heart and Lung Center between 2014 and 2021. I was lucky to find a place in the Acute Heart Failure study group, which has been doing research in the field for a long time. In this group I have found many friends, kind peer support in times of need, the best travel companions and fellow foodies for congress trips around the world, but also support for understanding the secrets of statistics, scientific discussions and lots of good laughs.

I am deeply indebted to my supervisor, Docent Johan Lassus, who not only supervised my thesis but also encouraged me to become a cardiologist. Johan has provided a model on how to be a scientist as well as a first-rate clinical cardiologist and has been a great inspiration to me. Johan has steered me both on my scientific and professional career, and as my chief medical officer has luckily been in a position to provide me with enough study leave so that I find myself in a position where I am able to write the acknowledgements for my thesis.

Another person to whom I owe my deepest gratitude is my second supervisor, Docent Veli-Pekka Harjola, who is the primary investigator of the CardShock study and the driving force of the Acute Heart Failure study group. Thank you for your expert advice on scientific writing, endless enthusiasm and finding the time in your extremely busy schedule to provide me with just the right amount of support.

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My sincere thanks to all my co-authors. Thank you for your patience, your support and your help in formulating the ideas of our collaboration into coherent scientific reports. I have been very fortunate to have collaborated with some of the great minds in cardiology. In particular, I would like to thank professor emeritus Markku S. Nieminen for leading me to study heart failure and allowing me to join the Acute Heart Failure study group, which he originally created, and Professor Alexandre Mebazaa for encouraging me to look behind the numbers at the biological processes involved.
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Latest and greatest, I would like to thank my spouse Krista and our children, Jaason, Joakim and Jesper, for having had the patience to let me work and study for what must seem like an eternity and still finding the energy to encourage and support me. I wish every person on Earth had a support team like you. I love you very much!
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<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ACS</td>
<td>acute coronary syndrome</td>
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<tr>
<td>AKI</td>
<td>acute kidney injury</td>
</tr>
<tr>
<td>AKI&lt;sub&gt; crea48h&lt;/sub&gt;</td>
<td>acute kidney injury defined by an increase in creatinine within 48 hours of baseline</td>
</tr>
<tr>
<td>ALP</td>
<td>alkaline phosphatase</td>
</tr>
<tr>
<td>ALT</td>
<td>alanine aminotransferase</td>
</tr>
<tr>
<td>APACHE</td>
<td>Acute Physiology and Chronic Health Evaluation</td>
</tr>
<tr>
<td>AU</td>
<td>arbitrary units</td>
</tr>
<tr>
<td>AUC</td>
<td>area under the curve</td>
</tr>
<tr>
<td>Bil</td>
<td>bilirubin</td>
</tr>
<tr>
<td>CABG</td>
<td>coronary artery bypass graft</td>
</tr>
<tr>
<td>CAD</td>
<td>coronary artery disease</td>
</tr>
<tr>
<td>CI</td>
<td>confidence interval</td>
</tr>
<tr>
<td>CKD-EPI</td>
<td>Chronic Kidney Disease Epidemiology Collaboration</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>CVP</td>
<td>central venous pressure</td>
</tr>
<tr>
<td>CysC</td>
<td>cystatin C</td>
</tr>
<tr>
<td>eGFR</td>
<td>estimated glomerular filtration rate</td>
</tr>
<tr>
<td>GFR</td>
<td>glomerular filtration rate</td>
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<tr>
<td>GGT</td>
<td>gamma-glutamyl transferase</td>
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<tr>
<td>HF</td>
<td>heart failure</td>
</tr>
<tr>
<td>HR</td>
<td>hazard ratio</td>
</tr>
<tr>
<td>hs-TnT</td>
<td>high-sensitivity troponin T</td>
</tr>
<tr>
<td>IABP</td>
<td>intra-aortic balloon pump</td>
</tr>
<tr>
<td>IABP-SHOCK II</td>
<td>Intraaortic Balloon Pump in Cardiogenic Shock II</td>
</tr>
<tr>
<td>ICU</td>
<td>intensive care unit</td>
</tr>
<tr>
<td>IQR</td>
<td>interquartile range</td>
</tr>
<tr>
<td>KDIGO</td>
<td>Kidney Disease: Improving Global Outcomes</td>
</tr>
<tr>
<td>LFT</td>
<td>liver function test</td>
</tr>
<tr>
<td>LVEF</td>
<td>left ventricular ejection fraction</td>
</tr>
<tr>
<td>MAP</td>
<td>mean arterial pressure</td>
</tr>
<tr>
<td>MI</td>
<td>myocardial infarction</td>
</tr>
<tr>
<td>miRNA</td>
<td>micro-ribonucleic acid</td>
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<tr>
<td>NGAL</td>
<td>neutrophil gelatinase-associated lipocalin</td>
</tr>
<tr>
<td>NRI</td>
<td>net reclassification improvement</td>
</tr>
<tr>
<td>NT-proBNP</td>
<td>N-terminal pro-B-type natriuretic peptide</td>
</tr>
<tr>
<td>OR</td>
<td>odds ratio</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<td>-------------</td>
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<tr>
<td>P-Alb</td>
<td>baseline plasma albumin</td>
</tr>
<tr>
<td>PCI</td>
<td>percutaneous coronary intervention</td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
</tr>
<tr>
<td>PENK</td>
<td>proenkephalin</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td>ROC</td>
<td>receiver operating characteristic</td>
</tr>
<tr>
<td>RRT</td>
<td>renal replacement therapy</td>
</tr>
<tr>
<td>( r_s )</td>
<td>Spearman correlation coefficient</td>
</tr>
<tr>
<td>SD</td>
<td>standard deviation</td>
</tr>
<tr>
<td>SHOCK</td>
<td>Should We Emergently Revascularize Occluded Coronaries for Cardiogenic Shock</td>
</tr>
<tr>
<td>SIRS</td>
<td>systemic inflammatory response syndrome</td>
</tr>
<tr>
<td>SOFA</td>
<td>Sepsis-related Organ Failure Assessment</td>
</tr>
<tr>
<td>STEMI</td>
<td>ST-elevation myocardial infarction</td>
</tr>
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</table>
2 INTRODUCTION

Cardiogenic shock is a state of systemic hypoperfusion and hypoxia caused by insufficient cardiac output. It is the most severe form of acute heart failure (HF). Cardiogenic shock is a complex clinical entity, with several different causes and presentations that result from the interaction of the acute cardiac insult and patients’ underlying medical and cardiac conditions. The most common cause of cardiogenic shock is acute myocardial infarction (MI) with left ventricular dysfunction. Other common etiologies are mechanical complications of acute MI, valve lesions, cardiomyopathies, acute exacerbation of chronic HF and myocarditis. The initial cardiac insult can vary from acute MI causing severe impairment of cardiac performance to more gradual progressive impairments such as those seen in patients with acute decompensation of chronic HF, who experience a decline in cardiac output as a result of precipitating factors.

Independent of the initial cardiac insult, the pathophysiology of cardiogenic shock depends on systemic hypoperfusion caused by insufficient cardiac output. Hypoperfusion and other physiological derangements, such as microcirculatory dysfunction and systemic inflammatory response, lead to end-organ injury, and if not managed in time, multi-organ failure and eventually death. The incidence, sequence and predisposing factors for organ injury and impairment have not been extensively studied in cardiogenic shock. Circulating biomarkers can provide information on the pathophysiology involved and help in tailoring patient-specific therapies.

Despite advances in treatment, mortality in cardiogenic shock remains high at 30%–50%. There are several advanced treatment options, such as mechanical circulatory support systems, that may help a select group of high-risk patients but also carry a high risk of complications. Risk assessment tools are necessary to select suitable patients for these costly, invasive and complication-prone therapies. In the absence of early predictors of poor outcomes, risk assessment remains largely based on individual clinical judgement. Without early predictors of end-organ dysfunction, organ injury and dysfunction may be evident too late in the course of cardiogenic shock and the damage may already have become extensive or irreversible, with little help provided even by advanced therapies.

The aim of this thesis was to study the prognostic capabilities of several biomarkers for assessing outcomes in patients enrolled in the prospective, observational, multinational CardShock study on cardiogenic shock, with focus on biomarkers associated with end-organ injury.
3 REVIEW OF THE LITERATURE

3.1 Cardiogenic shock

3.1.1 Definition, epidemiology and etiology

Cardiogenic shock is the most severe form of acute HF. There is currently no uniform definition of cardiogenic shock.\(^{18}\) Cardiogenic shock is a clinical diagnosis and is generally defined as a state in which impairment of cardiac output causes both clinical and biochemical manifestations of inadequate tissue perfusion.\(^{19}\) The clinical presentation is generally characterized by persistent hypotension not responsive to fluid replacement, with clinical signs of hypoperfusion, such as low urine output, cold extremities and altered mental status. As there are no generally agreed unequivocal signs or symptoms to define cardiogenic shock, studies of cardiogenic shock have used different definitions (Table 1). The most recent definition suggested by the Heart Failure Association of the European Society of Cardiology is ‘a syndrome caused by a primary cardiovascular disorder in which inadequate cardiac output results in a life-threatening state of tissue hypoperfusion associated with impairment of tissue oxygen metabolism and hyperlactatemia which, depending on its severity, may result in multi-organ dysfunction and death.’\(^{18}\) Recently, the Society for Cardiovascular Angiography and Interventions (SCAI) has suggested a more uniform scheme for defining and classifying different categories of cardiogenic shock.\(^{20}\) Based on this new definition, there are five categories of risk: pre-shock to extreme cardiogenic shock, labelled A–E (Figure 1). This classification system of states of cardiogenic shock will help make future trials of cardiogenic shock more comparable. The SCAI classification has been validated in a large cohort of unselected intensive care unit (ICU) patients with different etiologies of cardiogenic shock, showing robust mortality risk stratification.\(^{21}\)

The prevalence of cardiogenic shock varies based on the definition of cardiogenic shock, the clinical setting involved and, due to advances in cardiac treatment, also on the time when the study was conducted.\(^{2-5}\) Cardiogenic shock patients comprise 2–5% of patients in acute HF studies and registries and 14–16% of ICU patients.\(^{22,23}\)

Cardiogenic shock is caused by impaired cardiac output, which can be due to primary myocardial, valvular, electrical or pericardial abnormalities. The most common etiology of cardiogenic shock is acute coronary syndrome (ACS), which causes 50–80% of cardiogenic shock cases.\(^{23,24}\) In ACS, insufficient blood flow through the coronary arteries leads to myocardial ischemia and infarction. Most of the cardiogenic shock related to ACS is caused by ST-segment elevation MI, while a smaller proportion is due to non-ST-segment elevation MI.\(^{14,23}\) Other possible etiologies include acute exacerbation of chronic HF, mechanical complications
of ACS, valve lesions, cardiomyopathies, myocarditis, pulmonary embolism and Takotsubo syndrome. Cardiogenic shock is detected at admission in 30–40% of patients and occurs later in the course of hospitalization in 60–70% of patients.12,25

Table 1. Different pragmatic and clinical trial definitions of cardiogenic shock used.

<table>
<thead>
<tr>
<th>ESC Heart Failure Association position paper 22</th>
<th>SHOCK trial 26</th>
<th>IABP-SHOCK II 27</th>
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<tr>
<td>A syndrome caused by a primary cardiovascular disorder in which inadequate cardiac output results in a life-threatening state of tissue hypoperfusion associated with impairment of tissue oxygen metabolism and hyperlactatemia which, depending on its severity, may result in multi-organ dysfunction and death</td>
<td>Clinical criteria: SBP &lt;90 mmHg for ≥30 min OR Support to maintain SBP ≥90 mmHg AND End-organ hypoperfusion (urine output &lt;30 mL/h or cool extremities) Hemodynamic criteria: cardiac index of ≤2.2 L·min⁻¹·m⁻² AND PCWP ≥15 mmHg</td>
<td>Clinical criteria: SBP &lt;90 mmHg for ≥30 min OR Catecholamines to maintain SBP &gt;90 mmHg AND Clinical pulmonary congestion AND Impaired end-organ perfusion (altered mental status, cold/clammy skin and extremities, urine output &lt;30 mL/h or lactate &gt;2.0 mmol/L)</td>
</tr>
</tbody>
</table>

ESC = European Society of Cardiology; IABP-SHOCK II = Intraaortic Balloon Pump in Cardiogenic Shock II; PCWP = pulmonary capillary wedge pressure; SBP = systolic blood pressure; SHOCK = Should We Emergently Revascularize Occluded Coronaries for Cardiogenic Shock.

3.1.2 Pathophysiology

Despite varying etiologies, the pathophysologies of cardiogenic shock are quite similar. The cascade is started by an initial cardiac insult which causes cardiac output to decrease, leading to hemodynamic alterations, microcirculatory dysfunction and systemic inflammatory response syndrome (SIRS). These mechanisms lead to end-organ hypoperfusion, multi-organ injury and if the cascade is not stopped in time, ultimately to death.

The classical pathogenic mechanism of cardiogenic shock is a large acute MI leading to severe acute left ventricular failure. As a consequence, stroke volume and concomitant cardiac output are reduced, leading to low systemic blood pressure and increased left ventricular end-diastolic pressure.28 With the progression of cardiogenic shock, compensatory mechanisms are activated which may be maladaptive and lead to worsening shock. As the blood pressure drops, systemic vasoconstriction occurs, which may initially improve organ perfusion but leads to elevations in afterload and central venous pressure (CVP), increasing the load on the already failing heart.29 Microcirculatory dysfunction is also often present early in the course of cardiogenic shock,30 causes cellular hypoxia and is associated with organ injury and poor prognosis.31 Another mechanism often contributing to the pathophysiology of cardiogenic shock is systemic inflammatory response.30 Increased levels of several cytokines (interleukins 6, 7, 8 and 10) have been detected shortly after onset of cardiogenic shock and are associated with an increase in early
mortality. It has been estimated that SIRS is present in 20–40% of cardiogenic shock patients. In addition, complicating factors such as infection may be present in 20–30% of cardiogenic shock patients. Microcirculatory injury of the intestinal barrier causes increased bacterial translocation, leading to infection, cytokine release and inflammatory responses.

Figure 1. Pathophysiology of cardiogenic shock with staged abnormalities of clinical examination, hemodynamics, microcirculatory dysfunction, and organ failure. The SCAI classification is presented in the upper row. Reproduced from Wiley with permission.

3.1.3 Management of cardiogenic shock

Management of cardiogenic shock should start immediately after it has been identified. After basic life support, the initial steps in the management of cardiogenic shock should aim to find out the cause of the cardiogenic shock and, if possible, start specific treatment to reverse the cause of the cardiogenic shock and restore systemic perfusion. The most important initial investigations in determining the
etiology of cardiogenic shock are ECG and echocardiography. Further treatment decisions are guided by the suspected etiology of the cardiogenic shock.

As the most common etiology of cardiogenic shock is ACS, coronary angiography is warranted in most cases. Cardiac catheterization is both the definitive diagnostic investigation and guides therapeutic intervention in cardiogenic shock complicating acute MI.\(^{36}\) For cardiogenic shock caused by ACS, the initial treatment is rapid revascularization. The landmark Should We Emergently Revascularize Occluded Coronaries for Cardiogenic Shock (SHOCK) trial established the importance of rapid revascularization in the treatment of cardiogenic shock caused by ACS, showing that an early invasive strategy was associated with significantly lower all-cause mortality than initial stabilization.\(^{26}\) In the CULPRIT-SHOCK trial, it was further shown that culprit-vessel only revascularization in cardiogenic shock caused by ACS was superior to complete revascularization of all lesions that were considered hemodynamically significant.\(^{37}\) In cases where percutaneous procedures are not feasible, coronary artery bypass grafting should be considered. In a subanalysis of the SHOCK trial, coronary artery bypass graft (CABG) surgery and percutaneous coronary intervention (PCI) had similar 1-year mortality rates.\(^{38}\) For mechanical complications and valvular causes of cardiogenic shock, the treatment is surgical repair, if feasible.

Medical treatment aiming to restore adequate systemic perfusion should also be initiated as soon as possible, especially in patients with other etiologies of cardiogenic shock and in those in whom revascularization or surgical treatment is not considered feasible. This includes initial fluid resuscitation/challenge to correct for possible (relative) hypovolemia and initiation of vasoactive medications, such as norepinephrine, levosimendan, dobutamine and epinephrine. Since hypoperfusion and hypotension are central features, 80–90% of patients with cardiogenic shock require vasoactive medications.\(^{22}\) Regarding the choice of vasoactives, epinephrine use was associated with a significantly higher rate of refractory cardiogenic shock compared with norepinephrine in a recent randomized trial\(^{39}\) and was also associated with worse outcomes in a propensity-score matched analysis of the CardShock study patients\(^{40}\), as well as a threefold increase in mortality in a recent meta-analysis.\(^{41}\) Dopamine use in shock has also been associated with higher 28-day mortality and arrhythmias compared with norepinephrine.\(^{42}\) These data suggest using norepinephrine as the first-line vasoactive medication and to limit the dose and duration of vasoactive medications to the lowest possible according to current recommendations.\(^{43,44}\)

Mechanical circulatory support to improve cardiac output and systemic perfusion has an emerging role in the treatment of cardiogenic shock. However, the use of mechanical assist devices is associated with significant complications and high-quality evidence showing a net benefit in their use is largely absent. The routine use of intra-aortic balloon pumps (IABPs) has been studied in a
randomized study of cardiogenic shock patients with ACS etiology and failed to show benefit to mortality or any of the secondary end points. IABP use has not been studied in patients with other etiologies of cardiogenic shock and an IABP may be used in patients with mechanical complications or for stabilization in order to transport the patient into a tertiary centre. Impella, a microaxial pump that unloads the left ventricle by expelling blood flow to the aorta, did not show survival benefit in a propensity-matched study. In recent registries, Impella use has been associated with excess mortality, bleeding and access site complications, underlining the need for larger randomized trials before adopting Impella devices in clinical use.

### 3.2 Organ injury in cardiogenic shock

In cardiogenic shock, hypotension and global tissue hypoxia lead to organ injury. The extent of multi-organ injury is central in determining the prognosis of the patient. In a retrospective registry analysis of >400 000 patients with cardiogenic shock, there was a stepwise relationship between the number of dysfunctional organs and in-hospital mortality, a lower probability of home discharge and higher in-hospital cost. Several different organ systems can be affected (Figure 1). The digestive system and bowel appear to be among the first organs involved in multi-organ injury related to shock. Acute respiratory failure is almost invariably associated with cardiogenic shock, with hypoxaemia and hypercapnia caused by pulmonary congestion and decreased respiratory drive due to central nervous system hypoperfusion. The systemic hypoxia and microcirculatory failure affect several different organ systems simultaneously. In addition, functional impairment of one organ may exacerbate dysfunction of another organ, leading to a vicious cycle of multi-organ failure. Studies suggest that the prevention and correction of organ injuries are associated with better outcomes in acute HF. Thus, a central goal in the treatment of cardiogenic shock is the prevention and optimal management of multi-organ injury.

#### 3.2.1 Acute kidney injury

Acute kidney injury (AKI) in cardiogenic shock is caused by mechanisms similar to those in other organ injuries in cardiogenic shock. The mechanisms involved are decreased cardiac output, hypotension, venous congestion, microcirculatory failure, and systemic inflammatory response. Reduced cardiac output leads to hypoperfusion and venous congestion, which leads to the activation of the sympathetic nervous system and the renin–angiotensin–aldosterone system. The resulting vasoconstriction aims to raise blood pressure and temporarily helps to
maintain the glomerular filtration rate (GFR) by increasing the filtration in the kidney but results in decreased GFR and renal injury when the compensatory mechanisms fail. In a study of patients with cardiogenic shock who developed AKI, they were found to have higher CVP, lower mean arterial pressure (MAP) and needed higher doses of inotropic agents. Studies have also stressed the importance of venous congestion in the development of AKI in the setting of acute decompensated HF and chronic HF.

Currently, the diagnosis of AKI is made by an increase in serial serum creatinine measurements or by a decrease in urine output. Several different criteria for AKI have been used in studies, such as the RIFLE criteria, AKIN criteria and the, most contemporary, Kidney Disease: Improving Global Outcomes (KDIGO) criteria. Recently, a consensus statement on the use of AKI biomarkers has also been published. The use of different criteria has led to varying incidences of AKI in different studies. A study comparing the RIFLE and KDIGO criteria in the assessment of AKI in patients with acute MI found that the KDIGO criteria found more patients with AKI and was better at predicting outcomes compared with the RIFLE criteria.

A large registry analysis from the National Inpatient Sample, which used ICD diagnosis coding to detect cardiogenic shock and AKI, found that 35% of patients in cardiogenic shock developed AKI and 3.4% required renal replacement therapy. In another study of AKI in cardiogenic shock caused by ST-elevation myocardial infarction (STEMI) using a creatinine increase >25% from baseline to define AKI, the incidence of AKI was 55%. In this study, AKI was found to be the strongest independent predictor of mortality, and age >75 years, left ventricular ejection fraction (LVEF) <40%, mechanical ventilation, Sepsis-related Organ Failure Assessment (SOFA) score and the amount of intravenous contrast used were found to be associated with AKI. A third study of patients with cardiogenic shock after MI reported a 33% incidence of AKI defined as a urine volume <20 mL/h associated with an increase in serum creatinine level >0.5 mg/dL or >50% above the baseline value. The effect of AKI on survival in cardiogenic shock patients has been investigated in several studies. In a study of 118 patients with cardiogenic shock caused by acute MI, the in-hospital mortality for cardiogenic shock patients with AKI was 87% compared with 53% for patients who did not develop AKI, whereas a study of 97 patients with STEMI and cardiogenic shock found that AKI carried an in-hospital mortality rate of 50% versus 2.2% in patients without AKI. In the National Inpatient Sample, AKI in cardiogenic shock was associated with 1.3-fold higher mortality and if renal replacement therapy was required, mortality was 1.7-fold higher. In the CardShock study cohort, the incidence of AKI using the creatinine-based KDIGO criteria was 31% and had an unadjusted odds ratio (OR) for 90-day mortality of 7.5 (95% confidence interval [CI] 3.5-12.3), with 90-
day mortality reaching 70% for those with AKI compared with 24% for those without AKI. Thus, the presence of AKI identifies a patient population at a high risk of mortality. The importance of kidney function to mortality is underscored by the incorporation of measures of kidney function in several of the risk scores developed for cardiogenic shock. The Sleeper score from the SHOCK trial used creatinine >1.9 mg/dL, the Intraaortic Balloon Pump in Cardiogenic Shock II (IABP-SHOCK II) score includes creatinine >1.5 mg/dL at admission and the CardShock risk score stratifies patients by estimated glomerular filtration rate (eGFR).

Although AKI is usually defined by a decrease in urine output or GFR, there are also direct markers of tubular injury (such as neutrophil gelatinase-associated lipocalin [NGAL]) and other kidney injury biomarkers available which can be used either in the prediction of AKI or as prognostic biomarkers. In acute decompensated HF, high urinary NGAL levels (>32.5 μg/g of creatinine) at admission were found to predict AKI within 48 hours. For populations of cardiogenic shock patients, studies assessing the usefulness of kidney injury markers are sparse. In one study of 190 patients enrolled in the IABP-SHOCK II study, creatinine, NGAL and kidney injury molecule-1 were found to be higher in nonsurvivors but conferred no additional prognostic information compared with creatinine. However, the utility of NGAL in predicting AKI was not assessed in this study.

### 3.2.2 Acute liver injury

Liver injury and dysfunction are quite frequent in critically ill patients and are associated with poor outcomes. Liver injury in critical illness is caused by tissue hypoxaemia, leading to diffuse centrilobular liver injury. In the literature, the focus has been on hypoxic hepatitis, which is also variably called ischemic hepatitis or shock liver. Hypoxic hepatitis is characterized by a sharp elevation of transaminases and lactate dehydrogenase. There is no universally agreed definition of hypoxic hepatitis. A reasonable clinical definition is a syndrome with rapid and transient increases in alanine aminotransferase (ALT) to a level of more than 10 times the upper limit of normal in the setting of cardiac, circulatory or respiratory failure. However, this definition excludes smaller increases and changes in other liver function tests (LFTs) that may also be caused by inadequate liver perfusion due to cardiac causes. Transaminases usually reach their peak within 3 days of the hypoxic insult and return to normal levels within 10 days if the hypoxic state is reversed. In 114 critically ill patients with hypoxic hepatitis, the most common cause was cardiogenic shock (52%), followed by septic shock (32%).
Hypoxic hepatitis has been shown to be associated with poor prognosis also in cardiogenic shock. In a study of 172 patients with cardiogenic shock with ACS etiology from the IABP-SHOCK II study, hypoxic hepatitis (defined as a transaminase rise of >20 times the upper limit of normal) was present in 18% of the patients in the cohort. Patients with hypoxic hepatitis were found to have a significantly higher mortality compared with patients without hypoxic hepatitis (68% and 34%, respectively, \( p < 0.001 \)). Smaller increases have been described to have an effect on survival in acute HF and in patients with STEMI but have not been previously studied in cardiogenic shock patients.

Another form of liver injury and dysfunction in HF is the congestive hepatopathy associated with elevated CVPs, which is mostly characterized by elevated levels of bilirubin (Bil), gamma-glutamyl transferase (GGT) and alkaline phosphatase (ALP). In patients with acute decompensated HF, elevations in transaminases were associated with hypoperfusion, whereas elevations of ALP were associated with signs of systemic congestion and elevated right heart filling pressures. In hypoxic hepatitis, it has been shown that elevations in Bil occur later, 2–4 days after hypoxic hepatitis, and are also associated with an increased rate of complications and mortality. However, Bil elevations after hypoxic hepatitis were mostly associated with septic shock and occurred less often in patients with cardiogenic shock in this study. The effect of early changes in transaminases as well as the role of other LFT abnormalities in cardiogenic shock remain poorly studied.

### 3.3 Studied biomarkers

A biomarker is defined as ‘a defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes or responses to an exposure or intervention.’ Prognostic biomarkers are further defined as ‘biomarkers used to identify likelihood of a clinical event, disease recurrence or progression in patients who have the disease or medical condition of interest.’ A prognostic biomarker is one that indicates an increased (or decreased) likelihood of a future clinical event, disease recurrence or progression in an identified population. Prognostic biomarkers are measured at a defined baseline. When considering different treatments, prognostic biomarkers can contribute to decisions about whether or how aggressively to intervene with the treatment. Several interesting biomarkers related to hypoperfusion and organ injury have been studied in patients with acute MI and HF patients, but respective cardiogenic shock data are sparse. Here, a brief overview of the biomarkers studied in this thesis is presented.
### 3.3.1 Liver biomarkers

There are several commonly used biomarkers of liver function and injury. Liver biomarkers are usually classified as relating to predominantly liver cell necrosis (such as transaminases) or cholestasis (e.g. ALP, GGT and Bil).\(^{81,82}\)

Transaminases, such as ALT and aspartate aminotransferase, are enzymes which catalyse the transfer of amino groups from amino acids alanine and aspartate to ketoglutaric acid, generating components of the citric acid cycle. They are found especially in liver parenchymal cells, but small concentrations can be found in several other tissues, such as the muscles, kidneys, lung and heart.\(^{83}\) Aspartate aminotransferase is found in significant quantities in muscle cells as well as cardiomyocytes and has been one of the first biomarkers used to detect myocardial injury\(^{84}\), whereas plasma ALT is a relatively specific marker of hepatocyte injury.

ALP is an enzyme which transports liver metabolites across cell membranes found on the surface of bile duct epithelia. Hepatic and bone diseases are the most common causes of elevated ALP levels, although ALP is found in smaller quantities also in the kidneys, intestinal tract and leucocytes.\(^{85}\) Cholestasis increases the synthesis of ALP, whereas an accumulation of bile salts increases the release of ALP from bile duct cells, leading to a rise in plasma ALP concentrations in cholestatic conditions.\(^{86,87}\)

GGT is an enzyme catalysing the transfer of glutamyl groups to amino acids. It is found in several tissues but mostly in liver cells, biliary epithelial cells and kidney tubular cells as well as pancreatic cells and vascular endothelial cells.\(^{88}\) It is a sensitive indicator of extra- and intrahepatic cholestasis. In acute hepatitis it is elevated earlier than ALP and stays elevated for longer periods. GGT is not elevated in bone disease, making it more specific than ALP for cholestasis. GGT may, however, be elevated due to acute pancreatitis and chronic alcohol use or due to several medications (anti-epileptics, tricyclic antidepressants).

Bil is a breakdown product of the heme group in hemoglobin and other heme proteins such as myoglobin and cytochrome P450 isoenzymes. It is formed in macrophages in the spleen, bone marrow and liver.\(^{89}\) Unconjugated Bil is transferred from the circulation to hepatocytes, where it is conjugated with glucuronides in hepatocytic microsomes to make it more water soluble to allow its excretion in the bile. Bil levels can be elevated due to increased production (e.g. hemolysis) or a decreased rate of conjugation, which result in excess levels of unconjugated Bil, or decreased biliary secretion (cholestasis), which results in excess levels of conjugated Bil. Abnormal LFTs are a common finding in acute as well as chronic HF.\(^{49,78,90-93}\) Their association with worse outcomes has been well established for chronic HF\(^{94,95}\) but remains less well studied in patients with acute HF. The liver biomarkers most often found to be associated with worse prognosis in an acute HF setting are ALT\(^{90,78,93}\) and total Bil.\(^{92}\) Abnormal ALP
levels at baseline have also been shown to be associated with a higher 180-day mortality\textsuperscript{78} or the combined end point of mortality, or rehospitalization within 60 days.\textsuperscript{96} There have also been reports linking GGT with a worse prognosis.\textsuperscript{97,98} In chronic HF, GGT is frequently increased and is associated with disease severity, death or heart transplantation.\textsuperscript{99}

Most studies of acute HF have assessed only baseline liver biomarker tests, whereas the role of changes in LFTs during treatment has remained less well studied. Some investigations have found no connection between mortality and the changes in liver enzymes during treatment\textsuperscript{78,92,96}, whereas one investigation has shown an increase in mortality when ALT rises by more than 20% within 2 days of enrolment.\textsuperscript{89} The prevalence of LFT abnormalities and their association with prognosis in cardiogenic shock remains largely unstudied.

### 3.3.2 Albumin

Human serum albumin is a large protein of 585 amino acids with a molecular weight of 66 kDa. It is encoded in the human genome by the \textit{ALB} gene. Albumin is the most abundant plasma protein in mammals\textsuperscript{100}. The normal serum range of albumin is 35–45 g/L, with the total body content of a 70 kg human including about 300 g of albumin. Albumin is the main determinant of colloid osmotic pressure, representing about 80% of normal plasma colloid oncotic pressure and 50% of plasma protein content.\textsuperscript{101} The colloid osmotic pressure provided by albumin and other plasma proteins provides a balance between hydrostatic and colloid osmotic pressures, which is required to keep fluids inside the blood vessels and prevent the leakage of fluids into the interstitium and tissue oedema. Albumin has a daily degradation rate of 4% and a half-life of approximately 21 days\textsuperscript{101}, leading to a daily synthesis of approximately 200 mg of albumin/kg body weight per day. Albumin synthesis is decreased by several cytokines such as interleukins 1 and 6 and tumour necrosis factor alpha\textsuperscript{101,102}. Insulin is required for adequate synthesis, whereas corticosteroids increase both albumin synthesis and catabolism.\textsuperscript{101}

Hypoalbuminemia is a frequent finding both in chronic illness\textsuperscript{103} and acute conditions.\textsuperscript{104} In chronic illness, it has been thought that hypoalbuminemia may be the result of decreased synthesis in the liver due to wasting and cachexia. However, recent literature suggests that increased catabolism may be a more important contributor to hypoalbuminemia.\textsuperscript{105} In acute conditions the mechanisms of hypoalbuminemia differ from those in chronic disease, with capillary leakage into the interstitial spaces due to inflammatory processes being considered the largest contributor. Other mechanisms involved are decreased synthesis, hemodilution due to fluid administration, renal and gut losses due to congestion, and increased catabolism.\textsuperscript{106-108}
The effect of hypoalbuminemia on outcome has been studied in several different clinical settings but not in cardiogenic shock patients. The increase in mortality associated with hypoalbuminemia has been described in most detail for end-stage renal disease but hypoalbuminemia has also been shown to be associated with excess mortality in various different conditions such as trauma, critical illness, cancer, in the elderly and in chronic HF. It has also been suggested that the addition of plasma albumin to the Acute Physiology and Chronic Health Evaluation II (APACHE II) score would improve its prognostic abilities.

Considering acute cardiovascular disease, hypoalbuminemia has been shown to be associated with an increase in complications in acute MI as well as worse outcomes in both acute MI and acute HF. Interestingly, the Mini Nutritional Assessment revealed that 75% of patients with acute decompensated HF had malnutrition or were at risk of malnutrition, suggesting that malnutrition may play a part in the pathophysiology of acute HF and hypoalbuminemia.

### 3.3.3 Proenkephalin

Proenkephalin (PENK) is a small endogenous opioid peptide, with a molecular weight of approximately 4.5 kDa. There are endogenous opioid peptides found in the body, which are called endorphins, dynorphins and enkephalins. The two main forms of enkephalins, leucine-enkephalins and methionine-enkephalins, were discovered in 1975. Endogenous and exogenous opioids have numerous effects on the neural system, such as modulation of nociception, respiratory depression and addiction. Opioid receptors, especially the delta opioid receptor, are also expressed in peripheral organs, with the highest concentration found in the kidney, implying a possible direct effect of opioids on the kidney. Associations between endogenous opioids and kidney function were discovered in the 1980s, when the methionine-enkephalin D-Ala2-MePhe4-Met-(o)-enkephalin-ol was found to inhibit the secretion of antidiuretic hormone and induce diuresis. Enkephalins are also produced in the heart. The short half-life of enkephalins has made it difficult to assess their physiological functions. PENK, however, has a longer half-life and has been subsequently used as a surrogate marker for the activity of the endogenous opioid system. Transcription of the encoding gene PENK produces the precursor peptide preproenkephalin A, with a length of 267 amino acids. Preproenkephalin A contains both the functional peptides for leucine-enkephalin and methionine-enkephalin. Preproenkephalin A is further cleaved into functional enkephalins as well as several smaller peptides, one of which is PENK. PENK is formed by amino acids 119–159 of preproenkephalin A and has the benefit of being relatively stable in plasma, making it a good surrogate marker for the activity of the endogenous opioid system and enkephalins.
PENK has been used as a marker of kidney function. As a small peptide, PENK is freely filtrated in the glomerulus\textsuperscript{127,128} and is found in the urine. In a recent study of critically ill patients with septic shock, PENK levels were more accurate in determining true GFR assessed using plasma iohexol clearance than conventional creatinine-based eGFR calculations.\textsuperscript{129} PENK has also been demonstrated to predict AKI. In septic patients, PENK plasma concentrations were independently associated with AKI and related to AKI severity classified by the RIFLE criteria.\textsuperscript{127} In another study, PENK had a similar ability to detect AKI as estimated GFR when compared using receiver operating characteristic (ROC) curves.\textsuperscript{130} PENK has also been shown to predict AKI in cardiac surgery patients,\textsuperscript{131} as well as worsening renal function in a large cohort of patients with acute HF.\textsuperscript{132}

Elevated levels of PENK have also been shown to be associated with major cardiac events in patients with cardiac disease. In 1141 patients with acute MI, PENK levels were found to reflect cardiorenal status and elevated PENK levels were prognostic of death, recurrent acute MI and HF.\textsuperscript{133} In a cohort of 1908 patients with acute HF, PENK was associated with worsening renal function, in-hospital mortality as well as mortality during 1-year follow-up.\textsuperscript{134} In another study that included 1589 patients with acute HF, PENK was a predictor of 180-day mortality and HF hospitalization within 60 days.\textsuperscript{135} However, the possible association of PENK with outcomes has not been studied in cardiogenic shock.

### 3.3.4 Neutrophil gelatinase-associated lipocalin

NGAL (also known as lipocalin 2 or lcn2) is a small protein that was initially identified in mature neutrophil granules\textsuperscript{136} but has been subsequently found in numerous other cell types, including renal cells\textsuperscript{137} and cardiomyocytes.\textsuperscript{138} NGAL has been shown to take part in several biological processes, such as chemotaxis,\textsuperscript{139} iron transportation,\textsuperscript{140} and inhibition of bacterial growth.\textsuperscript{141} NGAL is also used as a biomarker of renal injury, as it is rapidly released in response to renal tubular cell damage.\textsuperscript{142,143} In animals, experimental ischemia-reperfusion of the kidney induces a 300-fold increase in circulating NGAL levels.\textsuperscript{144} Serum NGAL levels have also been shown to be sensitive markers of AKI in patients who have had cardiac surgery.\textsuperscript{145} NGAL is one of the most extensively studied biomarkers of AKI. It has been studied both in adult\textsuperscript{146,147} and paediatric\textsuperscript{148,149} populations and across different clinical settings, such as in patients who have had cardiac surgery,\textsuperscript{145-147} in critically ill patients,\textsuperscript{148,149} in contrast-media induced nephropathy after coronary angiography\textsuperscript{150,151} and in patients admitted to emergency care.\textsuperscript{152} Serum NGAL levels have also been shown to be independently related to the severity of AKI.\textsuperscript{153} A disadvantage of NGAL as a biomarker for AKI is that its plasma levels may be influenced by other medical conditions, such as inflammation, anaemia, ischemia, hypertension and malignancies.\textsuperscript{154,155}
Multiple studies have also shown elevated levels of circulating NGAL in patients with cardiovascular disease. Compared with healthy controls, serum NGAL levels are elevated in patients with acute MI\textsuperscript{156} and in patients with chronic HF\textsuperscript{157}. Studies also suggest that NGAL has prognostic value in patients with HF. High levels of circulating or urinary NGAL have been associated with renal complications\textsuperscript{158,159} and higher mortality\textsuperscript{160,161}. However, the association of circulating levels of NGAL with AKI and outcomes in cardiogenic shock remains unassessed.

### 3.3.5 MiRNAs in cardiac disease

Micro-ribonucleic acids (miRNAs) were originally discovered 40 years ago in the nematode \textit{Caenorhabditis elegans}\textsuperscript{162}. MiRNA genes are found throughout the genome.\textsuperscript{163} Subsequent studies have established that miRNAs are small, non-coding ribonucleic acids (RNAs) that regulate post-transcriptional gene expression by repressing messenger RNA translation through the RNA interference pathway.\textsuperscript{164} MiRNA genes are originally transcribed by the RNA polymerase II\textsuperscript{165}, leading to the formation of a primary miRNA transcript.\textsuperscript{166} The primary miRNA transcript is further modified by two endonuclease processing steps by the enzymes Drosha and Dicer before becoming a mature, active miRNA,\textsuperscript{167} which is a duplex of 2 RNA strings of approximately 21 nucleotides in length. The mature miRNA combines with the RNA induced silencing complex, which then seeks target messenger RNAs that have complementarity with the specific miRNA, leading to translational repression and degradation of target messenger RNAs.\textsuperscript{168} A single miRNA may regulate several messenger RNAs, as miRNA binding is mostly regulated by the seed region of nucleotides 2–7 of the miRNA, which may be complementary to several messenger RNAs.\textsuperscript{169,170} Today, over 2500 miRNAs have been annotated in the human genome.\textsuperscript{171}

MiRNAs have been shown to play an important part in several phases of cardiac development.\textsuperscript{172,173} Interestingly, fetal gene programming by miRNAs has also been implicated in the development of HF.\textsuperscript{174} It is currently thought that miRNAs have an intracellular effect in post-transcriptional gene expression. However, in 2008, miRNAs were also detected in the circulating blood\textsuperscript{175} and were found to be remarkably stable molecules.\textsuperscript{176} Several studies have shown that circulating miRNAs are taken up by target cells and regulate target gene expression in the uptaking cells.\textsuperscript{177-179}

The physiological role of circulating miRNAs remains to be elucidated, but their role as diagnostic and prognostic biomarkers has been established. The diagnostic and prognostic performance of circulating miRNAs has been assessed in patients with ACS and MI,\textsuperscript{180,181} as well as in patients with HF.\textsuperscript{182-187} Four miRNAs (miR-1, miR-133, miR-208 and miR-499) have been consistently found to be elevated in plasma within hours after the onset of acute MI.\textsuperscript{188-193} However, miRNAs are not
often tissue-specific, and miR-1, miR-133 and miR-499 are also expressed in the skeletal muscle.\textsuperscript{194,195}

MiR-423-5p has been consistently shown to be associated with the diagnosis and prognosis of HF\textsuperscript{182,185,186,196}, although some discrepancies exist\textsuperscript{184,197,198}. Furthermore, the expression of miR-423-5p increased in rat kidneys in response to ischemia/reperfusion injury and induced endoplasmic reticulum stress, oxidative stress and apoptosis of kidney cells, potentially leading to worsening of kidney injury.\textsuperscript{199} Similar induction of apoptosis by miR-423-5p has been shown in cardiomyocytes\textsuperscript{200}, suggesting that miR-423-5p may also have a role in the pathogenesis and progression of heart disease. Indeed, miR-423-5p has also been reported to be upregulated in failing human myocardium.\textsuperscript{174} Nevertheless, the role of elevated circulating levels of miR-423-5p in cardiogenic shock patients remains unstudied.

### 3.4 Assessing prognosis in cardiogenic shock

With the advent of novel invasive treatment options for cardiogenic shock, such as mechanical circulatory support systems and left ventricular assist devices, the need for accurate means of assessing patient prognosis has become increasingly important. Early risk assessment and patient profiling are needed to guide patient selection for advanced HF therapies, such as mechanical circulatory support systems and left ventricular assist devices. Risk assessment and patient risk stratification are also important for other aspects, such as for use in clinical trials, in classifying patients for enrolment, to compare treatment groups and to evaluate the effects of experimental treatments and procedures.\textsuperscript{201} Risk assessment has traditionally relied on clinical parameters and routine laboratory tests.

#### 3.4.1 Clinical risk scores

There are several mortality risk assessment scores developed for general ICU patients, such as the Simplified Acute Physiology Score II\textsuperscript{202}, APACHE II–IV\textsuperscript{115,203,204}, the Multiple Organ Dysfunction Score\textsuperscript{205} and the SOFA score\textsuperscript{206}. However, as they have not been developed especially for patients with cardiogenic shock, they may contain measures which are not generally available for all cardiogenic shock patients (such as CVP) and miss risk factors which are more relevant for them (e.g. LVEF).

Clinical risk scores have been developed for risk stratification and prognostic assessment specifically in cardiogenic shock. The first clinical risk score was the Sleeper risk score from the SHOCK trial population.\textsuperscript{17} Current risk scores developed in the contemporary era when PCI has been part of routine management include
the CardShock risk score, the IABP-SHOCK II score, the CLIP score, and CS4P. Of these, the CardShock risk score, IABP-SHOCK II score and CLIP score have been validated in other independent patient cohorts of cardiogenic shock, and only the CardShock risk score has been developed for patients with etiologies of cardiogenic shock other than ACS. Both the CardShock risk score and IABP-SHOCK II risk scores assess short-term mortality risk based on variables measured at baseline (Table 2).

**Table 2.** Validated clinical risk scores developed for cardiogenic shock used in studies I–IV.

<table>
<thead>
<tr>
<th>CardShock risk score</th>
<th>Points</th>
<th>IABP-SHOCK II score</th>
<th>Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age &gt;75 years</td>
<td>1</td>
<td>Age &gt;73 years</td>
<td>1</td>
</tr>
<tr>
<td>Altered mental status at presentation</td>
<td>1</td>
<td>History of stroke</td>
<td>2</td>
</tr>
<tr>
<td>Previous MI or CABG</td>
<td>1</td>
<td>Glucose &gt;10.6 mmol/L</td>
<td>1</td>
</tr>
<tr>
<td>ACS etiology</td>
<td>1</td>
<td>Creatinine &gt;132.6 µmol/L</td>
<td>1</td>
</tr>
<tr>
<td>LVEF &lt;40%</td>
<td>1</td>
<td>Blood lactate &gt;5 mmol/L</td>
<td>2</td>
</tr>
<tr>
<td>Blood lactate</td>
<td></td>
<td>TIMI flow grade &lt;3 after PCI</td>
<td>2</td>
</tr>
<tr>
<td>&lt;2 mmol/L</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2–4 mmol/L</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;4 mmol/L</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>eGFR</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>&gt;60 mL/min/1.73 m²</td>
<td>0</td>
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</tr>
<tr>
<td>30–60 mL/min/1.73 m²</td>
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<td></td>
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<td>&lt;30 mL/min/1.73 m²</td>
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<tr>
<td><strong>Maximum</strong></td>
<td>9</td>
<td><strong>Maximum</strong></td>
<td>9</td>
</tr>
</tbody>
</table>

ACS = acute coronary syndrome; CABG = coronary artery bypass graft; eGFR = estimated glomerular filtration rate using the Chronic Kidney Disease Epidemiology Collaboration equation; LVEF = left ventricular ejection fraction; MI = myocardial infarction; PCI = percutaneous coronary intervention; TIMI = thrombolysis in myocardial infarction.

CardShock risk score reproduced with permission from Wiley, IABP-SHOCK II risk score reproduced with permission from Elsevier.

### 3.4.2 Biomarkers in risk stratification

Biomarkers often form an integral part of risk stratification in critical illness. Several of the aforementioned risk scores also incorporate biomarkers in the risk score: the Multiple Organ Dysfunction Score includes platelet count, serum Bil and creatinine levels as biomarkers for mortality risk assessment, whereas the APACHE II score includes pH, sodium, potassium, creatinine, hematocrit and white blood cell count. Recently, a novel biomarker-only risk score for
cardiogenic shock including cystatin C (CysC) as a marker of renal function, lactate as a marker of tissue hypoxaemia, interleukin 6 as an inflammatory marker and N-terminal pro-B-type natriuretic peptide (NT-proBNP) as a marker of HF with good discrimination for 30-day mortality was described. Biomarkers may provide information for the recognition, prognostication and management of cardiogenic shock. One of the biomarkers included in many risk scores is lactate. Elevated lactate levels are a general marker for inadequate tissue oxygenation/metabolism, and serum lactate >2 mmol/L is one of the diagnostic criteria of cardiogenic shock.43 Lactate also has a strong prognostic role.209,210 In the National Cardiogenic Shock Initiative database, cardiac power and lactate at 12–24 hours were the best predictors of survival.211 Also, changes in lactate during the initial stages of treatment have been shown to be associated with prognosis.212

Several other biomarkers have shown prognostic value in addition to the clinical risk scores in cardiogenic shock. In the CardShock study cohort, early risk assessment combining ST2 and NT-proBNP measurements have been shown to provide prognostic value beyond clinical variables in patients with cardiogenic shock due to ACS.213 Adrenomedullin levels after 48 hours have also been found to predict 90-day mortality independently of the CardShock risk score and were associated with parameters of hemodynamic impairment.214 Admission blood glucose has also been shown to have prognostic significance in cardiogenic shock.215 Other biomarkers shown to affect prognosis in cardiogenic shock include growth differentiation factor 15,216,217 selenoprotein P,218 catalytic iron,219 angiopoietin 2,220 osteoprotegerin,217 fibroblast growth factor 23,221 procalcitonin,222 and IL-6.222
4 AIMS

The aims of this study were to investigate biomarkers associated with end-organ injury in cardiogenic shock and their prognostic importance in assessing outcomes in cardiogenic shock patients. The focus was on biomarkers associated with liver and kidney injury, with special interest in some novel biomarkers.

In more detail, the aims were:

1. To evaluate the frequency of abnormal LFTs and their effect on outcome in patients with cardiogenic shock, and to investigate the role of early changes in LFTs. (I)
2. To determine the prevalence and prognostic value of low plasma albumin on survival in cardiogenic shock and to determine the factors associated with hypoalbuminemia in cardiogenic shock patients. (II)
3. To assess the ability of plasma proenkephalin (P-PENK) and plasma neutrophil gelatinase-associated lipocalin (P-NGAL) to predict AKI and outcomes in cardiogenic shock. (III)
4. To examine the potential of miR-423-5p levels to predict mortality and associations of miR-423-5p with prognostic markers in cardiogenic shock. (IV)
5 SUBJECTS AND METHODS

5.1 The CardShock study

The CardShock study was a prospective, observational, multinational European study of cardiogenic shock patients, conducted in nine different locations (Helsinki, Barcelona, Copenhagen, Brno, Athens, Warsaw, Porto, Rome and Brescia) in eight countries. Patients were recruited from emergency departments, cardiac care units and ICUs as well as catheterization laboratories in tertiary hospitals. Patient recruitment was conducted between October 2010 and December 2012, during which time a total of 219 patients were enrolled in the study. The study was conducted in accordance with the Declaration of Helsinki and was accepted by local ethics committees in each of the participating centres, notwithstanding Copenhagen, where the study was approved by the Danish Protection Agency as according to Danish law ethical approval is not necessary for studies utilizing information from existing registries.

The CardShock study enrolled consecutive patients aged over 18 years within 6 hours of identification of cardiogenic shock. Informed consent was obtained from the patient or their next of kin if the patient was unable to provide consent. To be included in the study, the patient needed to have an acute cardiac cause of shock. In addition, the required inclusion criteria were 1) systolic blood pressure <90 mmHg (after adequate fluid challenge) for 30 min or need for vasopressor therapy to maintain systolic blood pressure >90 mmHg, and 2) signs of hypoperfusion (altered mental status, cold periphery, oliguria <0.5 m/kg/h for the previous 6 hours, or blood lactate >2 mmol/L). Patients were excluded from the study if the shock was caused by ongoing hemodynamically significant arrhythmias or after cardiac or noncardiac surgery. Cardiogenic shock etiology was determined by local investigators. ACS etiology was defined as shock caused by MI with or without ST-segment elevation. After study enrolment, patients were treated according to local practice, and treatment and procedures were registered. Demographic data and medical history were registered at baseline, and clinical signs and measurements were registered every 6–24 hours. Echocardiography was performed at baseline and at 72 hours. Routine laboratory samples were analysed locally. Invasive hemodynamic measurements were made in 79 patients.

Additionally, in 179 patients blood for later analysis was drawn at different time points between baseline and discharge from the cardiac care unit/ICU in centres participating in the biomarker arm of the study (Helsinki, Athens, Barcelona, Warsaw, Brno, Porto and Brescia). These patients form the population studied in this thesis (biomarker substudy arm of the CardShock study). After collection, the
blood samples were centrifuged, and separated plasma was immediately frozen in aliquots and stored at −80°C for later use.

Primary end points of the study were in-hospital, 90-day and 1-year all-cause mortality. Vital status of the study patients was determined through population and hospital registries or direct contact with the next of kin. Two patients were lost to follow-up and were left out of the survival analyses. The patient populations for studies I–IV can be seen in Figure 2.

5.2 Study outlines

5.2.1 Study I

Study I included 178 CardShock patients with baseline plasma samples available. Creatinine, C-reactive protein (CRP), high-sensitivity troponin T (hs-TnT), NT-proBNP, ALT, ALP, GGT and total Bil were analysed from serial blood samples drawn at baseline/0, 12 and 24 hours (all ±3 hours) in 178, 154 and 141 patients, respectively. The analyses were conducted at a central accredited laboratory (ISLAB, Kuopio, Finland) using standard commercial kits (Roche Diagnostics, Basel, Switzerland).

The cut-offs for abnormal values used by the central laboratory were >35 IU/L for ALT, >105 IU/L for ALP, >115 IU/L for GGT and >25 µmol/L for total Bil. eGFR was calculated from creatinine values using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. Arterial blood lactate and pH were analysed by local laboratories in the participating centres.
At least two separate LFT values within 24 hours were available for a total of 156/178 patients. An increase in LFT was determined as a rise of more than 20% from the first measurement to the highest value within 24 hours. This cut-off value was selected based on ROC curve analysis and has also been used in a previous investigation in acute HF. Patients with all LFT measurements in the normal range were not considered to have an LFT increase.

5.2.2 Study II

The study cohort for study II consisted of 178 CardShock study patients who had plasma samples taken at baseline. Additional plasma samples available for the same patients taken at 12, 24, 36, 48 and 72 hours (all ±3 hours) were also analysed. Creatinine, CRP, hs-TnT, NT-proBNP, ALT, total Bil and baseline plasma albumin (P-Alb) were analysed at a central accredited laboratory (ISLAB, Kuopio, Finland) using standard commercial laboratory kits (Roche Diagnostics, Basel, Switzerland). The cut-off used to determine abnormal values for P-Alb was <34 g/L, which is also the lower limit of normal for the commercial laboratory test kit used and has been used in several studies of HF. Arterial blood lactate and hemoglobin were analysed locally. eGFR was calculated from creatinine values using the CKD-EPI equation. Body mass index (BMI) was calculated as weight (kg) at admission / height (m)$^2$. Echocardiography was performed per protocol at study entry.

5.2.3 Study III

Study III included 154 CardShock study patients who had a baseline sample and at least one additional sample within 48 hours available. Creatinine, CRP, hs-TnT, NT-proBNP, ALT, ALP, total Bil and CysC were analysed from the plasma samples using commercially available standard kits (Abbott Laboratories, Abbott Park, IL, USA for CysC; Roche Diagnostics, Basel, Switzerland for all other tests) at a central accredited laboratory (ISLAB, Kuopio, Finland). Additionally, PENK concentrations in the plasma samples were obtained using Sphingotest® penKid immunoassay (SphingoTec, Hennigsdorf, Germany) commercial laboratory kits, and P-NGAL concentrations were determined using a particle-enhanced turbidimetric immunoassay (BioPorto Diagnostics, Hellerup, Denmark). eGFR was calculated from creatinine values with the CKD-EPI equation.

AKI was defined and staged according to the KDIGO criteria (Table 3). For AKI staging at baseline, a recently described staging classification which includes biomarker levels was used (Table 3). Main outcomes investigated in this study were AKI defined by an increase in creatinine of more than 26.5 μmol/L within 48 hours of study inclusion (AKI$_{\text{crea48h}}$) and 90-day all-cause mortality. AKI$_{\text{crea48h}}$
was selected a priori as one of the main outcomes studied as in cardiogenic shock patients $\text{AKI}_{\text{crea}48\text{h}}$ has been shown to be associated with worse prognosis, unlike KDIGO urine-output based definitions of AKI.$^{64}$

Assessment of $\text{AKI}_{\text{crea}48\text{h}}$ was based on changes in creatinine levels from baseline until 48 hours, and the largest increase within this time was used for staging. Urine output was recorded at 6, 12, 18 and 24 hours and used for urine-output based definitions of AKI. AKI staging by urine output was categorized according to the lowest urine output for a time interval within the first 24 hours. The Youden index was used to select the cut-offs of P-PENK and P-NGAL used for AKI and mortality prediction. Subclinical AKI was defined as biomarker positivity (either P-PENK or P-NGAL > cut-off) without $\text{AKI}_{\text{crea}48\text{h}}$. Two published risk scores (CardShock risk score$^6$ and IABP-SHOCK II score$^{65}$) that have been published and validated in cardiogenic shock patient populations were calculated for each patient to assess whether P-PENK or P-NGAL provided added value in risk prediction compared with the risk scores alone.

**Table 3.** Acute kidney injury (AKI) definitions and staging.

<table>
<thead>
<tr>
<th>Subclinical AKI</th>
<th>AKI stage 1*</th>
<th>AKI stage 2</th>
<th>AKI stage 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-PENK $&gt;$84.8 pmol/mL or NGAL $&gt;$104 ng/mL</td>
<td>Creatinine increase of $\geq 0.3$ mg/dL ($=26.5$ μmol/L) in 48h or Creatinine increase to 1.5–1.9 times baseline within 7 days or Urine output $&lt;$0.5 mL/kg/h for 6h</td>
<td>Creatinine increase to 2–2.9 times baseline within 7 days or Urine output $&lt;$0.5 mL/kg/h for 12h</td>
<td>Creatinine increase to 3 times baseline within 7 days or Creatinine increase to $\geq 4$ mg/dL ($=354$ μmol/L) or Urine output $&lt;$0.3 mL/kg/h for 24h or Anuria 12h or Initiation of RRT</td>
</tr>
</tbody>
</table>

*AKI stage IS
Subclinical AKI +, AKI stage 1 criteria -
AKI stage 1A
Subclinical AKI -, AKI stage 1 criteria +
AKI stage 1B
Subclinical AKI +, AKI stage 1 criteria +

NGAL = neutrophil gelatinase-associated lipocalin; P-PENK = plasma proenkephalin; RRT = renal replacement therapy.
5.2.4 Study IV

For study IV, circulating miR-423-5p levels were determined in 179 CardShock study patients with baseline plasma samples available. Blood samples obtained in ethylenediaminetetraacetic acid tubes were used. Total RNA was extracted from the baseline plasma samples using the mirVana PARIS kit (Ambion, Applied Biosystem, Lennik, Belgium). Synthetic *C. elegans* miR-39 (cel-miR-39) (Qiagen, Venlo, the Netherlands) was added to the plasma samples as a spike-in to correct for differences in miRNA extraction efficiency. After DNase treatment to remove potential genomic DNA contamination, reverse transcription of RNAs was carried out using the miScript reverse transcription kit (Qiagen). The resulting complementary DNA was diluted 10-fold before quantitative polymerase chain reaction (PCR) using the miScript SYBR Green PCR kit and miR-423-5p-specific miScript primer sets (Qiagen). To control for the absence of contaminating DNA and non-specific amplification, each PCR plate contained a control without reverse transcriptase and another control without RNA. Circulating miR-423-5p expression levels were normalized using the threshold cycle (Ct) of the cel-miR-39 control and calculated using the formula: $2^{(Ct\text{ cel-miR-39} – Ct\text{ miR-423-5p})}$. The results were expressed in arbitrary units (AU).

Additionally, creatinine, hs-ThT, NT-proBNP and ALT were analysed at a central accredited laboratory (ISLAB, Kuopio, Finland) using standard commercial kits (Roche Diagnostics, Basel, Switzerland). eGFR was calculated from creatinine values using the CKD-EPI equation.\(^\text{223}\) Arterial blood lactate was analysed locally.

5.3 Statistical analyses

Continuous variables are presented as mean and standard deviation (SD) for normally distributed variables or median and interquartile range (IQR) for non-normally distributed variables. Categorical variables are presented as numbers (N) and percentages (%). Group comparisons were performed using the Fisher’s exact test for categorical variables and the two-way analysis of variance test, Student’s t-test, Mann–Whitney *U* test or Mantel–Haenszel test for continuous variables, as appropriate. Associations between continuous variables were assessed using Spearman correlations for non-normally distributed variables and Pearson correlations for normally distributed values. To test for the significance of changes in biomarker levels between different time points, a paired samples t-test was used for normally distributed variables and the Wilcoxon signed-rank test was used for non-normally distributed variables. Differences in mortality were assessed by drawing Kaplan–Meier survival curves, which were compared with the log-rank test. Logistic regression analysis was used to identify variables associated with binary dependent variables. Survival analyses were made using
Cox proportional hazard models. Based on significant association with outcome in univariable analysis, multivariable modelling was performed. To select variables for multivariable modelling, variables with a univariable \( p < 0.1 \) were entered into a multivariable logistic regression model. Stepwise forward and backward likelihood ratio tests with significance <0.05 for inclusion and >0.1 for elimination were used to determine independent predictors of the dependent variable.

In study I, adjustment was made for the variables included in the CardShock risk score (age, history of MI or CABG, altered mental status at presentation, ACS etiology, LVEF, lactate and eGFR). In study IV, the model was adjusted additionally with hs-TnT and ALT at baseline. Log-minus-log plots were used to evaluate the adequacy of the proportional hazards assumption. Results from the regression analyses are presented as hazard ratios (HRs) with 95% CIs.

In study II, logistic regression analysis was used to identify variables associated with baseline hypoalbuminemia. Adjustments in multivariable models were made for 1) variables statistically significantly associated with hypoalbuminemia at baseline \( (p < 0.05) \), i.e. smoking status, comorbidities (HF with reduced ejection fraction, coronary artery disease [CAD], prior MI), calcium channel blocker use, lung oedema on X-ray, BMI, eGFR, hemoglobin, NT-proBNP, and CRP at baseline, and presence of multi-vessel disease in primary coronary angiography, as well as 2) CardShock risk score, IABP-SHOCK II score and combinations of 1) and 2).

In study III, differences in P-PENK and P-NGAL trajectories between different groups were assessed using linear mixed modelling.

In study IV, a general linear model was constructed to analyse the independent predictors of miR-423-5p as the dependent variable. For this analysis, miR-423-5p levels were log-transformed to normalize the distribution and the residuals.

To test for differences in discriminatory capabilities between different variables and predictive models, ROC curves were drawn and the area under the curve (AUC) was calculated. To assess whether incorporating a variable into the multivariable model provided additional prognostic value, the likelihood ratio test for nested models was used. In study II, discrimination was also assessed by the integrated discrimination index (IDI) and clinical risk stratification by net reclassification improvement (NRI).

A two-sided \( p < 0.05 \) was used as the limit for statistical significance. Data were analysed using the SPSS statistical package, versions 23–27 (IBM Corp, Armonk, NY), with the exception of the reclassification analyses, which were performed with R version 3.5.1 using packages Hmisc and pROC.
6 RESULTS

6.1 Patient characteristics

Table 4 shows the baseline characteristics of patient populations used in the studies compared with all patients included in the CardShock study. As can be seen, patients in the biomarker arm of the CardShock study are similar to the overall CardShock study population, without any significant differences in patient characteristics. In study III, 154 patients with a baseline sample and at least one other sample within 24 hours of the study baseline were selected, whereas for study I, patients with at least two samples within 24 hours were used. Two patients did not have baseline samples available but had 12-hour and 24-hour samples available, which is why the total number of patients in the second part of study I is 156 patients.

In general, 26% of the patients included in these studies were women, with a mean BMI of 26.9. Etiology of cardiogenic shock was determined to be ACS in 80% of the cases. Average MAP was 57 mmHg. The most common comorbidities were hypertension (61%), CAD (33%) and diabetes (30%), while a history of previous MI (25%) or HF (16%) was less common.

There were no statistically significant differences between the populations. It should be noted, however, that 90-day mortality in patients with at least two plasma samples within 24 hours was slightly lower due to survivor bias, as patients who died before the second sample could not be included in this group.
Table 4. Patient demographics and clinical characteristics at baseline.

<table>
<thead>
<tr>
<th></th>
<th>Total CardShock study population</th>
<th>Biomarker substudy population (studies I*, II* and IV)</th>
<th>Patients with 2 samples within 24 hours (studies I and III*)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=219</td>
<td>N=179</td>
<td>N=156</td>
</tr>
<tr>
<td>Age (years)</td>
<td>67 (12)</td>
<td>66 (12)</td>
<td>66 (12)</td>
</tr>
<tr>
<td>Women, n (%)</td>
<td>57 (26)</td>
<td>47 (26)</td>
<td>39 (25)</td>
</tr>
<tr>
<td>BMI, median (IQR)</td>
<td>26.8 (4.2)</td>
<td>26.9 (4.2)</td>
<td>26.9 (4.0)</td>
</tr>
<tr>
<td><strong>Medical history</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>76 (35)</td>
<td>59 (33)</td>
<td>53 (34)</td>
</tr>
<tr>
<td>Previous myocardial infarction</td>
<td>54 (25)</td>
<td>45 (25)</td>
<td>40 (26)</td>
</tr>
<tr>
<td>Prior revascularization</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCI</td>
<td>32 (15)</td>
<td>29 (16)</td>
<td>25 (16)</td>
</tr>
<tr>
<td>CABG</td>
<td>16 (7)</td>
<td>11 (6)</td>
<td>12 (8)</td>
</tr>
<tr>
<td>Heart failure</td>
<td>36 (16)</td>
<td>29 (16)</td>
<td>26 (17)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>132 (60)</td>
<td>109 (61)</td>
<td>97 (62)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>62 (28)</td>
<td>53 (30)</td>
<td>43 (28)</td>
</tr>
<tr>
<td>Renal insufficiency</td>
<td>25 (11)</td>
<td>22 (12)</td>
<td>17 (11)</td>
</tr>
<tr>
<td>Stroke/TIA</td>
<td>20 (9)</td>
<td>16 (9)</td>
<td>14 (9)</td>
</tr>
<tr>
<td>Smoker</td>
<td>87 (40)</td>
<td>72 (40)</td>
<td>62 (40)</td>
</tr>
<tr>
<td><strong>Presentation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sinus rhythm</td>
<td>159 (74)</td>
<td>129 (73)</td>
<td>113 (73)</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>34 (16)</td>
<td>27 (15)</td>
<td>21 (14)</td>
</tr>
<tr>
<td>ACS etiology of shock</td>
<td>177 (81)</td>
<td>143 (80)</td>
<td>124 (80)</td>
</tr>
<tr>
<td>Resuscitated from cardiac arrest</td>
<td>62 (28)</td>
<td>47 (26)</td>
<td>45 (29)</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>78 (14)</td>
<td>77 (12)</td>
<td>77 (12)</td>
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<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>47 (10)</td>
<td>47 (10)</td>
<td>47 (10)</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>57 (11)</td>
<td>57 (11)</td>
<td>57 (11)</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>90 (28)</td>
<td>88 (29)</td>
<td>87 (28)</td>
</tr>
<tr>
<td><strong>Clinical findings</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cold periphery</td>
<td>207 (95)</td>
<td>171 (96)</td>
<td>148 (96)</td>
</tr>
<tr>
<td>Confusion</td>
<td>148 (68)</td>
<td>118 (66)</td>
<td>103 (68)</td>
</tr>
<tr>
<td>Oliguria</td>
<td>121 (55)</td>
<td>94 (53)</td>
<td>80 (52)</td>
</tr>
<tr>
<td>Lactate &gt;2 mmol/L</td>
<td>155 (71)</td>
<td>125 (70)</td>
<td>104 (68)</td>
</tr>
<tr>
<td>Time from detection of shock to study baseline, min</td>
<td>120 (1-224)</td>
<td>120 (24-240)</td>
<td>120 (30-240)</td>
</tr>
<tr>
<td>Baseline investigations</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Echocardiography

<table>
<thead>
<tr>
<th></th>
<th>Value 1</th>
<th>Value 2</th>
<th>Value 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVEDD (mm)</td>
<td>52 (9)</td>
<td>52 (8)</td>
<td>52 (11)</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>33 (14)</td>
<td>33 (14)</td>
<td>33 (13)</td>
</tr>
</tbody>
</table>

### Biochemistry

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value 1</th>
<th>Value 2</th>
<th>Value 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood hemoglobin, g/L</td>
<td>127 (25)</td>
<td>129 (23)</td>
<td>130 (23)</td>
</tr>
<tr>
<td>Sodium, mmol/L</td>
<td>136 (7)</td>
<td>136 (8)</td>
<td>136 (8)</td>
</tr>
<tr>
<td>Potassium, mmol/L</td>
<td>4.1 (3.8–4.5)</td>
<td>4.4 (1.8)</td>
<td>4.3 (1.9)</td>
</tr>
<tr>
<td>Blood glucose, mmol/L</td>
<td>12.7 (6.6)</td>
<td>12.7 (6.4)</td>
<td>12.2 (6.1)</td>
</tr>
<tr>
<td>Arterial blood lactate, mmol/L</td>
<td>2.8 (1.7–5.8)</td>
<td>2.7 (1.7–5.8)</td>
<td>2.5 (1.6–5.1)</td>
</tr>
<tr>
<td>hs-TnT, ng/L</td>
<td>N/A</td>
<td>2190 (388–5418)</td>
<td>2290 (388–6186)</td>
</tr>
<tr>
<td>NT-proBNP, pg/mL</td>
<td>N/A</td>
<td>2710 (585–9434)</td>
<td>2236 (543–8408)</td>
</tr>
<tr>
<td>Creatinine (µmol/L)</td>
<td>N/A</td>
<td>104 (78–140)</td>
<td>98 (77–135)</td>
</tr>
<tr>
<td>eGFR, mL/min/1.73 m²</td>
<td>N/A</td>
<td>63 (30)</td>
<td>65 (28)</td>
</tr>
<tr>
<td>CRP, g/L</td>
<td>N/A</td>
<td>16 (4–54)</td>
<td>13 (4–48)</td>
</tr>
</tbody>
</table>

### Invasive hemodynamic measurements

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value 1</th>
<th>Value 2</th>
<th>Value 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central venous pressure, mmHg</td>
<td>13 (5)</td>
<td>13 (5)</td>
<td>13 (5)</td>
</tr>
<tr>
<td>Cardiac index</td>
<td>2.2 (0.9)</td>
<td>2.2 (0.9)</td>
<td>2.2 (0.9)</td>
</tr>
</tbody>
</table>

### Mortality

<table>
<thead>
<tr>
<th></th>
<th>Value 1</th>
<th>Value 2</th>
<th>Value 3</th>
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</thead>
<tbody>
<tr>
<td>In-hospital mortality</td>
<td>80 (37)</td>
<td>67 (37)</td>
<td>53 (34)</td>
</tr>
<tr>
<td>90-day mortality</td>
<td>89 (41)</td>
<td>74 (42)</td>
<td>59 (38)</td>
</tr>
</tbody>
</table>

*with minor variations

Results shown as counts (%) for categorical variables, and mean (SD) or median (IQR) for continuous variables.

ACS = acute coronary syndrome; BMI = body mass index; CABG = coronary artery bypass graft; CRP = C-reactive protein; eGFR = estimated glomerular filtration rate; hs-TnT = high-sensitivity troponin T; IQR = interquartile range; LVEDD = left ventricular end-diastolic diameter; LVEF = left ventricular ejection fraction; N/A = not applicable; NT-proBNP = N-terminal pro-B-type natriuretic peptide; PCI = percutaneous coronary intervention; SD = standard deviation; TIA = transient ischemic attack.

### 6.2 Liver biomarkers in cardiogenic shock (I)

Study I included 178 CardShock patients with all LFTs (ALT, ALP, GGT and Bil) available at baseline. At least two separate measurements within 24 hours from baseline were available for 156 patients, enabling assessment of the frequency and associations of early changes in LFTs.
6.2.1 ALT levels and changes in cardiogenic shock

Elevated levels of LFTs at baseline and at later time points were quite common (Table 5). Of the LFTs studied, ALT was the most frequently abnormal. Median ALT at baseline (ALT\(_{0h}\)) was 44 IU/L (IQR 20–92) and was abnormal (>35 IU/L) in 102 (58%) patients. Abnormal ALT\(_{0h}\) was more frequent among patients who died (68% compared with 51% in patients alive; \(p=0.03\)) during 90-day follow-up. As shown in Table 5, abnormal ALT levels were more frequent at all time points up to 24 hours after baseline in those deceased at 90 days.

In this cohort of cardiogenic shock, abnormal ALT\(_{0h}\) was associated with higher 90-day mortality (49% vs 32%, \(p=0.03\)). Abnormal ALT\(_{0h}\) was associated with several other baseline variables: higher levels of lactate (5.2 mmol/L vs 2.4 mmol/L in patients with normal ALT, \(p<0.001\)), oliguria prior to study baseline (65% vs 48%, \(p=0.03\)), higher hs-TnT (3592 ng/L vs 1568 ng/L, \(p=0.004\)) and lower eGFR (56 mL/min/1.73 m\(^2\) vs 66 mL/min/1.73 m\(^2\), \(p=0.03\)). In univariable Cox regression analysis, abnormal ALT\(_{0h}\) was associated with 90-day mortality with a HR of 1.7 (95% CI 1.1–2.8, \(p=0.03\)), but after adjusting for differences in baseline lactate levels the association was no longer statistically significant.

An increase in ALT>+20% within 24 hours of baseline (ΔALT>+20%) was observed in 24% (37/154) of patients with at least two ALT measurements available. In the ΔALT>+20% group, the median for the highest ALT level within 24 hours was 160 U/L (IQR 73–502), and the median absolute and relative increases in ALT within 24 hours were 69 U/L (IQR 30–329) and 100% (IQR 50%–530%), respectively.

ΔALT>+20% was associated with an over twofold increase in mortality compared with those without >20% increase in ALT (ALT stable): 70% and 28%, respectively (Figure 3). In the univariable Cox regression analysis, ΔALT>+20% was associated with 90-day mortality with a HR of 3.8 (95% CI 2.2–6.3, \(p<0.001\)). In the multivariable analysis, adjusting for the baseline level of ALT, highest hs-TnT level within 24 hours (PeakTnT) and the change in lactate levels within 24 hours, in addition to the risk factors included in the CardShock risk score, the association with 90-day mortality remained statistically significant (HR 3.0, 95% CI 1.6–5.6, \(p=0.001\)).

ΔALT>+20% was an important predictor of 90-day mortality regardless of the absolute baseline level (ALT\(_{0h}\)), as can be seen in the survival curves in Figure 4. ΔALT>+20% was associated with increased mortality both in patients with normal and abnormal baseline levels. The survival in the ALT stable group did not differ between those having normal and abnormal levels of baseline ALT. ΔALT>+20% was associated with oliguria and higher levels of lactate, higher PeakTnT as well as lower LVEF and eGFR (Table 6). There were, however, no significant differences in blood pressure, heart rate, previous medical history,
cardiovascular medications at admission, NT-proBNP or etiology of coronary syndrome between the ΔALT>+20% and ALT stable groups.

Of the 178 patients with baseline ALT values available, 17 patients (10%) fulfilled the criteria for hypoxic hepatitis (ALT >20 times the upper limit of normal [>700 U/L] within the first 24 hours). For the patients with hypoxic hepatitis, the 90-day mortality was 65% (11/17) (Jäntti et al., unpublished results). Even after the exclusion of patients who fulfilled the most commonly used criteria for hypoxic hepatitis (ALT >20 times the upper limit of normal) the association ΔALT>+20% and 90-day mortality remained independent (HR 3.4, 95% CI 1.9-5.9, p<0.001).

Serial invasive hemodynamic measurements, such as CVP and cardiac index, were available for a total of 79 and 50 patients, respectively. In the ΔALT>+20% group, the cardiac index was initially at the same level but recovered more slowly and was significantly lower between 24 hours and 48 hours compared with ALT stable (Figure 5a). In the ΔALT>+20% group, CVP was higher between 12 hours and 24 hours (p-values for pairwise comparisons 0.001 and 0.003), with a trend towards higher CVP up to 48 hours (Figure 5b).

### Table 5. Distribution of liver function test values at different time points.

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>90-day survivors</th>
<th>90-day nonsurvivors</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ALT (U/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 hours</td>
<td>44 (20–92)</td>
<td>38 (18–81)</td>
<td>58 (35–121)</td>
<td>0.03</td>
</tr>
<tr>
<td>12 hours</td>
<td>44 (17–110)</td>
<td>33 (15–70)</td>
<td>74 (34–159)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>24 hours</td>
<td>37 (18–85)</td>
<td>28 (16–63)</td>
<td>61 (29–129)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>ALP (U/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 hours</td>
<td>61 (49–81)</td>
<td>60 (47–78)</td>
<td>63 (49–82)</td>
<td>0.8</td>
</tr>
<tr>
<td>12 hours</td>
<td>59 (46–71)</td>
<td>60 (47–72)</td>
<td>57 (46–70)</td>
<td>0.7</td>
</tr>
<tr>
<td>24 hours</td>
<td>56 (42–70)</td>
<td>57 (43–71)</td>
<td>54 (42–69)</td>
<td>0.6</td>
</tr>
<tr>
<td><strong>GGT (U/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 hours</td>
<td>53 (31–104)</td>
<td>57 (34–106)</td>
<td>47 (28–79)</td>
<td>0.3</td>
</tr>
<tr>
<td>12 hours</td>
<td>53 (31–93)</td>
<td>55 (31–101)</td>
<td>46 (32–81)</td>
<td>0.3</td>
</tr>
<tr>
<td>24 hours</td>
<td>49 (31–87)</td>
<td>52 (31–99)</td>
<td>43 (31–76)</td>
<td>0.2</td>
</tr>
<tr>
<td><strong>Bil (µmol/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 hours</td>
<td>9.6 (5.7–15.4)</td>
<td>9.6 (6.0–16.6)</td>
<td>9.4 (5.6–15.4)</td>
<td>0.8</td>
</tr>
<tr>
<td>12 hours</td>
<td>9.7 (6.3–15.9)</td>
<td>9.9 (6.8–15.7)</td>
<td>9.2 (5.2–16.8)</td>
<td>0.2</td>
</tr>
<tr>
<td>24 hours</td>
<td>10.0 (6.6–16.6)</td>
<td>10.7 (7.2–16.3)</td>
<td>8.7 (5.6–18)</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Results presented as median (IQR).

ALP = alkaline phosphatase; ALT = alanine aminotransferase; Bil = bilirubin; GGT = gamma-glutamyl transferase; IQR = interquartile range.
Figure 3. Kaplan–Meier survival curves for ALT stable and ΔALT>+20%. ALT = alanine aminotransferase. Reproduced with permission from study I.227

Figure 4. Kaplan–Meier survival curves with respect to baseline ALT level and increase in ALT within the first 24 hours. ALT = alanine aminotransferase; NS = non-significant. Reproduced with permission from study I.227
Table 6. Baseline characteristics of study I patients with two plasma samples within 24 hours available, divided by ALT increase greater (ΔALT>+20%) or smaller than 20% (ALT stable) within 24 hours.

<table>
<thead>
<tr>
<th></th>
<th>All (N=156)</th>
<th>ALT stable (N=119)</th>
<th>ΔALT&gt;+20% (N=37)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVEF, %</td>
<td>33 (13)</td>
<td>35 (14)</td>
<td>28 (11)</td>
<td>0.01</td>
</tr>
<tr>
<td>ACS etiology</td>
<td>124 (80%)</td>
<td>91 (77%)</td>
<td>33 (89%)</td>
<td>0.11</td>
</tr>
<tr>
<td>Oliguria</td>
<td>80 (52%)</td>
<td>55 (47%)</td>
<td>25 (69%)</td>
<td>0.02</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>13 (4–48)</td>
<td>14 (5–59)</td>
<td>10 (3–27)</td>
<td>0.04</td>
</tr>
<tr>
<td>Highest hs-TnT within 24 hours, ng/L</td>
<td>4573 (1059–14956)</td>
<td>3677 (765–9843)</td>
<td>16963 (3572–35903)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ALT, U/L</td>
<td>42 (20–87)</td>
<td>38 (18–85)</td>
<td>62 (32–114)</td>
<td>0.06</td>
</tr>
<tr>
<td>Lactate, mmol/L</td>
<td>2.5 (1.6–5.1)</td>
<td>2.4 (1.5–3.7)</td>
<td>5.0 (2.4–7.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>eGFR, mL/min/1.73 m²</td>
<td>65 (28)</td>
<td>68 (28)</td>
<td>56 (25)</td>
<td>0.02</td>
</tr>
<tr>
<td>90-day mortality</td>
<td>59 (38%)</td>
<td>33 (28%)</td>
<td>26 (70%)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Results shown as counts (%) for categorical variables, and mean (SD) or median (IQR) for continuous variables.

ACS = acute coronary syndrome; ALT = alanine aminotransferase; CRP = C-reactive protein; eGFR = estimated glomerular filtration rate; hs-TnT = high-sensitivity troponin T; IQR = interquartile range; LVEF = left ventricular ejection fraction; SD = standard deviation.

Figure 5. Differences in cardiac index (a) and central venous pressure (b) between ALT stable and ΔALT>+20% groups. Reproduced with permission from study I.227

ALT = alanine aminotransferase; *p<0.01; § p<0.10.
6.2.2 Predictors of early ALT increase

In the multivariable logistic regression analysis, lower LVEF (OR 0.95, 95% CI 0.92-0.99, p=0.006), lower MAP (OR 0.95, 95% CI 0.90-0.99, p=0.03), lower CRP (OR 0.98, 95% CI 0.97-0.99, p=0.01) and lower eGFR (OR 0.98, 95% CI 0.96-0.99, p=0.002) were found to be independent baseline predictors of ΔALT>+20%.

6.2.3 Other liver biomarkers in cardiogenic shock

Compared with ALT, abnormal values in other LFTs were less frequent (ALP 11%, GGT 20%, total Bil 12%) and elevations more moderate (Table 5). There were no statistically significant differences in the levels of other LFTs obtained within 24 hours from enrolment between those who died and those who survived 90 days. Compared with patients with normal total Bil values at baseline, patients with elevated Bil levels were younger (60 vs 67 years for patients with normal Bil at baseline, p=0.02) and were more likely to have dilated cardiomyopathy (19% vs 3%, p=0.01) and a history of alcohol abuse (29% vs 12%, p=0.04). Similarly, patients with elevated GGT levels at baseline were more likely to have a history of alcohol abuse (31% vs 9% for patients with normal GGT levels, p=0.002). There were moderate levels of correlation between the liver biomarkers: ALP correlated moderately with GGT and ALT (Spearman correlation coefficient $r_s$ 0.34 and 0.25, p<0.001 for both), whereas GGT correlated with ALT and total Bil ($r_s$=0.39 and 0.28, p<0.001 for both). Total Bil levels also correlated with NT-proBNP levels ($r_s$=0.37, p<0.001).
6.3 Hypoalbuminemia in cardiogenic shock (II)

6.3.1 Characteristics of hypoalbuminemic patients

The study cohort for study II consisted of 178 patients from the CardShock study who had plasma samples available at baseline. Plasma albumin levels at baseline (P-Alb) were normally distributed. The mean plasma albumin for the whole cohort was 29.5 (SD 6.4) g/L. Observed P-Alb levels ranged from 10.6 g/L to 42.9 g/L. Baseline characteristics and clinical presentation of patients are shown in Table 7.

Compared with patients with normal plasma albumin levels, hypoalbuminemic patients had more comorbidities, such as prior MI ($p=0.01$), HF with reduced ejection fraction ($p=0.02$) and ischemic heart disease ($p=0.048$). In laboratory and imaging tests at baseline, patients with hypoalbuminemia were more often found to have lung oedema on X-ray, as well as higher levels of NT-proBNP and CRP, and lower eGFR and hemoglobin levels (Table 7). Concerning previous medication use, fewer patients in the hypoalbuminemic group were using calcium channel blockers. Notably, BMI was lower in the hypoalbuminemic group compared with the group with normal albumin levels. In coronary angiography on admission, patients with hypoalbuminemia were more likely to have multi-vessel disease. There were no significant differences in baseline LFTs (ALT and total Bil) between the two albumin groups.

Independent predictors of low plasma albumin levels in multivariable logistic regression analysis are shown in Table 8. In multivariable analysis, independent predictors of hypoalbuminemia were higher CRP at baseline, pulmonary oedema on chest X-ray, history of HF with reduced ejection fraction, older age and calcium channel blocker use prior to admission.
Table 7. Patient characteristics, laboratory results, angiographic findings, and mortality in normoalbuminemic and hypoalbuminemic cardiogenic shock patients.

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>Normoalbuminemia (P-Alb ≥34 g/L)</th>
<th>Hypoalbuminemia (P-Alb &lt;34 g/L)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographics (N=178)</td>
<td></td>
<td>(N=178)</td>
<td>(N=178)</td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td>72 (41)</td>
<td>23 (54)</td>
<td>49 (37)</td>
<td>0.05</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27.0 (4)</td>
<td>28.2 (4)</td>
<td>26.6 (4)</td>
<td>0.03</td>
</tr>
<tr>
<td>Medical history</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>58 (33)</td>
<td>9 (21)</td>
<td>49 (37)</td>
<td>0.05</td>
</tr>
<tr>
<td>Previous myocardal infarction</td>
<td>45 (25)</td>
<td>5 (11)</td>
<td>40 (30)</td>
<td>0.01</td>
</tr>
<tr>
<td>History of HFrEF</td>
<td>22 (13)</td>
<td>1 (2)</td>
<td>21 (16)</td>
<td>0.02</td>
</tr>
<tr>
<td>Calcium channel blocker use</td>
<td>22 (12)</td>
<td>10 (23)</td>
<td>13 (10)</td>
<td>0.04</td>
</tr>
<tr>
<td>Clinical presentation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung oedema on X-ray</td>
<td>60 (36)</td>
<td>10 (23)</td>
<td>50 (40)</td>
<td>0.04</td>
</tr>
<tr>
<td>LVEF, %</td>
<td>33 (14)</td>
<td>35 (13)</td>
<td>32 (14)</td>
<td>0.09</td>
</tr>
<tr>
<td>Mortality</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>90-day mortality</td>
<td>74 (42)</td>
<td>10 (23)</td>
<td>64 (48)</td>
<td>0.004</td>
</tr>
<tr>
<td>Laboratory test results at baseline</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>eGFR, mL/min/1.73 m²</td>
<td>63 (30)</td>
<td>69 (26)</td>
<td>60 (30)</td>
<td>0.04</td>
</tr>
<tr>
<td>NT-proBNP, ng/L</td>
<td>2710 (585–9434)</td>
<td>866 (226–5029)</td>
<td>3769 (1037–11745)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>16 (4–54)</td>
<td>7 (2–19)</td>
<td>25 (5–75)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Leucocytes, 10E9</td>
<td>14.0 (5.4)</td>
<td>14.7 (6.0)</td>
<td>13.8 (5.3)</td>
<td>0.30</td>
</tr>
<tr>
<td>Hemoglobin, g/L</td>
<td>129 (23)</td>
<td>139 (20)</td>
<td>125 (23)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Albumin, g/L</td>
<td>29.5 (6.4)</td>
<td>37.2 (2.3)</td>
<td>27.0 (5.1)</td>
<td></td>
</tr>
<tr>
<td>Lactate, mmol/L</td>
<td>2.7 (1.7–5.8)</td>
<td>2.4 (1.5–5.1)</td>
<td>2.9 (1.7–5.9)</td>
<td>0.15</td>
</tr>
<tr>
<td>Change in albumin between baseline and 72h, g/L</td>
<td>-5.0 (6.4)</td>
<td>-10.2 (6.2)</td>
<td>-2.5 (4.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Angiographic findings</td>
<td>(n=136)</td>
<td>(n=36)</td>
<td>(n=100)</td>
<td></td>
</tr>
<tr>
<td>Multi-vessel disease</td>
<td>93 (68)</td>
<td>18 (50)</td>
<td>75 (75)</td>
<td>0.006</td>
</tr>
<tr>
<td>TIMI flow &lt;3 post PCI</td>
<td>37 (30)</td>
<td>6 (18)</td>
<td>31 (35)</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Results shown as N (%) for categorical variables, and mean (SD) or median (IQR) for continuous variables.

BMI = body mass index; CRP = C-reactive protein; eGFR = estimated glomerular filtration rate; HFrEF = heart failure with reduced ejection fraction; IQR = interquartile range; LVEF = left ventricular ejection fraction; NT-proBNP = N-terminal pro-B-type natriuretic peptide; P-Alb = baseline plasma albumin; PCI = percutaneous coronary intervention; SD = standard deviation; TIMI = thrombolysis in myocardial infarction.
Table 8. Factors independently associated with hypoalbuminemia at baseline.

<table>
<thead>
<tr>
<th></th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP at baseline, mg/L</td>
<td>1.02</td>
<td>1.003–1.03</td>
<td>0.01</td>
</tr>
<tr>
<td>Lung oedema on chest X-ray</td>
<td>2.9</td>
<td>1.2–7.1</td>
<td>0.02</td>
</tr>
<tr>
<td>History of HFrEF</td>
<td>11.7</td>
<td>1.4–98</td>
<td>0.02</td>
</tr>
<tr>
<td>Age, years</td>
<td>1.04</td>
<td>1.01–1.07</td>
<td>0.02</td>
</tr>
<tr>
<td>Use of calcium channel</td>
<td>0.3</td>
<td>0.1–0.9</td>
<td>0.03</td>
</tr>
<tr>
<td>blocking medication</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CI = confidence interval; CRP = C-reactive protein; HFrEF = heart failure with reduced ejection fraction.

6.3.2 Frequency and prognostic effect of hypoalbuminemia

Hypoalbuminemia (P-Alb <34 g/L) was observed quite frequently already at baseline in 134/178 patients (75%). Hypoalbuminemia at baseline was associated with higher 90-day mortality (48.1% vs 23.3%, p=0.004). Figure 6 shows that 90-day mortality also increased across plasma albumin quartiles (p=0.001 for trend).

In univariable logistic regression analysis, baseline P-Alb was found to have an OR of 2.4 per 10 g/L decrease (95% CI 1.5-4.1, p=0.001). In multivariate logistic regression analysis, adjusting for the CardShock risk score, comorbidities (HF with reduced ejection fraction, ischemic heart disease), smoking status, calcium channel blocker use, lung oedema on X-ray, BMI, hemoglobin, NT-proBNP and CRP at baseline as well as presence of multi-vessel disease in primary coronary angiography, P-Alb was found to have an adjusted OR of 2.9 per 10 g/L decrease (95% CI 1.02-8.4, p=0.045). The association of baseline P-Alb remained independent also after adjusting for either the CardShock risk score or the IABP-SHOCK II score. Addition of P-Alb to the risk prediction model improved the discriminatory capabilities of both the CardShock risk score and the IABP-SHOCK II score alone ($\chi^2 = 5.301$, p=0.02 and $\chi^2 = 7.088$, p=0.008 for comparison of nested models, respectively). Discrimination was also assessed using the IDI and clinical risk stratification by NRI (Table 9).
Figure 6. 90-day mortality by baseline albumin quartiles. Reproduced with permission from study II.228

The baseline plasma albumin ranges for the quartiles were 34.0–42.9 g/L for the 1st quartile, 30.0–33.9 g/L for the 2nd quartile, 25.9–29.9 g/L for the 3rd quartile and 10.4–25.9 g/L for the 4th quartile.

Table 9. Comparison of risk score models with and without plasma albumin. Reproduced with permission from study II.228

<table>
<thead>
<tr>
<th>Model</th>
<th>AUC (95% CI)</th>
<th>Continuous NRI (95% CI)</th>
<th>IDI (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CardShock risk score</td>
<td>0.798 (0.734–0.862)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CardShock risk score + P-Alb</td>
<td>0.819 (0.757–0.881)</td>
<td>0.297 (−0.006 to 0.600)</td>
<td>0.027 (0.003–0.051)</td>
</tr>
<tr>
<td>IABP-SHOCK II score</td>
<td>0.719 (0.629–0.808)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IABP-SHOCK II score + P-Alb</td>
<td>0.750 (0.661–0.839)</td>
<td>0.355 (−0.004 to 0.715)</td>
<td>0.054 (0.013–0.095)</td>
</tr>
</tbody>
</table>

AUC = area under the curve; CI = confidence interval; IABP-SHOCK II = Intraaortic Balloon Pump in Cardiogenic Shock II; IDI = integrated discrimination index; NRI = net reclassification improvement; P-Alb = baseline plasma albumin.

6.3.3 Changes in plasma albumin concentration during hospitalization

Plasma albumin level decreased during hospitalization both in survivors and nonsurvivors with a similar rate between baseline and 72 hours (−4.6 g/L vs −5.4 g/L, \( p=0.5 \)). Figure 7 shows the trajectories of albumin concentrations for 90-day survivors and nonsurvivors, as well as for patients with and without hypoalbuminemia at baseline. The observed P-Alb levels were significantly lower (\( p<0.05 \)) at each time point in nonsurvivors compared with survivors throughout the follow-up period up to 72 hours, except for the 48-hour time point, for which a trend towards statistical significance existed (\( p=0.09 \)). For those with normal plasma albumin levels at baseline, the decrease between baseline and 72 hours
was greater than for patients with hypoalbuminemia at baseline. However, the rate of albumin decrease was not associated with mortality. The rate of albumin decrease was negatively correlated with fluid balance and CRP at 72 hours, and a positive correlation was found for ALP and Bil. CVP at 72 hours had a negative correlation with the rate of decrease of albumin with borderline statistical significance (correlation coefficient −0.26, \( p=0.051 \)).

Figure 7. (a) Mean plasma albumin at different time points during hospitalization in 90-day survivors and nonsurvivors of cardiogenic shock. Mean change between 0h and 72h −4.6 g/L for survivors, −5.4 g/L for nonsurvivors; \( p=0.54 \). (b) Plasma albumin at different time points during hospitalization in patients with normoalbuminemia or hypoalbuminemia at baseline. Mean change between 0h and 72 hours −10.8 mg/L for normoalbuminemic patients and −2.5 mg/L for hypoalbuminemic patients; \( p<0.001 \). Reproduced with permission from study II.228

* \( p<0.05 \), § \( p<0.10 \) for the difference in baseline plasma albumin (P-Alb) between groups at this time point. Error bar = standard deviation.
6.4 P-PENK and P-NGAL in cardiogenic shock (III)

For study III, patients with baseline plasma sample and at least one other sample within 24 hours were selected. Baseline characteristics of patients (n=154) divided by occurrence of AKI defined by an increase in creatinine within 48 hours (\( \text{AKI}_{\text{crea48h}} \)) can be seen in Table 10. \( \text{AKI}_{\text{crea48h}} \) was observed in 47/154 (31%) patients. Patients with \( \text{AKI}_{\text{crea48h}} \) were older and more often had oliguria at baseline, as well as lower eGFR at baseline. Creatinine, lactate and ALT levels were higher in patients who developed \( \text{AKI}_{\text{crea48h}} \), and they had higher CardShock and IABP-SHOCK II risk scores. P-PENK and P-NGAL were also significantly higher at all time points in patients who developed AKI.

At baseline, P-PENK was measured in 152/154 patients and P-NGAL was measured in 146/154 patients. Median baseline PENK was 105 (IQR 71–167) pmol/mL, and median baseline P-NGAL was 138 ng/mL (84–214). There were no statistically significant gender-based differences in the baseline levels of either P-PENK or P-NGAL.

Patients with P-PENK and P-NGAL levels above median were older and had worse renal function (history of renal insufficiency, higher creatinine, lower eGFR) and lower hemoglobin at baseline. They also developed \( \text{AKI}_{\text{crea48h}} \) within 48 hours more frequently and their mortality was higher.

Higher P-PENK and P-NGAL were associated with higher levels of NT-proBNP and lactate as well as higher CardShock and IABP-SHOCK II risk scores (Table 11).
Table 10. Patient characteristics divided by occurrence of $\text{AKI}_{\text{crea}48h}$.

<table>
<thead>
<tr>
<th></th>
<th>All (N=154)</th>
<th>No $\text{AKI}_{\text{crea}48h}$ (N=107)</th>
<th>$\text{AKI}_{\text{crea}48h}$ (N=47)</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>66 (12)</td>
<td>65 (12)</td>
<td>69 (12)</td>
<td>0.05</td>
</tr>
<tr>
<td>Renal insufficiency</td>
<td>47 (31%)</td>
<td>8 (8%)</td>
<td>9 (19%)</td>
<td>0.03</td>
</tr>
<tr>
<td>Medications in use at admission</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACEI</td>
<td>44 (29)</td>
<td>35 (33%)</td>
<td>9 (19%)</td>
<td>0.09</td>
</tr>
<tr>
<td>ARB</td>
<td>23 (15%)</td>
<td>14 (13%)</td>
<td>9 (19%)</td>
<td>0.46</td>
</tr>
<tr>
<td>Diuretics</td>
<td>43 (28%)</td>
<td>25 (24%)</td>
<td>18 (38%)</td>
<td>0.08</td>
</tr>
<tr>
<td>Clinical presentation at baseline</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cold periphery</td>
<td>146 (95%)</td>
<td>100 (94%)</td>
<td>46 (98%)</td>
<td>0.44</td>
</tr>
<tr>
<td>Confusion</td>
<td>103 (68%)</td>
<td>69 (66%)</td>
<td>34 (72%)</td>
<td>0.46</td>
</tr>
<tr>
<td>Oliguria</td>
<td>79 (52%)</td>
<td>47 (45%)</td>
<td>32 (70%)</td>
<td>0.01</td>
</tr>
<tr>
<td>CardShock risk score, points</td>
<td>4.2 (1.8)</td>
<td>3.9 (1.7)</td>
<td>5.0 (1.8)</td>
<td>0.001</td>
</tr>
<tr>
<td>IABP-SHOCK II risk score, points</td>
<td>2.2 (1.7)</td>
<td>1.8 (1.5)</td>
<td>2.9 (1.8)</td>
<td>0.001</td>
</tr>
<tr>
<td>Laboratory test results at baseline</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>eGFR, mL/min/1.73 m²</td>
<td>65 (28)</td>
<td>69 (29)</td>
<td>55 (24)</td>
<td>0.005</td>
</tr>
<tr>
<td>Creatinine, μmol/L</td>
<td>99 (77–136)</td>
<td>94 (70–131)</td>
<td>108 (85–141)</td>
<td>0.02</td>
</tr>
<tr>
<td>ALT, U/L</td>
<td>42 (20–87)</td>
<td>33 (18–78)</td>
<td>71 (36–120)</td>
<td>0.01</td>
</tr>
<tr>
<td>Lactate, mmol/L</td>
<td>2.6 (1.6–5.2)</td>
<td>2.4 (1.4–4.2)</td>
<td>3.6 (2.3–7.0)</td>
<td>0.003</td>
</tr>
<tr>
<td>90-day mortality</td>
<td>58 (38%)</td>
<td>25 (24%)</td>
<td>33 (70%)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Results shown as n (%) for categorical variables, and mean (SD) or median (IQR) for continuous variables.

ACEI = angiotensin converting enzyme inhibitor; AKI = acute kidney injury; ALT = alanine aminotransferase; ARB = angiotensin receptor blocker; eGFR = estimated glomerular filtration rate; IABP-SHOCK II = Intraaortic Balloon Pump in Cardiogenic Shock II; IQR = interquartile range; SD = standard deviation.
Table 11. Baseline characteristics, renal outcomes, interventions and mortality stratified by P-PENK and P-NGAL cut-offs at baseline.

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>PENK_{0h} &lt;84.8</th>
<th>PENK_{0h} &gt;84.8</th>
<th>p-value</th>
<th>P-NGAL_{0h} &lt;104</th>
<th>P-NGAL_{0h} &gt;104</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(N=154)</td>
<td>(N=61)</td>
<td>(N=91)</td>
<td>(N=49)</td>
<td>(N=97)</td>
<td>(N=61)</td>
<td>(N=91)</td>
<td>(N=49)</td>
</tr>
<tr>
<td>Age, years</td>
<td>66 (12)</td>
<td>61 (12)</td>
<td>70 (11)</td>
<td>&lt;0.001</td>
<td>61 (13)</td>
<td>69 (11)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Medical history</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>96 (62%)</td>
<td>33 (54%)</td>
<td>61 (67%)</td>
<td>0.13</td>
<td>24 (49%)</td>
<td>68 (70%)</td>
<td>0.02</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>51 (33%)</td>
<td>16 (26%)</td>
<td>35 (39%)</td>
<td>0.16</td>
<td>13 (27%)</td>
<td>38 (39%)</td>
<td>0.15</td>
</tr>
<tr>
<td>Heart failure</td>
<td>24 (16%)</td>
<td>6 (10%)</td>
<td>18 (20%)</td>
<td>0.11</td>
<td>2 (4%)</td>
<td>22 (23%)</td>
<td>0.004</td>
</tr>
<tr>
<td>Renal insufficiency</td>
<td>17 (11%)</td>
<td>1 (2%)</td>
<td>16 (18%)</td>
<td>0.003</td>
<td>0 (0%)</td>
<td>17 (18%)</td>
<td>0.002</td>
</tr>
<tr>
<td>AKI stage at baseline</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage 0</td>
<td>21 (14%)</td>
<td>21 (14%)</td>
<td>0 (0%)</td>
<td>&lt;0.001</td>
<td>17 (12%)</td>
<td>0/0%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Stage 1S</td>
<td>51 (34%)</td>
<td>12 (8%)</td>
<td>38 (26%)</td>
<td>13 (9%)</td>
<td>36 (25%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage 1A</td>
<td>16 (11%)</td>
<td>16 (11%)</td>
<td>0 (0%)</td>
<td>15 (10%)</td>
<td>0 (0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage 1B</td>
<td>63 (42%)</td>
<td>11 (7%)</td>
<td>51 (34%)</td>
<td>3 (2%)</td>
<td>60 (42%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Detection of AKI by increase in creatinine after baseline</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AKI_{crea} &lt;24 hours</td>
<td>35 (23%)</td>
<td>7 (12%)</td>
<td>27 (30%)</td>
<td>0.02</td>
<td>6 (12%)</td>
<td>29 (30%)</td>
<td>0.005</td>
</tr>
<tr>
<td>AKI_{crea} 24–48 hours</td>
<td>12 (8%)</td>
<td>4 (7%)</td>
<td>8 (9%)</td>
<td>1 (2%)</td>
<td>10 (10%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AKI CysC &lt;48 hours</td>
<td>49 (33%)</td>
<td>17 (29%)</td>
<td>32 (36%)</td>
<td>0.32</td>
<td>9 (18%)</td>
<td>40 (42%)</td>
<td>0.005</td>
</tr>
<tr>
<td>AKI by urine output after baseline</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No AKI</td>
<td>75 (50%)</td>
<td>37 (61%)</td>
<td>38 (42%)</td>
<td>0.04</td>
<td>31 (63%)</td>
<td>42 (44%)</td>
<td>0.02</td>
</tr>
<tr>
<td>Stage 1</td>
<td>33 (22%)</td>
<td>13 (21%)</td>
<td>20 (22%)</td>
<td>11 (22%)</td>
<td>19 (20%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage 2 or 3</td>
<td>43 (29%)</td>
<td>11 (18%)</td>
<td>32 (36%)</td>
<td>7 (14%)</td>
<td>35 (37%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AKI severity by creatinine (RRT excluded from staging)</td>
<td>0.02</td>
<td>0.009</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No AKI</td>
<td>106 (70%)</td>
<td>50 (82%)</td>
<td>56 (62%)</td>
<td>42 (86%)</td>
<td>58 (60%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage 1</td>
<td>27 (18%)</td>
<td>9 (15%)</td>
<td>18 (20%)</td>
<td>5 (10%)</td>
<td>22 (23%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage 2</td>
<td>10 (7%)</td>
<td>2 (3%)</td>
<td>8 (9%)</td>
<td>2 (4%)</td>
<td>7 (7%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage 3</td>
<td>9 (6%)</td>
<td>0 (0%)</td>
<td>9 (10%)</td>
<td>0 (0%)</td>
<td>10 (10%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interventions</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renal replacement therapy</td>
<td>22 (14%)</td>
<td>7 (12%)</td>
<td>15 (17%)</td>
<td>0.49</td>
<td>3 (6%)</td>
<td>19 (20%)</td>
<td>0.048</td>
</tr>
<tr>
<td>Use of adrenaline</td>
<td>21 (14%)</td>
<td>7 (12%)</td>
<td>14 (15%)</td>
<td>0.63</td>
<td>2 (4%)</td>
<td>18 (19%)</td>
<td>0.02</td>
</tr>
<tr>
<td>Coronary angiography</td>
<td>128 (83%)</td>
<td>55 (90%)</td>
<td>71 (78%)</td>
<td>0.08</td>
<td>45 (92%)</td>
<td>75 (77%)</td>
<td>0.04</td>
</tr>
<tr>
<td>90-day mortality</td>
<td>58 (38%)</td>
<td>15 (25%)</td>
<td>42 (47%)</td>
<td>0.006</td>
<td>9 (18%)</td>
<td>49 (52%)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Results shown as n (%) for categorical variables, and mean (SD) for continuous variables.

AKI = acute kidney injury; crea = creatinine; CysC = cystatin C; P-NGAL = plasma neutrophil gelatinase-associated lipocalin; P-PENK = plasma proenkephalin; RRT = renal replacement therapy; SD = standard deviation.
6.4.1 Temporal changes in P-PENK and P-NGAL

Temporal changes in the biomarkers between baseline and 48 hours are shown in Figure 8. Patients who developed AKI had significantly higher P-PENK and P-NGAL levels at all time points. Levels of P-PENK and P-NGAL between baseline and 48 hours for patients who developed AKI<sub>crea48h</sub> can be seen in Figure 9 and for patients who died within 90 days in Figure 10. There was a statistically significant increase in P-NGAL (<i>p</i>=0.003) between baseline and 24 hours in nonsurvivors, whereas P-PENK levels remained stable or slightly declined in both survivors and nonsurvivors. At all time points tested, high levels of P-PENK and P-NGAL were associated both with AKI<sub>crea48h</sub> and 90-day mortality.

![Figure 8](image1.png)

**Figure 8.** Temporal changes of plasma proenkephalin (P-PENK) (A) and plasma neutrophil gelatinase-associated lipocalin (P-NGAL) (B) from baseline to 48 hours. Median, error bars = 95% confidence interval.

![Figure 9](image2.png)

**Figure 9.** Plasma proenkephalin (P-PENK) (A) and plasma neutrophil gelatinase-associated lipocalin (P-NGAL) (B) median levels at different time points separated by occurrence of AKI<sub>crea48h</sub>. Reproduced with permission from study III.229

Error bars = 95% confidence interval. AKI = acute kidney injury.
Figure 10. Plasma proenkephalin (P-PENK) (A) and plasma neutrophil gelatinase-associated lipocalin (P-NGAL) (B) median levels at different time points separated by 90-day mortality. Reproduced with permission from study III.229

Error bars = 95% confidence interval.

6.4.2 Determinants of P-PENK and P-NGAL

Univariate general linear models were constructed to find independent predictors of P-PENK and P-NGAL levels. The independent predictors of P-PENK levels in descending order according to variance were found to be eGFR (F-statistic 82.8, \( p < 0.001 \)) and log-normalized lactate levels at baseline (F-statistic 5.0, \( p = 0.03 \)), which accounted for 47% of the variance in P-PENK with eGFR alone accounting for 36% of the total variance. Independent predictors of P-NGAL levels in descending order according to variance were found to be eGFR (F-statistic 84.9, \( p < 0.001 \)), log-normalized lactate levels at baseline (F-statistic 16.4, \( p < 0.001 \)), BMI (F-statistic 6.0, \( p = 0.02 \)) and ALP (F-statistic 5.0, \( p = 0.03 \)). These variables together were found to represent 56% of the variance in P-NGAL with eGFR alone accounting for 39% of the observed variance.

6.4.3 Association of P-PENK and P-NGAL levels with renal outcomes and interventions

At baseline, the AUC for AKI\textsubscript{\text{crea48h}} was 0.684 for P-PENK and 0.701 for P-NGAL. The optimal cut-off for predicting AKI\textsubscript{\text{crea48h}} was 84.8 pmol/mL for P-PENK and 104 ng/mL for P-NGAL. A Venn diagram showing the relationships between AKI\textsubscript{\text{crea48h}}, 90-day mortality, P-PENK and P-NGAL higher than this cut-off is shown in Figure 11. Differences in renal and mortality outcomes using these P-PENK and P-NGAL cut-offs are presented in Table 11.
Both P-PENK and P-NGAL higher than cut-off were associated with the development of AKI_{crea48h} in univariable logistic regression (OR 2.4, 95% CI 1.3-6.2, \(p=0.01\) for P-PENKₙₙ, OR 4.0, 95% CI 1.6-9.9, \(p=0.002\) for P-NGALₙₙ). Multivariable models were constructed to assess whether the association of high PENK and NGAL was independent of other variables associated with AKI_{crea48h}. Other independent predictors of AKI_{crea48h} were the use of diuretics and arterial pH at baseline. The association of P-PENK and P-NGAL higher than cut-off at baseline with AKI_{crea48h} remained independent despite adjustments for these variables (HRs 2.2, 95% CI 1.1-4.4 \(p=0.03\) for P-PENKₙₙ and 2.8, 95% CI 1.2-6.5, \(p=0.01\) for P-NGALₙₙ). Table 11 shows AKI severity, time to detection of AKI as well as clinical outcomes and procedures stratified by the baseline P-PENK and P-NGAL cut-offs for AKI_{crea48h} prediction. In most cases, AKI could already be detected within 12 hours of the baseline. There were 51 (34%) patients who fulfilled the criteria for AKI stage 1S. In patients with oliguria at baseline but without high PENK or NGAL (stage 1A) only 2/16 (13%) patients developed AKI_{crea48h} compared with 13/51 (25%) patients in stage 1S and 33/63 (52%) in stage 1B (\(p=0.001\)). Only 1/21 (5%) patients in stage 0 developed AKI_{crea48h}. High P-NGAL was associated both with early (≤24 hours from baseline) and late (>24 hours from baseline) AKI (\(p<0.05\) for subsets), whereas high P-PENK was associated only with early AKI. Both high P-PENK and high P-NGAL were associated with AKI severity stratified by urine output according to the KDIGO criteria, while only high P-NGAL was associated with AKI as defined by an increase in CysC. Only high P-NGAL was associated with the use of renal replacement therapy. Coronary angiography was performed less often, and adrenaline use was more frequent in the high P-NGAL group.
Figure 11. Venn diagram of plasma proenkephalin (P-PENK), plasma neutrophil gelatinase-associated lipocalin (P-NGAL) at baseline, acute kidney injury and 90-day mortality. PENK high = P-PENK < 84.8 pmol/mL at baseline. P-NGAL high = P-NGAL > 104 ng/mL at baseline. For illustrative purposes. Areas not proportional and all possible overlaps not shown. Reproduced with permission from study III.229

6.4.4 Association of P-PENK and P-NGAL levels with mortality

P-PENK$_{0h}$ > 84.8 pmol/mL as well as P-NGAL$_{0h}$ > 104 ng/mL were associated with higher 90-day mortality (Table 11). 90-day mortality also differed significantly between AKI stages at admission (7% for stage 0, 19% for stage 1S, 5% for stage 1A and 68% for stage 1B, \( p < 0.001 \)). Kaplan–Meier survival curves for patients with and without AKI$_{\text{crea}48h}$ separated by baseline P-PENK and baseline P-NGAL higher or lower than the optimal cut-off for AKI prediction (84.8 pmol/mL for P-PENK$_{0h}$, 104 ng/mL for P-NGAL$_{0h}$) are presented in Figure 12. P-NGAL$_{0h}$ > 104 ng/mL further stratified both patients with and without AKI$_{\text{crea}48h}$ into high and low mortality risk groups: 90-day mortality 76.9% vs 42.9% for patients who developed AKI$_{\text{crea}48h}$, 33.9% vs 14.3% for patients who did not develop AKI$_{\text{crea}48h}$ (\( p < 0.05 \) for both, Figure 12B). In comparison, for patients without AKI$_{\text{crea}48h}$ and P-PENK$_{0h}$ > 84.8 pmol/mL the 90-day mortality was 31.5% compared with 16.0% for patients without AKI$_{\text{crea}48h}$ but with P-PENK$_{0h}$ < 84.8 pmol/mL (\( p = 0.07 \), Figure 12A).
Although both P-PENK and P-NGAL were associated with higher mortality at all time points examined, the discriminatory capabilities for mortality as assessed by AUC was highest for both at 24 hours. At 24 hours, the optimal cut-off for mortality was determined as 105.7 pmol/mL for P-PENK24h and 151 ng/mL for P-NGAL24h. Using these cut-offs, Kaplan–Meier survival curves showed significant differences in mortality: 90-day mortality for patients with P-PENK24h >105.7 pmol/mL was 68.2% compared with 17.4% for patients with P-PENK24h <105.7 pmol/mL, whereas 90-day mortality for patients with P-NGAL24h >151 ng/mL was 63.5% compared with 17.7% for patients with P-NGAL24h <151 ng/mL (p<0.001 for all).

In univariable Cox regression, PENK24h >105.7 pmol/mL was found to have a HR of 5.6 (95% CI 3.1-10.7, p<0.001) and NGAL24h >151 ng/mL a HR of 5.2 (95% CI 2.8-9.8, p<0.001) for 90-day mortality. In multivariable analysis, the association of both PENK24h >105.7 pmol/mL and NGAL24h >151 ng/mL with 90-day mortality remained independent despite adjustment for CardShock risk score (HR 4.5, 95% CI 2.3-8.7, p<0.001 for PENK24h and HR 3.4, 95% CI 1.7-6.8, p=0.001 for NGAL24h) or IABP-SHOCK II score (HR 4.3, 95% CI 2.0-9.0, p<0.001 for PENK24h and HR 4.2, 95% CI 2.0-8.9, p<0.001 for NGAL24h). The AUCs for 90-day mortality for CardShock risk score and IABP-SHOCK II risk score at 24 hours were 0.778 and 0.707.

Figure 12. Kaplan–Meier survival curves for patients with and without AKIcrea48h separated by plasma proenkephalin (P-PENK) higher or lower than 84.8 pmol/mL at baseline (A) and plasma neutrophil gelatinase-associated lipocalin (P-NGAL) higher or lower than 104 ng/mL at baseline (B). Reproduced with permission from study III.229

§ p=0.07 * p<0.05 ** p<0.001
AKI = acute kidney injury defined by an increase in creatinine within 48 hours of baseline.
6.5 Circulating levels of miR-423-5p in cardiogenic shock (IV)

The study cohort for study IV consisted of 179 patients from the CardShock study who had plasma samples available at baseline. MiR-423-5p levels at baseline varied between 0.001 and 0.2819 AU, with a median of 0.0049 (IQR 0.0023–0.1316). The main etiology of cardiogenic shock was ACS (78%). Non-ACS causes consisted mainly of worsening chronic HF (10%), valvular and other mechanical causes (7%), and myocarditis (2%). The 90-day all-cause mortality was 42%. Patients with a miR-423-5p level above median had higher levels of lactate and ALT, whereas eGFR and cardiac index at baseline were lower compared with patients with a miR-423-5p level below median (Table 12).

6.5.1 Predictors of high miR-423-5p levels

A miR-423-5p level at baseline correlated with lactate ($r_s=0.28$, $p<0.001$), ALT ($r_s=0.38$, $p<0.001$) and creatinine ($r_s=0.19$, $p=0.01$). Using a general linear model, independent predictors of miR-423-5p levels were found to be ACS etiology ($p=0.001$), high ALT ($p=0.02$) and high blood lactate at baseline ($p=0.02$). To examine whether miR-423-5p level at baseline was associated with the amount of myocardial injury, we tested for correlation between miR-423-5p and hs-TnT at baseline and at 24 hours. A statistically significant but modest correlation between miR-423-5p at baseline and hs-TnT at 24 hours was found for ACS patients ($r_s=0.27$, $p=0.003$) but not for patients with non-ACS etiology of cardiogenic shock ($r_s=−0.04$, $p=0.87$). No correlation was found between miR-423-5p level and hs-TnT at baseline for either ACS or non-ACS patients ($r_s=0.06$, $p=0.46$ for ACS patients, $r_s=0.10$, $p=0.58$ for non-ACS patients).
Table 12. Clinical and biochemical characteristics of patients stratified by miR-423-5p level at baseline.

<table>
<thead>
<tr>
<th></th>
<th>All (N=179)</th>
<th>miR-423-5p below median (N=90)</th>
<th>miR-423-5p above median (N=89)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>66 (12)</td>
<td>66 (12)</td>
<td>66 (12)</td>
<td>0.8</td>
</tr>
<tr>
<td>Women</td>
<td>47 (26)</td>
<td>23 (26)</td>
<td>24 (27)</td>
<td>0.9</td>
</tr>
<tr>
<td>Previous MI or CABG</td>
<td>46 (26)</td>
<td>23 (26)</td>
<td>23 (26)</td>
<td>&gt;0.9</td>
</tr>
<tr>
<td>ACS etiology</td>
<td>143 (80)</td>
<td>69 (77)</td>
<td>74 (83)</td>
<td>0.4</td>
</tr>
<tr>
<td>eGFR, mL/min/1.73 m²</td>
<td>63 (30)</td>
<td>70 (30)</td>
<td>56 (27)</td>
<td>0.002</td>
</tr>
<tr>
<td>Lactate, mmol/L</td>
<td>2.7 (1.7–5.8)</td>
<td>2.4 (1.4–3.5)</td>
<td>3.7 (2.0–6.7)</td>
<td>0.001</td>
</tr>
<tr>
<td>ALT, U/L</td>
<td>44 (4-54)</td>
<td>35 (16-66)</td>
<td>68 (27-133)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>hs-TnT, ng/L</td>
<td>2190 (388–5418)</td>
<td>1635 (402–5127)</td>
<td>2565 (366–6870)</td>
<td>0.3</td>
</tr>
<tr>
<td>hs-TnT at 24 hours, ng/L</td>
<td>3848 (943–12756)</td>
<td>2599 (727–10310)</td>
<td>5217 (1575–17019)</td>
<td>0.02</td>
</tr>
<tr>
<td>Cardiac index, L/min/m²</td>
<td>2.2 (0.9)</td>
<td>2.4 (1.0)</td>
<td>1.8 (0.6)</td>
<td>0.04</td>
</tr>
<tr>
<td>90-day mortality</td>
<td>74 (42)</td>
<td>29 (33)</td>
<td>45 (51)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Results shown as n (%) for categorical variables, and mean (SD) or median (IQR) for continuous variables.

ACS = acute coronary syndrome; ALT = alanine aminotransferase; CABG = coronary artery bypass graft; eGFR = estimated glomerular filtration rate; hs-TnT = high-sensitivity troponin T; IQR = interquartile range; MI = myocardial infarction; SD = standard deviation. *n=37

6.5.2 Association of high miR-423-5p levels with mortality

Median miR-423-5p level at baseline was twofold higher in nonsurvivors (0.008 AU, IQR 0.003–0.017 in nonsurvivors compared with 0.004 AU, IQR 0.002–0.009 in survivors, p=0.003). Kaplan–Meier survival curves differed between patients with miR-423-5p level above or below median (Figure 13A, p=0.004). A miR-423-5p level above median was associated with 90-day all-cause mortality with an unadjusted HR of 1.9 (95% CI 1.2-3.1, p=0.006) in Cox regression analysis. In a multivariable Cox regression model after adjustment for the variables included in the CardShock risk score (i.e. age, prior MI or CABG, altered mental status at presentation, ACS etiology, LVEF, lactate and estimated glomerular filtration fraction) as well as hs-TnT and ALT at baseline, a miR-423-5p level above median was independently associated with 90-day all-cause mortality (HR 1.9, 95% CI 1.2-3.2, p=0.01, Figure 13B). In the adjusted model, lactate, prior MI or CABG, and LVEF were also independently associated with 90-day mortality. Nested Cox regression models were compared using the likelihood test ratio, which showed that the addition of a miR-423-5p level above median as a variable to the CardShock risk score model improved the model’s predictive power on 90-day all-cause mortality (χ² 7.2, p=0.007) and improved the c-statistic from 0.776 to 0.790.

We also explored the association of miR-423-5p levels with mortality depending on the etiology of cardiogenic shock. Comparing Kaplan–Meier curves, a miR-423-
5p level above median was associated with higher mortality in both ACS (\(p=0.04\)) and non-ACS (\(p=0.03\)) patients. Patients with ACS etiology of cardiogenic shock had higher miR-423-5p levels compared with non-ACS patients (median 0.005 AU, IQR 0.003–0.016 vs 0.003 AU, IQR 0.001–0.008; \(p=0.01\)). The difference in miR-423-5p levels between nonsurvivors and survivors was significant in non-ACS patients (median 0.010 AU, IQR 0.003–0.022 for nonsurvivors vs 0.002 AU, IQR 0.001–0.007 for survivors, \(p=0.006\)) and had a trend towards statistical significance in ACS patients (median 0.007 AU, IQR 0.004–0.016 for nonsurvivors vs 0.005 AU, IQR 0.002–0.016 for survivors; \(p=0.08\)).

\[\text{Figure 13.} \quad \text{Kaplan–Meier survival curves for patients with miR-423-5p below (black line) and above (grey line) median at baseline.} \]

\(\text{B) Hazard ratios and 95\% confidence intervals (in parentheses) of the multivariable model including CardShock risk score variables and other variables associated with miR-423-5p level. Reproduced with permission from study IV.}^{230}\]

\(*p<0.05 \quad **p=0.001\).

ACS = acute coronary syndrome; ALT = alanine aminotransferase; CABG = coronary artery bypass graft; eGFR = estimated glomerular filtration rate; HR = hazard ratio; hs-TnT = high-sensitivity troponin T; LV = left ventricle; MI = myocardial infarction.
6.6 Multi-organ dysfunction and biomarkers in cardiogenic shock

Figure 14 shows the impact of the number of organ dysfunctions on prognosis in cardiogenic shock (Jäntti et al., unpublished data). Organ dysfunctions were defined as A) ΔALT>+20% (the definition used in study I), B) AKI_{crea48h} (using the same definition as study III) and C) altered mental status at presentation (assessed by the local investigator). For patients with only one organ dysfunction, the most common organ dysfunction was altered mental status on presentation. For patients with two simultaneous organ dysfunctions, the most common combination was altered mental status and AKI_{crea48h}.

Figure 15 shows the effect of the number of biomarker abnormalities on 90-day all-cause mortality in cardiogenic shock (Jäntti et al., unpublished data). The number of biomarker abnormalities was associated with higher 90-day mortality, with the 90-day mortality ranging from 7.7% for patients without any of the biomarker abnormalities to 88.9% for patients with all four biomarker abnormalities. The definitions of biomarker abnormalities are the same as used in studies I–IV: ΔALT>+20% as in study I, hypoalbuminemia as in study II, NGAL_{24h} >151 ng/mL as in study III and miR-423-5p above median as in study IV.

Only 10% of patients had none of the investigated biomarker abnormalities present, whereas 38% had only one biomarker abnormality. Of the markers studied, the most common biomarker abnormality was hypoalbuminemia, which was present in 75% of all the patients. It was also the most common biomarker abnormality for patients with only one biomarker abnormality, accounting for 64% of these cases, followed by miR-423-5p above median (18%) and NGAL_{24h} >151 ng/mL (16%). For patients with hypoalbuminemia, it was combined with one other biomarker abnormality in 22% of cases and two other biomarker abnormalities in 27% of cases. In patients with two biomarker abnormalities, the most common combinations were hypoalbuminemia and miR-423-5p above median (50%) followed by hypoalbuminemia and NGAL_{24h} >151 ng/mL (33%). ΔALT>+20% was seldom present on its own (2%) and was usually associated with one or more other biomarker abnormalities. Interestingly, hypoalbuminemia was associated with ΔALT>+20% (29% vs 12% in normoalbuminemic patients, \(p=0.03\)) but not with AKI_{crea48h} (32% vs 26%, \(p=0.6\)).
Figure 14. Kaplan–Meier survival curve showing the impact of the number of organ injuries or impairments ($\Delta$ALT+$\geq$20%, acute kidney injury [AKIcrea48h] and/or altered mental status at presentation) detected on survival in patients included in the CardShock study.

AKIcrea48h = acute kidney injury defined by an increase in creatinine within 48 hours of baseline; ALT = alanine aminotransferase.
No. of biomarker abnormalities | Hypoalbuminemia | NGAL\(_{24h}\) >151 ng/mL | miR-423-5p above median | ΔALT>+20% | 90-day mortality
--- | --- | --- | --- | --- | ---
0 (N=13) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 1 (8%)
1 (N=50) | 32 (64%) | 8 (16%) | 9 (18%) | 1 (2%) | 11 (22%)
2 (N=28) | 24 (86%) | 11 (39%) | 16 (57%) | 5 (18%) | 8 (30%)
3 (N=32) | 30 (94%) | 25 (78%) | 25 (78%) | 16 (50%) | 19 (59%)
4 (N=9) | 9 (100%) | 9 (100%) | 9 (100%) | 9 (100%) | 8 (89%)

**Figure 15.** Kaplan–Meier survival curve showing the impact of the number of biomarker abnormalities (ΔALT>+20%, P-Alb <34 g/L, NGAL\(_{24h}\) >151 ng/mL and/or miR-423-5p above median) on survival in patients included in the CardShock study.

ALT = alanine aminotransferase; NGAL = neutrophil gelatinase-associated lipocalin; P-Alb = baseline plasma albumin.
7 DISCUSSION

7.1 Liver biomarkers in cardiogenic shock

Study I assessed the role of liver biomarker abnormalities and early changes of liver biomarkers associated with mortality in cardiogenic shock. We found that ALT was quite frequently abnormal already at baseline (51% of patients) and was more frequently elevated in patients who subsequently died within 90 days of follow-up. GGT was also frequently elevated already at baseline (in 46% of patients), but the proportion of patients with abnormal GGT was similar in survivors and nonsurvivors. Baseline elevations in other liver biomarkers tested (ALP, total Bil) were infrequent and were not associated with 90-day mortality. Abnormal ALT levels at baseline were associated with higher mortality, but the association with mortality was not independent of the level of lactate, which is in line with previous findings in acute HF suggesting that ALT can be used as a surrogate marker for hemodynamics but is not independently associated with mortality.90

7.1.1 Early increases in ALT levels

While elevated baseline ALT was not a predictor of outcome, early increases of >20% in ALT were found in a quarter of patients and were associated with a marked increase in mortality. An ALT increase >20% was associated with findings of hypoperfusion (higher lactate, oliguria, lower LVEF) and predicted mortality independently of CardShock risk score, peak hs-TnT level and changes in lactate levels within 24 hours. Furthermore, ΔALT>+20% was an important predictor of 90-day mortality regardless of the baseline level of ALT. Cardiogenic shock is a syndrome characterized by an acute drop in cardiac output, resulting in systemic hypoperfusion and organ injury. Due to the abrupt nature of cardiogenic shock, it may be that initial changes in LFTs are more informative than their absolute levels at presentation, depending on the kinetics of the LFTs involved. Transaminases, such as ALT, have been shown to increase rapidly in case of hypoxic shock due to liver cell necrosis.81,82 It may be that in some cases baseline elevations of ALT already represent acute liver injury due to hypoperfusion but in other cases may be caused by other factors (e.g. pre-existing liver disease, drug induced ALT increases), in which case further increases after baseline may help identify patients with prolonged hypoperfusion and progressive acute liver injury at higher risk of death.

In study I, independent baseline predictors of ΔALT>+20% were lower LVEF, lower MAP, lower CRP and lower eGFR at baseline. Impaired LVEF and low MAP are the main contributors to hypoperfusion induced liver injury, causing
an increase in ALT. On the other hand, lower CRP may be explained by decreased hepatic production of the dysfunctional hypoperfused liver. In the ALT+20% group, there were also more patients who developed AKI. Lower eGFR was associated with ΔALT+20% but is also a strong risk factor for AKI. In a study of hypoxic hepatitis in cardiogenic shock, admission serum creatinine was a predictor of hypoxic hepatitis in univariable analysis. Thus, low eGFR may predispose to both AKI and liver dysfunction. In addition to pre-existing renal dysfunction, similar pathophysiological mechanisms in cardiogenic shock may cause both AKI and liver dysfunction, resulting in multi-organ injury. Interestingly, acute liver injury was rarely an isolated finding but most often related to other organ injuries (altered mental status, AKI) and occurred concomitantly with hypoalbuminemia.

Even quite small increases in ALT within 24 hours were associated with excess mortality. Previous investigations into the effect of transaminase elevations in critically ill patients have focused on more severe elevations. In a meta-analysis that included 24 studies of hypoxic hepatitis, the cut-off for the transaminase elevations used to detect hypoxic hepatitis varied from >300 to >3000 between studies. Smaller increases have been associated with adverse outcomes in acute HF and in patients with acute STEMI, but they have not been previously reported for cardiogenic shock patients.

In study I, mortality for patients with hypoxic hepatitis as defined by ALT levels >700 U/L (20 times the upper limit of normal) was similar to a previous study in cardiogenic shock patients, confirming the prognostic role of hypoxic hepatitis in cardiogenic shock. However, mortality in patients with ΔALT+20% was as high as in hypoxic hepatitis, showing that even smaller ALT increases are of clinical importance and are associated with significantly worse prognosis in cardiogenic shock patients. An early ALT increase was detected in more patients than hypoxic hepatitis, suggesting that ΔALT+20% may be a more sensitive marker of liver injury and poor prognosis. Using only the criteria for hypoxic hepatitis will underestimate both the proportion of patients with liver injury and the increased risk of death associated with it. Since ΔALT+20% was associated with mortality independently of lactate and other covariates, it is probable that liver injury caused by cardiogenic shock is in itself associated with worse prognosis and is not just a surrogate marker for poor hemodynamics and hypoperfusion.

7.1.2 Other liver biomarkers in cardiogenic shock

No statistically significant differences between 90-day survivors and nonsurvivors were found in the levels of other LFTs studied (ALP, GGT, Bil). There have been previous reports on both acute as well as chronic HF where high levels of total Bil have been associated with worse prognosis. Studies have also shown an association between high levels of GGT and worse prognosis. On the other hand,
in a study comprising 234 patients with acute decompensated HF, no association between total Bil and mortality was found.\textsuperscript{96} The differences in associations of other LFTs and mortality between our study and these studies of acute and chronic HF may arise from differences in study populations and mechanisms leading to elevation of LFTs. In most of the previous studies\textsuperscript{89,78,92,96} cardiogenic shock patients have been excluded or been present in small numbers and thus are not representative of cardiogenic shock. Patients with HF mostly present with hemodynamic congestion, while cardiogenic shock is characterized by a sudden drop in cardiac output, low blood pressure and end-organ hypoperfusion. While hypoperfusion primarily leads to hepatocyte injury and elevation of transaminases, congestion may manifest as cholestasis and cause elevation of cholestatic markers (ALP, Bil).\textsuperscript{78}

Previous investigations have also shown associations between liver biomarkers and invasive hemodynamic measurements such as CVP and cardiac index.\textsuperscript{90,91,233} Van Deursen et al. showed that in patients hospitalized for worsening HF, ALT, ALP, GGT and total Bil measured 1–3 days prior to right-sided heart catheterization, all correlated with CVP, whereas only total Bil and transaminases such as ALT correlated with cardiac index.\textsuperscript{90} We found an association between ALT and cardiac index, as well as ALT and CVP, but not with Bil, ALP or GGT. This might be due to the smaller population size and likely also because patients with cardiogenic shock have sudden onset of disease. It is possible that changes in Bil reflect more chronic or subacute changes in hemodynamics, whereas elevated transaminases are a sign of more recent perturbations. In a study of 206 critically ill patients with hypoxic hepatitis, it was found that jaundice and elevations of Bil developed later in the course of hypoxic hepatitis (3–8 days) and occurred less often in patients with cardiogenic shock in this study.\textsuperscript{234} Transaminases, such as ALT, have been shown to increase rapidly in case of hypoxic shock due to liver cell necrosis.\textsuperscript{235} ALP and Bil elevations, on the other hand, are thought to result from bile canaliculi compression,\textsuperscript{78} not liver cell necrosis. It is likely that these kinds of changes are not as rapid as transaminase elevations resulting from direct liver cell injury and thus may not be apparent within a few days of follow-up.

### 7.2 Hypoalbuminemia in cardiogenic shock

#### 7.2.1 Causes of hypoalbuminemia in cardiogenic shock

In study II, it was found that in cardiogenic shock patients, hypoalbuminemia was present in most of the patients (75%) already at baseline. Plasma albumin levels also decreased during hospitalization in all patients. The rate of decrease was greater for patients with normoalbuminemia at baseline but was not associated
with 90-day mortality. Factors found to be independently associated with hypoalbuminemia were age, history of HF with reduced ejection fraction, use of calcium channel blockers at baseline, pulmonary oedema on X-ray at baseline, and CRP at baseline.

The prevalence of hypoalbuminemia in cardiogenic shock was strikingly high. Various factors may simultaneously contribute to the observed low levels of plasma albumin. First, hypoalbuminemia may be a pre-existing condition that is associated with comorbidities, nutritional status, and frailty, as patients with hypoalbuminemia were found to have lower BMI, lower hemoglobin and a higher frequency of comorbidities, such as CAD, prior MI, and HF with a reduced ejection fraction. Previous investigations into acute HF and ACS have also found that hypoalbuminemia is associated with conditions such as previous HF, peripheral artery disease, diabetes mellitus and hypertension, as well as multi-vessel CAD. However, the plasma albumin levels encountered in this cohort of cardiogenic shock patients seem to be lower than in previous studies of acute HF and ACS.

In study II as well as in previous investigations elevated CRP levels were associated with hypoalbuminemia. Causes of elevated CRP could be either acute infection or systemic inflammatory response, which is often involved in the pathogenesis of cardiogenic shock, but also pre-existing chronic illness resulting in low-grade inflammation, leading to anaemia of chronic illness and hypoalbuminemia. Inflammation could lead to hypoalbuminemia through capillary leakage of albumin, causing a decrease in intravascular colloid osmotic pressures and further aggravating vascular permeability. Indeed, we found that hypoalbuminemia was also associated with pulmonary oedema on chest X-ray at admission.

In an acute setting, circulating albumin levels can diminish quite precipitously. This is thought to be caused by increased vascular permeability and the redistribution of albumin, termed the transcapillary escape rate. Degradation of albumin is not thought to play an important role, as the rate of catabolism is less than 1/10th of the transcapillary escape rate. One possible cause could be the systemic inflammatory response that often accompanies cardiogenic shock, as the transcapillary escape rate of albumin in the circulation, generally estimated to be around 5–6%/hour in healthy individuals, can be increased by up to 300% in the presence of septic shock.

A third mechanism leading to high observed levels of hypoalbuminemia has been suggested in a study of STEMI patients, where hypoalbuminemia at admission was associated with poorer post-procedural myocardial reperfusion and procedure-related complications, including cardiogenic shock. If hypoalbuminemia predisposes ACS patients to cardiogenic shock, this would lead to a higher prevalence of hypoalbuminemia in cardiogenic shock populations.
compared with STEMI populations (75% in study II compared with 30% in the study by Oduncu et al.\textsuperscript{17}).

Another factor contributing to the observed low plasma albumin levels could be hemodilution due to aggressive fluid administration. Unfortunately, data on the amount of fluids received by the patients in the cohort before study enrolment were not available, and thus we were unable to take into account this potential confounding factor. However, we found no statistically significant differences in fluid balance between patients with or without hypoalbuminemia at baseline after study enrolment.

In this study, we found no association between the rate of decrease in P-Alb during the first 72 hours and 90-day mortality. The significance of changes in albumin has been explored in ICU patients, but data are conflicting.\textsuperscript{10,107} We found that the rate of decrease of P-Alb was associated with the baseline level and differed between normo- and hypoalbuminemic patients. Correlations between changes in P-Alb between baseline and 72 hours with CRP, Bil, ALP, CVP and fluid balance at 72 hours suggest that inflammation, cholestatic liver injury and congestion may be involved in the rate of decrease of P-Alb in cardiogenic shock.

7.2.2 Association of hypoalbuminemia with mortality

Hypoalbuminemia has been shown to be associated with worse outcomes in ACS,\textsuperscript{116,117} acute HF\textsuperscript{192,119} and critical illness.\textsuperscript{107,115,239} In study II, increased mortality was associated in a linear fashion with decreasing baseline P-Alb levels in cardiogenic shock patients. Hypoalbuminemia at baseline was associated with increased mortality independently of other variables shown to be associated with mortality in cardiogenic shock. Considering the high mortality in cardiogenic shock, those with normal plasma albumin at baseline had a relatively favourable prognosis with an overall 90-day mortality of 23%. The mortality increased across lowering albumin quartiles with the lowest quartile (P-Alb levels <25.8 g/L) having a mortality rate of 57%. A meta-analysis of acutely ill patients estimated that each 10 g/L decrease in serum albumin concentration increased the odds of mortality by 137%.\textsuperscript{104} In study II, the unadjusted odds of 90-day mortality increased by 140% for each 10 g/L decrement, which is in line with this estimation.

Hypoalbuminemia was associated with several variables previously shown to be associated with worse outcomes, such as age and previous MI.\textsuperscript{6} However, the independent association of P-Alb at baseline with 90-day mortality even after adjusting for the CardShock risk score, the comorbidities at baseline, CRP and hemoglobin suggests that albumin may have effects on mortality which are not explained by these confounding variables. In a study of the prognostic value of albumin in patients with acute STEMI undergoing primary PCI, hypoalbuminemic patients less frequently achieved TIMI grade 3 flow after primary PCI, and
the authors suggested that hypoalbuminemia may play a direct role in poor reperfusion after PCI.\textsuperscript{117} Interestingly in this regard, albumin has been shown to exert anticoagulative properties\textsuperscript{240}, which might make patients with low plasma albumin more predisposed to poor flow after reperfusion therapy. It has also been suggested that varying levels of hypoalbuminemia may be associated not just with the presence or absence of a comorbidity but also with disease severity, in which case categorizing pre-existing diseases as binary variables may lead to attributing the risk caused by disease severity to albumin.\textsuperscript{103}

7.3 P-PENK and P-NGAL in cardiogenic shock

7.3.1 P-PENK and P-NGAL trajectories in AKI and nonsurvivors

Overall, levels of both PENK and NGAL decreased between 0 and 48 hours. However, for patients who developed AKI within 48 hours or died within 90 days, the levels at all time points were higher for both P-PENK and P-NGAL. For patients who developed AKI within 48 hours, P-PENK and NGAL levels initially increased and later declined; after 12 hours for P-PENK and after 24 hours for P-NGAL. For P-PENK, the trajectories were similar for 90-day survivors and nonsurvivors, but the overall levels were consistently higher in all time points for nonsurvivors. In contrast, for P-NGAL, the levels continued to increase up to 48 hours for nonsurvivors, while they were stable or declined in survivors. These findings most likely represent differences in the kinetics of the two biomarkers. P-NGAL expression has been shown to be upregulated in the proximal kidney tubular cells early in response to renal ischemia with a peak at 12 hours post-ischemia,\textsuperscript{144} which could explain the initial increase observed for P-NGAL in nonsurvivors and patients who developed AKI.\textsuperscript{crea48h} P-PENK, on the other hand, is a marker of the endogenous opioid system activity, which could be higher in those worse off in the beginning but whose activity might not increase over time.

7.3.2 Correlation of P-PENK and P-NGAL with other biomarkers

In previous investigations in patients with MI or HF, PENK was found to correlate with age, renal function (creatinine, blood urea nitrogen, urinary albumin excretion, CysC and GFR), heart rate, blood pressure, ejection fraction, TnT and natriuretic peptides but not with urinary tubular markers (N-acetyl-beta-D-glucosaminidase, NGAL and KIM-1).\textsuperscript{134,135} In patients with cardiogenic shock caused mostly by ACS (study III), PENK and NGAL both correlated strongly with eGFR but also with lactate. The correlation with eGFR was slightly stronger than with creatinine and further correlations with age, NT-proBNP and ALP were observed.
eGFR and lactate were strong independent predictors of P-PENK and P-NGAL levels, with variation in eGFR explaining 36% of the variation in P-PENK and 39% of the variation in P-NGAL. This is in accordance with previous reports in acute HF, where eGFR and urea explained 47% of the variation in PENK. Indeed, according to a recent report, in critically ill patients PENK was found to be a better marker of true GFR than eGFR calculated using creatinine.

NGAL was further correlated with markers of infection and inflammation. NGAL correlated with CRP, and although no correlation was observed with leucocytes, leucocytes differed between P-NGAL quartiles. NGAL, as the name suggests, is also found in neutrophils and has been shown to be elevated in SIRS, which is a frequent finding in cardiogenic shock.

### 7.3.3 P-PENK and P-NGAL as predictors of AKI

High levels of P-PENK and P-NGAL were both independently associated with the development of AKI and had similar discriminatory properties in AUC analysis. Several studies have shown high levels of P-NGAL to be predictive of AKI after cardiac surgery, in critically ill children, and in adults, with cut-offs used for AKI prediction ranging from 100 ng/mL to 270 ng/mL. In a recent meta-analysis comparing blood NGAL, urine NGAL and serum CysC, elevated blood NGAL was found to be the earliest marker of contrast induced nephropathy. Of the two markers investigated in study III, only P-NGAL was associated with renal replacement therapy and AKI assessed by changes in CysC plasma concentrations, suggesting it may be a more robust indicator of clinically relevant renal outcomes. Interestingly, coronary angiography was performed less often in the high P-NGAL group. A possible explanation for this observation might be a reluctance of treating physicians to give contrast to patients deemed to be at high risk of AKI. More frequent use of adrenaline also suggests that the patients in the high NGAL group developed more severe hypoperfusion, as adrenaline is usually reserved for the patients who deteriorate despite vasopressor/inotrope treatment.

### 7.3.4 Association of P-PENK and P-NGAL with mortality

Both P-PENK and P-NGAL showed good discriminatory capabilities for 90-day mortality at all time points up to 48 hours, with the highest AUCs for both at 24 hours. High levels of both P-PENK and P-NGAL were independently associated with 90-day mortality after adjusting for CardShock risk score, IABP-SHOCK II risk score or AKI. This finding suggests that the association with mortality for P-PENK and P-NGAL is not only due to association with AKI and that high levels of P-PENK and P-NGAL may represent other causes of increased mortality.
in the CardShock study population that are not included in the relevant risk scores. In fact, the AUC for P-PENK and P-NGAL at 24 hours was similar or better than that of the risk scores alone. This finding is in contrast with a recent report on acute HF, which found that serum NGAL at admission was independently associated with worse outcomes at 30 days but not at 60 days. In the GALLANT study, P-NGAL at discharge was associated with the composite end point of HF readmission and 30-day all-cause mortality with an AUC of 0.73. There have been concerns that elevations in P-NGAL are not very specific for AKI, as NGAL is also expressed at low levels in several human tissues, including kidney, lung, stomach and colon, as well as neutrophils. Serum NGAL has been shown to increase also with ischemia and inflammation. This might be a drawback in AKI prediction but might help explain the high discriminatory capability in 90-day all-cause mortality, in which SIRS and organ injuries may play a part. Unlike the risk scores developed for risk stratification at the time of detection of cardiogenic shock, P-PENK and P-NGAL could be useful in assessing mortality risk at later time points for which suitable risk markers have so far not been largely assessed.

7.3.5 Subclinical AKI

The advent of biomarkers associated with kidney injury has led to the creation of a new concept of subclinical AKI, in which there is an increase in biomarkers but without clinical AKI. A recent study in critically ill patients found that high levels of P-PENK in patients without AKI were associated with increased mortality and suggested that elevated P-PENK may be a marker of subclinical AKI. In study III, we also found that high levels of P-PENK and P-NGAL at baseline were associated with increased mortality in patients who did not develop AKI. Interestingly, low levels of P-NGAL at baseline were also associated with lower 90-day mortality for patients who did develop AKI. It could be hypothesized that this might be due to less severe AKI, where despite an increase in creatinine the renal injury is smaller, or a rise in creatinine due to other reasons, thus misclassifying patients as having AKI (pseudo-AKI).

Study III also showed the usefulness of the proposed new AKI staging using biomarkers on admission. We found that for cardiogenic shock patients with oliguria before study enrolment, 90-day mortality differed significantly between patients with low and high P-PENK/P-NGAL at baseline (5% vs 68%, p<0.001). Thus, oliguria before study inclusion was associated with worse outcomes only if combined with high baseline levels of P-PENK or P-NGAL. Subclinical AKI at baseline was associated with increased mortality both in patients with oliguria before study enrolment as well as in patients without AKI. Interestingly, we also found that AKI occurred almost solely if oliguria before study inclusion was combined with subclinical AKI at baseline. Oliguria is also part of
a physiological response to stress and hypotension/hypovolemia, and some studies have also shown that many patients with oliguria do not develop AKI defined by an increase in creatinine.\textsuperscript{251-253}

7.4 Circulating levels of miR-423-5p in cardiogenic shock

7.4.1 Association with mortality

Study IV showed that above median levels of miR-423-5p were associated with markers of organ injury and impairment (elevated ALT, lower eGFR), as well as markers of hypoperfusion (higher levels of lactate, lower cardiac index). These associations with previously known risk factors for worse prognosis may partly explain why patients with above median levels of circulating miR-423-5p had significantly higher 90-day mortality. However, miR-423-5p levels predicted 90-day all-cause mortality independently of established risk factors for mortality in cardiogenic shock (i.e. age, prior MI or CABG, ACS etiology, eGFR, lactate, altered mental status at presentation and LVEF), as well as hs-TnT and ALT. Thus, there are probably other, unidentified causes of increased mortality that are represented by elevated miR-423-5p levels. The association of above median levels of miR-423-5p with 90-day all-cause mortality was also independent of the etiology of cardiogenic shock. One possible hypothesis is that miR-423-5p may be a general marker for hypoperfusion induced organ injury that could represent organ injury in other organs as well, such as the lungs, gut and brain.

7.4.2 Differences in miR-423-5p levels by Etiology of cardiogenic shock

Study IV also found that miR-423-5p levels and correlations with hs-TnT at 24 hours differed between ACS and non-ACS patients. Differences in circulating miR-423-5p levels and correlations seem to suggest that the mechanisms leading to elevated levels of circulating miR-423-5p may differ between ACS and non-ACS patients. The origin and mechanisms leading to elevated levels of circulating miRNAs have not been fully elucidated.\textsuperscript{254} Although above median levels of miR-423-5p were associated with a higher 90-day mortality in both ACS and non-ACS patients in Kaplan–Meier analysis, ACS patients in general had higher miR-423-5p levels than non-ACS patients. For ACS patients, miR-423-5p levels also correlated positively with hs-TnT levels at 24 hours. This is compatible with a cardiac release of miR-423-5p. There is evidence that at least some of the circulating miR-423-5p may have a cardiac origin, as miR-423-5p has been shown to be enriched in the coronary circulation of HF patients.\textsuperscript{255} Circulating levels of miR-423-5p
have also been shown to be elevated early in acute MI. However, as miRNAs are generally not thought to be very tissue-specific, it may be hypothesized that circulating miR-423-5p could have other sources as well. As miR-423-5p correlated with markers of hypoperfusion and organ injury, miR-423-5p could be a general marker for hypoperfusion induced cellular damage in cardiogenic shock. Further studies are needed to validate our findings and elucidate the role of miR-423-5p in cardiogenic shock.

7.5 Multi-organ dysfunction and biomarkers in cardiogenic shock

Taken together, the biomarker abnormalities examined in studies I–IV seem to provide incremental prognostic information, with 90-day mortality increasing with the number of biomarker abnormalities detected. Interestingly, as the mortality increased with the number of biomarker abnormalities, it seems that the number of biomarker abnormalities provides additional information either on the severity or different aspects of the disease processes which cause the observed increase in mortality. The same is true for the number of different organ dysfunctions (liver, kidney, central nervous system), for which the surrogate markers of > +20% increase in ALT within 24 hours, AKI and altered mental status at presentation were used. These findings are in agreement with an analysis from the large National Inpatient Sample registry (>440,000 patients), which found that in patients with cardiogenic shock caused by acute MI, noncardiac (respiratory, renal, hepatic, neurological or hematologic) organ failure was present in 64% of patients and half of these had failure in two or more organ systems (32% of the total population). The most common types of organ failure associated with cardiogenic shock in this study were found to be respiratory (43%) and renal failure (35%), each of which was associated with a nearly 70% increased risk of in-hospital death in multivariable analysis. Hepatic failure was less common but was also strongly associated with the risk of death (OR 1.98; 95% CI 1.94-2.04). There was a clear stepwise increase in mortality for each additional organ system failure.

Monitoring renal and hepatic injury and dysfunction is essential for the management and mortality risk assessment of patients in cardiogenic shock. The studied biomarkers are associated with end-organ injury and can be used alone and in combination with clinical risk scores to aid in the prognostic evaluation of patients. Many of the risk scores already include some measures of organ injury and dysfunction. The CardShock risk score includes altered mental status at presentation as a marker for central nervous system dysfunction and eGFR as a measure of kidney function, while the IABP-SHOCK II score has creatinine as a measure of kidney function.
The SOFA score,\(^{206}\) on the other hand, solely comprises markers of organ failure without taking into account previous medical conditions or other risk factors. A recent study found that in unselected patients in an intensive cardiac care unit, the SOFA score on the first day of treatment had good discrimination for in-hospital mortality, but the discrimination was improved by calculating day-to-day changes or using mean or maximum values of multiple daily SOFA scores.\(^{259}\) An observational study at an ICU also found that the change in SOFA score was an important predictor of mortality.\(^{260}\) Thus, patient response and changes during treatment also have an effect on survival. A study comparing the CardShock, IABP-SHOCK II, APACHE II and SOFA risk scores found that all had modest prognostic accuracy, with the CardShock and SOFA risk scores having the highest AUCs of 0.76 (95% CI 0.72-0.81 for both).\(^{261}\) Unfortunately, we did not have data for all the variables included in the SOFA score for the CardShock study patients and thus were unable to calculate SOFA scores for comparison. Potentially in the future, a risk score that combines both baseline patient characteristics, biomarkers and changes during treatment could be developed to improve the prognostic performance.

### 7.6 Limitations

The studies included in this thesis had some limitations. First, due to missing samples, not all patients from the CardShock cohort could be included in these studies, with patient numbers varying between the studies. Especially for studies I and III, where changes in biomarker levels were assessed, survivor bias must be kept in mind when interpreting the results. Specifically, patients who died immediately after study inclusion were not included in the analysis as they could not have plasma samples beyond the baseline. Nevertheless, considering the general challenges with serial sampling in acute cardiac care, we believe that the final cohort available for analysis is representative of the cardiogenic shock patient population included in the study and the results are valid. Second, due to the observational nature of these studies, these findings should be considered hypothesis-generating and the associations found do not prove causality. Cardiogenic shock is a complex syndrome, and although adjustments for several relevant available variables were performed in multivariable analyses, unaccounted differences and unmeasured confounding variables between survivors and nonsurvivors may remain. Third, invasive hemodynamic measurements were available only for a relatively small proportion of patients. Routine invasive hemodynamic monitoring is not advocated, as it has not been shown to be beneficial in randomized clinical trials.\(^{262,263}\) The use of pulmonary artery catheter and CVP measurements was at the discretion of the treating physician and we believe that our cohort reflects
current clinical practice. Also, the cut-offs used for some of the biomarkers have been extrapolated from the same CardShock patient cohort in which they are applied and may overestimate the reported associations compared with other cardiogenic shock populations. Thus, the results should be validated in other cardiogenic shock patient populations before adopting them in clinical use. This is especially true concerning the results of biomarker combinations presented in section 5.6. It should also be noted that dichotomizing biomarkers using a single cut-off may lead to information loss as continuous variables contain information not captured using a single cut-off value (i.e. patients with very high biomarker levels may be more susceptible to adverse outcomes compared with patients with biomarker levels slightly above the designated cut-off).

Specific limitations concerning studies II and III were that we did not have data on the patients’ albumin or creatinine levels before study enrolment to determine whether the observed hypoalbuminemia or renal dysfunction was pre-existing or not. Also, we did not have information on the fluid administration before study enrolment, as hemodilution due to fluid resuscitation could be one of the causes of hypoalbuminemia.

7.7 Clinical implications and future directions

One of the strengths of the CardShock study is that it represents a prospectively recruited cohort that describes cardiogenic shock patients with various etiologies in the contemporary era, where coronary angiography and percutaneous revascularization are part of routine management. Most of the other studies of cardiogenic shock have either been based on registry data, with often dissimilar patient profiles (ICU, cardiac care units), been conducted before modern revascularization therapies, or included only patients with ACS etiology. The prospective nature of the CardShock study enables the comprehensive collection of prespecified data using common definitions, ensuring the reliability of the data. It provides a glimpse into current clinical treatment and prognosis of cardiogenic shock patients across several European countries. Finally, serial plasma sampling in a prospective cohort of unselected cardiogenic shock patients makes the CardShock study rather unique and allows analysis and investigation of various novel biomarkers, as presented in this thesis.

Clinical risk scores provide useful prognostic information using easily obtainable clinical variables. Many include biomarkers in addition to clinical variables. However, most of the variables included in the risk scores represent clinical, unmodifiable risk markers at baseline (i.e. age, prior medical history) and do not take into account changes in clinical status or responses to therapies during treatment. Risk scores developed for cardiogenic shock have been validated
at baseline\textsuperscript{6,17,65} and it is unknown how well they predict outcomes at later time points. Ideally, risk assessment should be updated during treatment taking into account additional information gathered during treatment and should also assess the response to treatment. Biomarkers and their evolution with time could provide additional information at later time points, making it possible to refine the risk assessment at later time points considering changes in patient status in response to therapies, and could possibly in the future be incorporated into machine learning algorithms to create continuously updating prognostic models.

Study I underscores the mortality risk associated with even relatively small increases in ALT, which likely represent liver injury due to hypoperfusion caused by cardiogenic shock. Larger elevations, as seen in hypoxic hepatitis with values \( >20 \) times the upper limit of normal are widely known to be an ominous sign in critical illness, but the prognostic importance of smaller changes is not universally recognized. We show that relatively small increases in ALT may be a marker of liver injury and a sign of poor prognosis in cardiogenic shock. In fact, in study I the mortality for patients with smaller increases was similar to those with hypoxic hepatitis. ALT is a routine laboratory test which is widely available, and serial ALT testing can be easily adopted in clinical practice. Serial ALT testing provides an easy-to-use, widely available method to identify acute liver injury and to stratify those patients who survive the first 24 hours into low (28\% mortality in this investigation) and high-risk (70\% mortality in this investigation) groups to aid in selecting patients for more intensive treatment options. Serial measurement of ALT should be part of the clinical assessment of every patient with cardiogenic shock.

Hypoalbuminemia is also recognized as a quite common finding in critical illness, but the fact that it is so prevalent already at baseline in cardiogenic shock was surprising. The upstream causes of hypoalbuminemia in cardiogenic shock are still largely unknown and merit further study to determine whether there are specific treatments and therapeutic options to reduce the mortality associated with hypoalbuminemia as, specifically, intravenous albumin administration has not been shown to be beneficial in these patients\textsuperscript{264\textendash}265. Nevertheless, low plasma albumin at baseline was associated with a higher 90-day mortality and should be incorporated in risk assessment in cardiogenic shock patients.

PENK and NGAL are not yet in routine clinical use, but evidence on their clinical utility in AKI prediction and assessment of kidney function and injury is mounting. Prediction of AKI would be important, as AKI in the setting of cardiogenic shock is frequent and carries a high mortality.\textsuperscript{64} Recognizing patients at risk of AKI as early as possible would be important to avoid therapies and treatments that might cause AKI (i.e. large amounts of intravenous contrast media) in these patients and for future studies regarding possible kidney-protective therapies. AKI definition and diagnosis is still evolving, as illustrated by the recent position
Previous work on traditional KDIGO AKI definitions suggested that urine output may not be the most specific variable to determine AKI. Other studies have also shown that many patients with oliguria do not develop AKI defined by an increase in creatinine. Our study provides information on the usefulness of the suggested new classification system, which includes predictive biomarkers in AKI classification, as oliguria before study inclusion was associated with worse outcomes only if combined with high baseline levels of P-PENK or P-NGAL. Also, subclinical AKI in which predictive biomarkers are positive without the development of clinical AKI was associated with worse outcomes. Thus, measurement of P-PENK and P-NGAL in cardiogenic shock patients at presentation and/or after 24 hours of treatment could help identify patients at increased risk of AKI and mortality.

Novel biomarkers, such as miR-423-5p, although not yet in clinical use, may help elucidate mechanisms and pathophysiology in cardiogenic shock. Even though miRNAs are found mostly inside cells, large quantities of miRNAs can also be found in circulating blood. In this respect, the fact that circulating miR-423-5p levels above median were associated with higher mortality independently of risk factors included in the CardShock risk score and organ injury markers such as ALT merits further study to elucidate the mechanisms behind this association, as the mechanisms leading to high circulating levels of miR-423-5p could be tissue damage, apoptosis, necrosis or active passage in microvesicles, exosomes or through bonding to a protein, or a combination of these. Also, as circulating miRNAs play an important part in intercellular signalling, the effects of high levels of circulating miR-423-5p on the body remain to be elucidated. However, as results between studies of the association of miRNAs with clinical variables have been found to vary, further research is warranted before these results can be applied in clinical practice.
CONCLUSIONS

The aim of this thesis was to assess several biomarkers and their association with organ injury and prognosis in cardiogenic shock patients. Specifically, LFTs, novel markers of kidney injury and function, as well as a candidate miRNA were investigated.

This thesis identified several biomarkers associated with organ dysfunction and poor outcomes in cardiogenic shock. We found that although ALT levels were frequently elevated already at baseline, they were not independently associated with 90-day mortality, whereas an increase in ALT of 20% or more within the first 24 hours as a putative marker of ongoing liver injury was strongly and independently associated with higher 90-day mortality. (I) Findings in study I suggest that early increases in ALT in patients in cardiogenic shock are related to organ hypoperfusion, which was supported by associations with lower cardiac index and clinical markers of hypoperfusion. The prevalence of hypoalbuminemia already in the early phase of cardiogenic shock was striking (II) and warrants further investigation. Furthermore, plasma albumin at baseline was independently associated with 90-day all-cause mortality, with mortality increasing across lower albumin quartiles in a linear fashion. Based on these results, we suggest that serial ALT measurements, as well as baseline albumin measurement should be incorporated in the clinical assessment of cardiogenic shock patients.

Study III showed that P-PENK and P-NGAL levels differed between patients who did and did not develop AKI_{crea48h} and between 90-day survivors and nonsurvivors. High baseline levels of both P-PENK and P-NGAL were able to predict AKI_{crea48h} with reasonable accuracy. High levels of P-PENK and P-NGAL at 24 hours were independently associated with higher 90-day all-cause mortality. Thus, P-PENK and P-NGAL seem useful biomarkers in the early prediction of AKI and outcomes in cardiogenic shock populations.

In study IV, a more novel biomarker, circulating miR-423-5p, was studied. Above median levels of circulating mir-423-5p were found to be associated with markers of hypoperfusion, organ injury and dysfunction. Moreover, a miR-423-5p level above median predicted 90-day mortality independently of established risk factors in cardiogenic shock patients. However, these results must be interpreted as preliminary and should be confirmed in another cardiogenic shock population with mixed etiologies of cardiogenic shock.

Although all studied biomarkers were associated with markers and clinical signs of hypoperfusion, they provided incremental prognostic information, with 90-day all-cause mortality increasing with the number of biomarker abnormalities detected. The studied biomarkers are associated with end-organ injury and can be used either separately or in combination with clinical risk scores to aid in the evaluation of patients in cardiogenic shock.
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