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NOVEL BIOMARKERS FOR DETECTION OF EARLY ACUTE KIDNEY INJURY, RENAL RECOVERY AND BACTERIAL INFECTIONS IN CRITICALLY ILL PATIENTS

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Cover page: Hypothetical spaghetti plot of biomarkers over time, increasing and decreasing in relation to an event, e.g. infection or AKI. Illustration by Niklas Jonsson and Oskar Lindström. Graphically inspired by the famous stacked plot of the first discovered pulsating star: Ostriker, Jeremiah P. The Nature of Pulsars. Scientific American Vol. 224, No. 1, 1971, pp. 48-63.

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Novel biomarkers for detection of early acute kidney injury, renal recovery and bacterial infections in critically ill patients

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"Everything you know or think you know is bullshit until proven otherwise."

Professor Rinaldo Bellomo, during a personal conversation on a break of Das SMACC, Berlin, June 26th 2017.

I dedicate this book to my family,
with love and ever-lasting gratitude

ABSTRACT

Diagnosis of infection in the intensive care unit (ICU) is challenging because the signs and symptoms normally attributed to infection are quite common also in ICU patients without infection. This is a problem as delayed antibiotic therapy may increase the risk of organ failure and ultimately, death. One example of organ failure is acute kidney injury (AKI), which affects more than 1/3 of ICU patients. Diagnosis of AKI and decision to initiate supportive treatment (e.g. renal replacement therapy, RRT) is largely based on markers of kidney dysfunction - rather than markers of kidney damage. Moreover, markers to predict successful discontinuation of RRT are currently lacking. It is possible that we in a foreseeable future will be able to detect and treat both infection and AKI in the ICU earlier than we can today. A method that has been suggested is the use of biomarkers - biological markers that we can measure in the patient's blood or urine. The aim of this thesis is to study a number of potential biomarkers to predict AKI development and renal recovery (studies I and IV) and to detect infection (studies II and III) in ICU patients.

Study I examined if endostatin – a potential marker of renal epithelial and endothelial damage – could predict the development of AKI within 72 hours after ICU admission. Of the 93 studied patients, 21 developed AKI within 72 hours. We also created a clinical risk prediction model based on age, APACHE II score and early oliguria. The statistical model predicted outcome with fair accuracy. Adding endostatin to the model increased prediction accuracy.

In study II we measured daily plasma calprotectin levels in 110 ICU patients in order to assess calprotectin as an early marker of infection in the ICU. Altogether, 58 patients developed infection. The study showed that, in ICU patients, plasma calprotectin was as good as C-reactive protein (CRP) in predicting infection and better than white blood cell count (WBC) and procalcitonin.

In study III we examined dimeric neutrophil-gelatinase associated lipocalin (dNGAL), a protein released from activated neutrophils, as an early marker of infection in the ICU and its response to antibiotic therapy. Daily plasma dNGAL was measured in 198 ICU patients. We found that infection, but not AKI, was independently associated with greater dimeric NGAL levels. However, its value as an early marker of bacterial infection was limited. Following initiation of appropriate antibiotic therapy, dNGAL decreased more rapidly than the traditional biomarkers CRP and PCT.

In study IV we studied 135 ICU patients with AKI requiring RRT. We assessed if biomarker concentrations in plasma and urine (NGAL, endostatin, cystatin C, creatinine, urea), before and during RRT, alone and together with a clinical prediction model, could improve prediction of renal recovery within 60 days of ICU admission (alive and without need for RRT). By day 60, renal recovery was found in 98 of the 135 patients. The individual biomarkers in plasma or urine were poor predictors of renal recovery. The clinical prediction model, based on patient age and daily urine output, predicted renal recovery with reasonable accuracy.

LIST OF SCIENTIFIC PAPERS

I. Plasma endostatin may improve acute kidney injury risk prediction in critically ill patients.
Johan Mårtensson, Niklas Jonsson, Neil J. Glassford, Max Bell, Claes-Roland Martling, Rinaldo Bellomo, Anders Larsson
Annals of Intensive Care 2016 Dec; 6:6

II. Plasma calprotectin as an early biomarker of bacterial infections in critically ill patients.
Niklas Jonsson, Tom Nilsen, Patrik Gille-Johnson, Max Bell, Claes-Roland Martling, Anders Larsson, Johan Mårtensson
Crit Care Resusc. 2017 Sep;19(3):205-213

III. Performance of plasma measurement of neutrophil gelatinase-associated lipocalin as a biomarker of bacterial infections in the intensive care unit.
Niklas Jonsson, Patrik Gille-Johnson, Claes-Roland Martling, Shengyuan Xu, Per Venge, Johan Mårtensson
Manuscript, submitted for publication

IV. Biomarkers and renal recovery in critically ill patients with severe acute kidney injury requiring renal replacement therapy.
Niklas Jonsson, Bo Ravn, Max Bell, Claes-Roland Martling, Anders Larsson, Johan Mårtensson
Manuscript, submitted for publication

CONTENTS

LIST OF ABBREVIATIONS

AKI	Acute kidney injury
ATP	Adenosine tri-phospate
APACHE	Acute physiology and chronic health evaluation
AUC ROC	Area under receiver operating characteristic curve
CI	Confidence interval
CKD	Chronic kidney disease
CRP	C-reactive protein
DAMP	Damage-associated molecular pattern
eGFR	Estimated glomerular filtration rate
ELISA	Enzyme-linked immunosorbent assay
ESRD	End-stage renal disease
GFR	Glomerular filtration rate
ICU	Intensive care unit
IQR	Interquartile range
KDIGO	Kidney Disease: Improving Global Outcomes
MDRD	Modification of diet in renal disease
NGAL	Neutrophil gelatinase-associated lipocalin
PAMP	Pathogen-associated molecular pattern
PCT	Procalcitonin
PETIA	Particle-enhanced turbidimetric immunoassay
PRR	Pattern recognition receptor
RIFLE	Risk, injury, failure, loss, end-stage
ROS	Reactive oxygen species
RRT	Renal replacement therapy
SIRS	Systemic inflammatory response syndrome
SOFA	Sequential organ failure assessment
WBC	White blood cell count

1 INTRODUCTION

Globally, sepsis is the cause of someone's death every four seconds (1). In the world's most advanced healthcare systems, sepsis has a mortality rate between 15 - 20 % (2). A European patient struck with sepsis is five times more likely to die than a patient suffering from a stroke or a heart attack (3). Sepsis claims more lives than bowel and breast cancer combined (4). Sepsis is a clinical syndrome caused by an invasive bacterial infection triggering a number of host responses which result in organ dysfunction, such as renal failure and increased endothelial permeability. The host response in sepsis is quite similar to the host response in sterile inflammation, clearly differentiated only by the presence of an underlying infection. Patients admitted to the Intensive Care Unit (ICU) invariably have concomitant conditions that elicit a host response, such as trauma, pancreatitis, autoimmune diseases, burns, which also affect the patients vital signs and attenuate the efficacy of the traditional biomarkers used to detect bacterial infection, i.e. C-reactive protein and Procalcitonin (5, 6). The phenotype of a sterile inflammation is in many cases indistinguishable from the inflammation caused by an infection (7). Confirmation of bacterial infections - positive microbiological cultures - come to hand post festum and are often inconclusive (8).

Early treatment with antibiotics has been shown to improve outcome in sepsis (9, 10) and is recommended by the Surviving Sepsis Campaign collaborators (11). Efforts should also be made to avoid inappropriate and unduly prolonged therapy, as it too may cause increased morbidity and mortality (12). A study of agreement among clinicians in diagnosing SIRS or sepsis in critical care patients, showed that ~ 60 % of patients ultimately classified as having a sterile inflammatory response, were given empiric antibiotic therapy on admission to the ICU (7). A priority in developing sepsis care is therefore to find early and accurate methods to identify invasive infections in ICU patients.

Acute kidney injury (AKI) is also a major clinical problem in patients treated in the ICU and sepsis is the cause of approximately half of all AKI cases in the ICU (13). In such cases, early antibiotic treatment is associated with better outcome (14). AKI, whether it is caused by sepsis or any other disease or event is characterized by a rapid loss of kidney function, together with high mortality and morbidity (15).

Biological markers (biomarkers) are molecules we can measure in biological samples - in this study plasma or urine. We hypothesize that measurements of the biomarkers mirror the pathological processes or pharmacological responses we are studying and that they may provide help for decision-making. A reliable biomarker should, in order to be of use in the ICU, be able to differentiate between an inflammatory process started by an infection, from one triggered by severe trauma or be able to accurately tell us when an organ has been so damaged that it will affect the patients outcome. All of the above must work in a mix of critically ill patients suffering from severe trauma, burns, heart failure, AKI, liver failure, bone marrow depression, autoimmune disease, etc.

2 BACKGROUND

2.1 DEFINITION OF SEPSIS

The ancient Greek word sêpsis can be encountered in Homer's Iliad, were it means "decay of organic matter" A definition produced around 2700 years later, at the Global Sepsis Alliance meeting in 2010 was: “Sepsis is a life-threatening condition that arises when the body's response to an infection injures its own tissues and organs”(16). An infection is, according to the International Sepsis Definitions Conference in 2001, "a pathologic process caused by the invasion of normally sterile tissue, fluid or body cavity by pathogenic or potentially pathogenic microorganisms"(17).

In the real world of intensive care medicine, early diagnosis of sepsis is typically based on non-specific clinical signs such as fever, tachycardia, and/or hypotension together with equally non-specific biomarkers such as white cell count and C-reactive protein (CRP) and later on in the disease process on bacterial cultures.

A definition of sepsis for the use in both clinical work and research setting was made in 1991 by the American College of Chest Physicians and the American Society of Critical Care Medicine (18). According to the definition, the patient needed to fit at least two Systemic Inflammatory Response Syndrome (SIRS) criteria: elevated heart rate, high or low body temperature, increased respiratory rate and abnormal white blood cell count, together with a suspected or confirmed infection. The sepsis definition also contained a stratification of sepsis severity ranging from "sepsis" to "severe sepsis" to "septic shock" (Table 1&2). In 2001, the sepsis definition was updated (to Sepsis-2) with an expanded list of signs and symptoms to help the clinician at the bedside and to make the sepsis definition more specific (17).

Even with the Sepsis-2 definition, an inherent problem with the SIRS criteria is that they just as well may be caused by sterile insults to the body, such as ischemia, inflammation, trauma, surgery or several insults combined. Contrariwise, SIRS criteria excludes one in eight patients with clinically significant infection (19). The present-day sepsis definition (Sepsis-3) was published in 2016 proposing a construct based on a suspected or confirmed infection together with an increase from baseline of two or more points in the SOFA score (Sequential Organ Failure Assessment). Nonetheless, the Sepsis-3 collaborators state that: "there are, as yet, no simple and unambiguous clinical or biological criteria, imaging or laboratory features that uniquely identify a septic patient." (20).

Table 1. Definition of sepsis from 1991.

Sepsis is a systemic inflammatory response to a suspected or confirmed infection, together with two or more of the following criteria (which should be a direct systemic response to an infection and in absence of other known causes):

1. Core temperature	> 38° C or < 36° C
2. Tachycardia	> 90 beats/min, except in patients with a medical condition known to increase the heart rate or those receiving treatment that would prevent tachycardia
3. Tachypnea	Respiratory rate > 20 breaths/min or $PaCO_2$ < 4.3 kPa or the use of mechanical ventilation for an acute respiratory failure
4. White blood cell count	> 12 x 10^9/L or < 4 x 10^9/L or > 10 % immature forms

Severe sepsis

Sepsis and at least one criteria for acute organ dysfunction (see table 2) or hypoperfusion abnormality, such as acute altered mental status.

Septic shock

Sepsis and patient requiring inotropic agent or vasopressor to compensate for cardiovascular dysfunction.

Table 2. Criteria for organ dysfunction.

One or more of the following criteria (organ dysfunction not explained by underlying disease or effects of concomitant therapy):

Cardiovascular dysfunction	Arterial systolic blood pressure < 90 mm Hg or mean arterial pressure < 70 mm Hg for at least 1 hour despite adequate fluid resuscitation, adequate intravascular volume status or the use of inotropic agent or vasopressors in an attempt to maintain a systolic blood pressure of > 90 mm Hg or a mean arterial pressure of > 70 mm Hg.
Kidney dysfunction	Urine output < 0.5 ml/kg of body weight/hour for 2 hours, despite adequate fluid resuscitation
Respiratory dysfunction	Ratio of PaO_2 to FiO_2 (PFI) < 27.
Hematologic dysfunction	Platelet count < 80 x 10^9/L or decrease by 50 % in the 3 days preceding enrollment.
Unexplained metabolic acidosis	pH < 7.30 or base deficit > 5.0 mmol/L in association with a plasma lactate level > 3.0 mmol/L.

PaO_2 partial pressure of arterial oxygen, and FiO_2 fraction of inspired oxygen.

2.2 INCIDENCE AND OUTCOMES OF SEPSIS

An observational study of sepsis occurrence in acutely ill patients (SOAP) in European ICUs during two weeks in 2002, showed an average prevalence of 30 % of severe sepsis and a 32 % ICU-mortality among those with severe sepsis (3). An international study of prevalence of infection in the ICU (EPIC II), showed that on a random day in may 2007, 51 % of patients were considered infected. Follow up of these patients showed higher ICU and hospital mortality, compared to other ICU patients (21). An observational study of severe sepsis in the ICU in England, Wales and Northern Ireland demonstrated that (due to longer stay in the ICU, compared to other patients) these patients accounted for 46 % of all ICU bed days (22). An observational study from Australia and New Zealand of the frequency of ICU admission diagnosis sepsis together with fulfillment of severe sepsis criteria, showed a 11.1 % incidence in 2012 with a 18.4 % hospital mortality. In 2000, the first observed year of the study, the incidence was 7.2 % and the hospital mortality was 35 %, thus showing an increase in incidence and decrease in mortality (2).

2.3 PATHOPHYSIOLOGY OF SEPSIS

The immune system is commonly divided into an innate and adaptive system, which both have humoral and cellular components. A crucial part of the host response is to activate the receptors of the innate immune system. The innate immune system can recognize patterns on the pathogen's molecular surface without having encountered them before, using pattern recognition receptors, PRRs (as opposed to the adaptive immune system, which reacts to pathogens it has aquired memory of). Not only immune cells have PRRs, also endothelial and parenchymal cells do (23).

The microbe's molecular surface patterns that are identified by the innate immune system are called pathogen-associated molecular patterns (PAMPs). Examples of PAMPs include lipopolysaccharide (LPS) on Gram-negative bacteria, peptidoglycans and lipoteichoic acid (LTA) on Gram-positive bacteria, flagellin and bacterial or viral DNA or RNA (24).

Cellular damage following mechanical trauma, infection or other harm can elicit a secretion of molecules such as endogenous DNA, ATP, uric acid, high mobility group box 1 protein (HMBG1) and reactive oxygen species (ROS). Under such circumstances these molecules act as danger signals and have been named damage-associated molecular patterns (DAMPs). At least to some extent, DAMPs bind to the same PRRs as PAMPs but also to other receptors (25). Subsequently, a sterile danger signal may activate the same response as a microbe will - the phenotype of infection is therefore almost indistinguishable from the response to a sterile pancreatitis or severe trauma (26, 27). In advanced stages of infection, the body is flooded with PAMPs and DAMPs, which may cause tissue malfunction (28). At present, more research is focusing on the host response to infection, rather than on the pathogen itself - and it may be the host response that is the primary determinant of outcome.

2.4 BIOMARKERS OF INFECTION IN THE ICU

A biomarker of infection should aid the clinician caring for the patients in the ICU to predict infection and to differentiate the infected patient from the non-infected patient. More than 170 infection biomarkers have been described in the literature, but not one has shown sufficient sensitivity and specificity in the mixed patient group of the ICU (29). The suggested biomarkers of infection in the ICU studied in this thesis are detailed in table 3.

Table 3. Suggested biomarkers of infection in the ICU in this study.

Biomarker	Synonyms	Studies	Cellular origins	Known molecular functions
Calprotectin	p8, 14; S100A8/A9: leukocyte L1 protein	II	Neutrophil granulocyte, monocyte	Zn and Mn chelation, TLR4-ligand, amplifies inflammation, apoptosis
C-reactive protein	CRP	II, III	Hepatocyte	Soluble PRR, surveillance molecule, activates complement, acts as opsonin
Total NGAL	Human neutrophil lipocalin (HNL), lipocalin 2	III, IV	Mainly neutrophil granulocyte and tubular epithelial cells	Bacteriostatic, iron sequestering, modulate oxidative stress, cell growth
Dimeric NGAL	Dimeric HNL	III	Neutrophil granulocyte	Bacteriostatic, iron sequestering
Procalcitonin	PCT	II, III	C-cells in the thyroid, liver cells, kidney cells, adipocytes, and muscle cells	Modulation of NO synthesis, calcium metabolism, pain relieving effects
White blood cell	Leukocyte	II, III	Hematopoietic stem cells	Circulating immune cells of the blood and lymph

Calprotectin

Calprotectin is complex of two calcium-binding proteins in the S100 family with a total molecular mass of 35 kDa. It is released from the cytosol of activated neutrophils, where it constitutes ~45 % of all proteins (30). Once released into the extracellular space it is bacteriocidal and fungicidal and has been suggested to increase early accumulation of neutrophils and a subsequent monocyte predominance in infected tissue (31). It's bacteriocidal and fungicidal properties may be related to sequestering of zink and manganese - essential metal ions for most living organisms (32).

C-reactive protein

C-reactive protein or CRP is a soluble pattern recognition receptor (PRR) in the innate immune system. It is released from hepatocytes after activation by interleukin-6. In the presence of pathogens, CRP activates complement, opzonisation and induction of phagocytosis and may act as a link between the innate and adaptive immune system (33).

Neutrophil gelatinase-associated lipocalin

NGAL, also known as human neutrophil lipocalin, is stored in specific granulae in neutrophils and is released when the neutrophil is activated (34). It exists as a monomer, homodimer and a heterodimer and has bacteriostatic properties that may be related to sequestering of iron - an essential metal ion for bacterial survival. Dimeric NGAL has a molecular mass of 45-kDa and is the predominant form of NGAL released by circulating neutrophils in response to bacterial infections. Dimeric NGAL seems to be unique to the neutrophils (35, 36).

Procalcitonin

A prohormone of calcitonin with multiple cellular origins. Circulating levels are low in healthy individuals and elevated levels are associated with bacterial infection. The expression of PCT is linked to IL-6, TNF and bacterial endotoxin (37).

White blood cell count

WBC is a count of leukocytes (basophils, eosinophils, lymphocytes, neutrophils and monocytes) per mm^3 of blood, performed with cytometry.

2.5 DEFINITION OF ACUTE KIDNEY INJURY

An early description of what we today call acute kidney injury, was published by John Abercrombie in 1821: "The disease seems, in general, to come suddenly . . . The peculiar symptom is a sudden diminution of secretion of urine, which soon amounts to a complete suspension of it. The affliction is probably first considered as retention; but the catheter being employed, the bladder is found to be empty. . . the symptoms now go on for several days; after which, the patient begins to talk a little incoherently, and shows a tendency to stupor. This increases gradually to perfect coma, which in a few days more is fatal. . . . the occurrence of coma may be expected from the fourth or fifth day from the time when the secretion of urine became completely suspended"(38).

In the end of the 20th century there was an abundance of definitions of kidney failure, but not one that all researchers and clinicians would prefer. In 2004 the Acute Dialysis Quality Initiative group (ADQI) proposed the RIFLE classification, which led to a consensus agreement (39). RIFLE is an acronym for Risk Injury Failure Loss End-stage renal disease. The RIFLE classification defines and grades AKI using the patient's serum creatinine increase (above her baseline value), together with her urine output.

In 2007 the Acute Kidney Injury Network (AKIN) proposed an update of the classification where classes L and E were discarded and classes R, I and F were replaced with stages 1-3. The new definition also included an addition of a minimum absolute creatinine increase, occurring within 48 hours. Finally, the initiation of renal replacement therapy (regardless of creatinine or urine output) was included as a marker of stage 3 AKI (40). In 2012 the Kidney Disease: Improving Global Outcomes (KDIGO) proposed a further update of the classification with the addition that the relative change in creatinine should occur within seven days (41). RIFLE, AKIN and KDIGO are presented in detail in tables 4-6.

Table 4. RIFLE AKI staging.

AKI severity	Plasma creatinine criteria	Urinary output criteria
RIFLE: Risk Injury Failure Loss End-stage renal disease		
Risk	≥ 1.5-fold increase in serum creatinine from baseline*	< 0.5 ml/kg/h for ≥ 6 hours
Injury	≥ 2.0-fold increase in serum creatinine from baseline*	< 0.5 ml/kg/h for ≥ 12 hours
Failure	≥ 3.0-fold increase in serum creatinine from baseline* or 44 μmol/l increase if baseline ≥ 354 μmol/l	< 0.3 ml/kg/h for ≥ 24 hours or anuria for ≥ 12 hours
Loss of kidney function	Complete loss of kidney function > 4 weeks	
End-stage renal disease	End-stage renal disease > 3 months	

*When baseline creatinine is unknown it is recommended to estimate baseline using the simplified Modification of Diet in Renal Disease (MDRD) equation, assuming a GFR of 75 ml/min/1.73 m^2.

Table 5. AKIN AKI staging KDIGO

AKIN: Acute Kidney Injury Network

Stage 1	≥ 1.5-fold increase in serum creatinine from baseline* or an absolute rise in serum creatinine of ≥ 26.4 μmol/l within 48 hours	< 0.5 ml/kg/h for ≥ 6 hours
Stage 2	≥ 2.0-fold increase in serum creatinine from baseline*	< 0.5 ml/kg/h for ≥ 12 hours
Stage 3	≥ 3.0-fold increase in serum creatinine from baseline* or initiation of renal replacement therapy	< 0.3 ml/kg/h for ≥ 24 hours or anuria for ≥ 12 hours

Table 6. KDIGO AKI staging

KDIGO: Kidney Disease: Improving Global Outcomes

Stage 1	1.5–1.9-fold increase in serum creatinine from baseline* within 7 days or ≥ 26.5 μmol/l increase within 48 hours	< 0.5 ml/kg/h for 6–12 hours
Stage 2	2.0–2.9-fold increase within 7 days	< 0.5 ml/kg/h for ≥ 12 hours
Stage 3	3.0 times baseline* or Increase in serum creatinine to ≥ 353.6 μmol/l within 7 days or initiation of renal replacement therapy	< 0.3 ml/kg/h for ≥ 24 hours or anuria for ≥ 12 hours

*When baseline creatinine is unknown it is recommended to estimate baseline using the simplified Modification of Diet in Renal Disease (MDRD) equation, assuming a GFR of 75 ml/min/1.73 m^2.

2.6 INCIDENCE AND OUTCOMES OF ACUTE KIDNEY INJURY

Incidence of ICU AKI varies depending on the population studied. In a nationwide Finnish multicenter study from 2013, including 17 ICUs, the incidence (95 % CI) was 39.3 % (37.5 - 41.1 %) and 10.2 % (9.1 -11.3 %) required RRT and 33.7 % (30.9 - 36.5 %) were dead within 90 days of ICU admission.

2.7 PATHOPHYSIOLOGY OF ACUTE KIDNEY INJURY

The emergence of biomarkers of kidney injury has led to a paradigm shift in the understanding of AKI pathophysiology (42). Historically, the taxonomy of AKI was based on anatomy (i.e. pre-, intra- and post-renal). Contemporary classification is based on aetiology (i.e. sepsis-associated, cardiorenal, hepatorenal, nephrotoxic, traumatic). The current understanding is that inflammation, initiated and propagated by DAMPs and PAMPs together with disturbances in the microcirculation are key factors (43). In sepsis-associated AKI it has been proposed that the tubular cell's response to danger may be a temporary adaptive response to maintain energy balance and avoid DNA damage (44). This view is supported by studies of cell-cycle arrest biomarkers and the lack of tubular necrosis and/or apoptosis in autopsies in patients who died after sepsis with AKI (45, 46). AKI after cardiac surgery is likely caused by hemodynamic, nephrotoxic and inflammatory factors together (47, 48).

2.8 RENAL RECOVERY

Failure to recover from AKI is often defined by RRT dependence, i.e. end-stage renal disease (ESRD) and death. A cohort study using the Swedish intensive care register from 2005 - 2011, showed that ICU patients (without previous chronic kidney disease) who developed AKI had a 1-year mortality of 49 %. After 1 year 2 % of the survivors were RRT dependent. Patients with acute-on chronic kidney disease had a 1 year mortality of 54 % and 25 % of survivors were RRT dependent (49). It is not fully understood how AKI progresses to end-stage renal disease. However, it has been shown that tubular cells subjected to cell-cycle arrest may adopt a proinflammatory and profibrotic phenotype (50).

2.9 BIOMARKERS OF ACUTE KIDNEY INJURY AND RENAL RECOVERY

An optimal biomarker of AKI should be able to 1) identify early signs of "stress" in the kidneys before kidney function declines and before GFR drops, 2) predict poor outcome (death and end-stage renal disease), 3) Provide high diagnostic and predictive accuracy 4) be fast and cheap to measure, 5) have a reasonable half-life in plasma as to provide information about AKI onset and 6) change expression early as the condition progresses (i.e. tell us if the condition is healing or worsening and if we need to continue RRT tomorrow or if we can wean the patient from RRT). In the years to come, perhaps we will have biomarkers that differentiate between different aetiologies of AKI and will help us chose the correct intervention (51). The prevailing AKI definition relies heavily on creatinine - a biomarker of kidney function and urine output - another marker of kidney function. In recent years, biomarker studies have demonstrated that a subgroup of patients who do not classify as AKI

according to KDIGO AKI (or show any other signs or symptoms of AKI), have elevated levels of biomarkers we believe are related to tubular injury (NGAL and KIM-1). Patients with this condition, which has been labeled subclinical AKI, appear to have an increased risk of death and need for RRT, compared to patients with normal biomarkers (52, 53). It is likely that the current concept and classification of AKI will be re-assessed in the years to come, as to include biomarkers of kidney injury. The current classification also lacks a definition of the timing of AKI onset. A biomarker of kidney injury, as opposed to a biomarker of kidney function, could be prove useful in determining onset and trajectory of AKI as well as renal recovery. Cellular origin and known molecular functions of the biomarkers used in studies I-IV are detailed in table 6.

Table 6. Biomarkers of AKI studied in this thesis.

Biomarker	Studies	Cellular origins	Known molecular functions
Creatinine (plasma and urine)	1-IV	Skeletal muscle	Byproduct of creatine metabolism
Cystatin C (plasma)	IV	All nucleated cells	Inhibitor of cysteine protease
Endostatin (plasma and urine)	I, IV	Epithelial and endothelial cells. Generated when collagen XVIII is cleaved.	Anti-angiogenesis
NGAL (plasma and urine)	I, III, IV	Neutrophil granulocyte, tubular cells, liver, lung	Iron sequestering, renal protection, bacteriostatic, apoptosis
Urea (plasma and urine)	IV	Hepatocytes	Byproduct of protein metabolism

3 AIMS OF THE STUDY

To investigate the ability of several biomarkers to predict acute kidney injury in critically ill patients.

To gain knowledge and generate hypotheses of the pathophysiology of acute kidney injury in critically ill patients.

To investigate the ability of several biomarkers to predict infection in critically ill patients.

To investigate the ability of several biomarkers to predict renal recovery after acute kidney injury and renal replacement therapy in critically ill patients.

To gain knowledge and generate hypotheses of the pathophysiology of renal recovery after acute kidney injury in critically ill patients.

4 SUBJECTS AND METHODS

4.1 ETHICAL CONSIDERATIONS

The Stockholm regional ethics committee approved studies I-IV, which were performed in accordance with the ethical standards laid down in the Declaration of Helsinki in 1964, and it's later amendments. Several ethical aspects were considered within the scope of this thesis. Patients were only enrolled in the studies if we had their consent or the consent of next of kin. Taking blood and urine samples from the patients was not considered a significant medical risk. All data were kept in a coded manner and the codes locked in a safety cabinet. Treating clinicians did not have access to study sample results, hence study participation did not affect the treatment the patients received. Enrolled patients were not given any financial compensation.

Table 7. Summary of subjects and methods used in studies I-II.

	Study I	**Study II**
Data source	PEAK	PEAK
Design	Prospective cohort	Prospective cohort
Study period	2007-2010	2007-2013
Participants (n)	93	188
Exposure	P-endostatin, Cystatin C, NGAL	P-calprotectin, CRP, PCT, WBC
Outcome	Prediction of AKI	Diagnosis and prediction of bacterial infection
Statistical analyses	Mann-Whitney U test, Fisher's exact test, RM-ANOVA, logistic regression, Spearman correlation, AUC ROC, NRI, IDI	Mann-Whitney U test, Fisher's exact test, X^2 test, RM-ANOVA, logistic regression, AUC ROC, DeLong test, Youden index

AKI Acute kidney injury, NGAL Neutrophil gelatinase-associated lipocalin, CRP C-reactive protein, PCT Procalcitonin, WBC White blood cell count, RM-ANOVA Repeated measures analysis of variance, AUC ROC Area under receiver operating characteristic curve, NRI Net reclassification improvement, IDI Integrated discrimination improvement.

Table 8. Summary of subjects and methods used in studies III-IV.

	Study III	**Study IV**
Data source	PEAK	EXCRETe
Design	Prospective cohort	Prospective cohort
Study period	2007-2014	2008-2016
Participants (n)	198+145	143
Exposure	P-dimeric NGAL, total NGAL, PCT, CRP, WBC	P/U-NGAL, endostatin, creatinine, urea, p-Cystatin C
Outcome	Diagnosis and prediction of bacterial infection. Biomarker kinetics after antibiotic therapy	Prediction of renal recovery
Statistical analyses	Kruskal-Wallis test, Mann-Whitney U test, Fisher's exact test, X^2 test, GLMM, linear regression, AUC ROC	Mann-Whitney U test, Fisher's exact test, X^2 test, AUC ROC, logistic regression, DeLong test

AKI Acute kidney injury, NGAL Neutrophil gelatinase-associated lipocalin, CRP C-reactive protein, PCT Procalcitonin, WBC White blood cell count, RM-ANOVA Repeated measures analysis of variance, AUC ROC Area under receiver operating characteristic curve, GLMM Generalized linear mixed model.

4.2 REGISTERS AND DATABASES

All databases exclusively include patients referred to the general ICU at the Karolinska University Hospital, Solna.

The Predicting Early Acute Kidney injury (PEAK) database (Studies I-III)

Patients with an expected length of stay of more than 24 hours and an estimated glomerular filtration rate (eGFR) of more than 60 mL/min/1.73 m^2 (modification of diet in renal disease [MDRD] equation) on ICU admission, were included in the Predicting Early Acute Kidney injury (PEAK) database between 2007 and 2014. Blood and urine samples were collected on study inclusion and twice daily thereafter until ICU discharge, death or start of renal replacement therapy. We defined AKI as a ≥ 50 % increase in plasma creatinine from baseline or an increase in plasma creatinine by ≥ 26.5 μmol/L within 48 h and/or a urine output less than 0.5 mL/kg/h for at least 6 consecutive hours according to the Kidney Disease: Improving Global Outcomes (KDIGO) criteria. We used the lowest creatinine level obtained within 3 months before ICU admission as baseline for the KDIGO classification. Missing baseline creatinine was imputed using the MDRD formula and an eGFR of 75 mL/min/1.73 m^2.

We defined the systemic inflammatory response syndrome (SIRS) using three or more SIRS criteria (Table 1). Sepsis was defined as a suspected or confirmed infection together with SIRS. The presence or absence of SIRS and sepsis was recorded in the database on each ICU day. Baseline characteristics, Acute Physiology And Chronic health Evaluation (APACHE II) score, ICU admission diagnosis, ICU length of stay and mortality were recorded. Physiological parameters (e.g. urinary output, arterial blood pressure), biomarker concentrations, vasopressor dose, ventilator settings and antibiotic therapy were recorded in the database. Information about co-morbid conditions and 30-day mortality was collected retrospectively from the hospital-based electronic case-record system.

The Bio-X database (Study III)

Altogether we included 145 healthy controls who were over 18 years old with no fever or other signs or symptoms of infection. Patient characteristics were recorded and blood was drawn.

The EXtracorporeal Clearance & REsidual renal function during renal replacement Therapy (EXCRETe) database (Study IV)

Patients with AKI, who were prescribed acute RRT with a duration likely to be ≥ 24 h during their stay, were included in the database between 2008 and 2016. Patients with end-stage renal disease were not included. Plasma and urine were collected once daily (except when patients were anuric). AKI and SIRS/sepsis were defined and recorded in the same way as in the PEAK database. Baseline characteristics, APACHE II score, ICU admission diagnosis, ICU length of stay and mortality was recorded. Physiological parameters (urinary output, arterial blood pressure), biomarker concentrations, vasopressor dose, ventilator settings and

antibiotic therapy were recorded in the database. Information about co-morbid conditions, length of RRT, dialysis dependence and mortality at 60 days was collected retrospectively from the hospital-based electronic case-record system.

4.3 SCORING AND STAGING METHODS

Severity of acute kidney injury

KDIGO AKI staging, used in studies I-IV, is based on relative increase of plasma creatinine within 7 days or absolute increase in plasma creatinine within 48 hours or initiation of RRT or an episode of oliguria/anuria. AKI staging criteria from 2004 up to this day are detailed in tables 4-6.

SIRS/sepsis scoring

We used a modification of the SIRS criteria (also applied by the Protein C Worldwide Evaluation in Severe Sepsis [PROWESS] study group) - i.e. at least three (instead of two) out of four criteria had to be fulfilled (54). The SIRS and sepsis classifications used in studies I-IV are detailed in table 1.

Allocation of infection site and probability of infection

Definitions proposed by the International Sepsis Forum (ISF) (55) were used to allocate specific infection sites and probability of infection. An infectious disease specialist blinded to study biomarker results (but not blinded to routine biomarkers i.e. CRP, PCT and WBC) classified patients as having no infection, probable infection, possible infection or confirmed infection according to the ISF criteria. Summarised ISF criteria are found in table 9.

Table 9. Summarized allocation of infection site and probability of infection, from the International Sepsis Forum consensus conference of infection in the ICU (ISF).

Foci of infection			
Pneumonia	Primary bloodstream infection	Secondary bloodstream infection	Catheter-related infection
Skin- and soft tissue infection	Urosepsis	Primary, secondary and tertiary peritonitis	Intra abdominal abscess
Endocarditis	Meningitis	Epiglottitis	
Probability of infection (criteria have been simplified and condensed for all foci, for brevity)			
Possible	Positive culture likely attributable to clinical infection	Clinical and radiographical findings in absence of positive culture	Positive culture of surgical site or exit site of catheter
Probable	Positive culture from adequate specimen indicative but below threshold level	Surgical evidence of infection or drainage of pus without positive culture	Strong clinical, radiographic or surgical evidence without positive culture
Microbiologically confirmed	Positive culture in uncontaminated specimen	Culture from peritoneum or blood > 24 h after GI perforation	Catheter tip- and blood culture from same pathogen

4.4 LABORATORY ASSAYS

Blood samples taken as a part of routine care were analysed at the Department of Clinical Chemistry, Karolinska University Hospital, Solna. Study samples were immediately centrifuged at 1500 g at 4˚C for 10 min. The supernatant plasma and urine were aliquoted into cryovials and stored at -80˚C and later analysed at the Department of Clinical Chemistry, Uppsala University Hospital, Uppsala, Sweden. In study III, NGAL was analysed by Diagnostics Development, Uppsala, Sweden. Laboratory personnel were blinded to clinical patient data. Assay characteristics are described in detail below and in table 10.

Enzyme-linked immunosorbent assay (ELISA)

In study III we quantified dimeric and total NGAL concentrations using two different so called "solid phase sandwich ELISAs": Polystyrene microtiter plates were coated with the monoclonal anti-NGAL antibody clone 763 ("capture antibody" from Diagnostics Development, Uppsala, Sweden) at 4˚C overnight. Additional binding sites were blocked with carbonate-bicarbonate buffer (Ivitrogen Corp., UK) at 37˚ for 1 hour. 100 µl of the sample (plasma or urine) were diluted with assay solution and incubated at room temperature for 2 hours. In the total NGAL assay, 100 µl of the monoclonal antibody clone 764 ("detection antibody" from Diagnostics Development, Uppsala, Sweden) were added and incubated at room temperature for 1 hour, followed by incubation with 100 µl of diluted horseradish peroxidase-conjugated streptavidin (GE Healthcare, UK) for 1 hour at room temperature. In the dimeric NGAL assay the "detecting antibody" was the monoclonal antibody clone 765 (Diagnostics Development, Uppsala, Sweden). In the final step, 100 µl of tetramethylbenzidine solution were added to visualize the enzyme reaction. A microplate reader (SPECTRA-max 250, GMI Inc., USA) measured absorbance.

Particle-enhanced turbidimetric immunoassay (PETIA)

PETIAs are faster and less labour intensive than ELISAs and can be run continuously as samples arrive to the laboratory. The basic principle of immunoturbidimetry is measuring the light scattering effect caused by antigen-antibody reactions in a solution such as plasma - light absorption is measured before the reaction starts and after the reaction has occurred and the difference in signal is referred to as the signal. Antibodies are prepared by attaching them to polystyrene particles. In study II we used antibodies against human calprotectin, purified from eggs from immunized hens. Avian antibodies do not react with the human complement system, rheumatoid factors or anti-mouse IgG antibodies - which could otherwise cause erroneous test results. An assay buffer is chosen to maximise reaction conditions. Cystatin C in study I and IV and CRP in all studies were also measured with PETIAs. All PETIAs have lower quantitation limits and antigen excess limitations.

Table 10. Assay characteristics.

Biomarker	Study	Immunoassay	Analyser	Total CV %
Endostatin	I, IV	ELISA	Cobas EE	6 %
NGAL	I, IV	ELISA	Cobas EE	6 %
Cystatin C	I	PETIA	Architect Ci8200	1.5 % at 0.8 mg/L
Cystatin C	IV	PETIA	Mindray BS-380	1.5 % at 0.8 mg/L
Calprotectin	II	PETIA	Mindray BS-380	1.5 % at 1.3 mg/L
Procalcitonin	II, III	ELISA	Cobas EE	CV 6 % at 0.25 ng/mL
CRP	I, II, III	PETIA	Architect Ci8200	4 %
dNGAL/total NGAL	III	ELISA	Cobas EE	6 %
Creatinine	I-IV	Alkaline picrate colorimetry	Mindray BS-380	1.2 % at 90 µmol/L
Urea	IV	Alkaline picrate colorimetry	Mindray BS-380	1.1 %
White blood cell count	I, II, III	Flow cytometry	Sysmex XN-9000	1.5 %

Endostatin and NGAL in study I

We used commercially available (R&D Systems, Minneapolis, MN) enzyme-linked immunosorbent assays (ELISAs): DY1098 for endostatin and DY1757 for NGAL. The assays had a total coefficient of variation (CV) of approximately 6 %.

Cystatin C in studies I and IV

In study I, Cystatin C was measured with a particle-enhanced turbidimetric immunoassay (PETIA) on the Architect Ci8200 analyzer (Abbott Laboratories, Abbott Park, IL) with reagents from Gentian (Moss, Norway). In study IV Cystatin C was measured with a PETIA on the Mindray BS-380 (Mindray Medical International, Shenzhen, China). CV for the cystatin C method was 1.5 % at 0.8 mg/L.

Calprotectin in study II

The calprotectin assay was a PETIA on the Mindray BS-380 with reagents from Gentian (Moss, Norway). CV for the Calprotectin method was 1.5 % at 1.3 mg/L.

Procalcitonin in studies II, III

PCT was analysed using an ELISA (CV 6 % at 0.25 ng/mL and 3 % at 10.4 ng/mL) on the Cobas EE (Roche Diagnostics, Mannheim, Germany). The expected normal PCT level was < 0.05 ng/mL.

C-reactive protein in studies II, III

CRP was analysed with a PETIA on the Architect Ci8200 (CV 4 %) (Abbott Laboratories, Abbott Park, IL). The expected normal CRP level was < 5mg/L.

Dimeric NGAL and total NGAL in study III

We quantified dimeric and total NGAL concentrations using two different ELISAs. The antibody configurations of the ELISAs were as follows: in both assays the microtiter plates were coated with the monoclonal antibody clone 763 (Diagnostics Development, Uppsala, Sweden). In the total NGAL assay the detecting antibody was the monoclonal antibody clone 764 (Diagnostics Development, Uppsala, Sweden) and in the dimeric NGAL assay the detecting antibody was the monoclonal antibody clone 765 (Diagnostics Development, Uppsala, Sweden).

4.5 STATISTICAL METHODS

Averages (median, arithmetic and geometric mean)

The average or central tendency aims to describe the entire data with a single value and can be calculated in many different ways. The median is the middle element of the data if they are ordered from smallest to largest, i.e. the 50^{th} percentile of the data. It is sometimes a convenient method of defining the average because it ignores outliers (good for data with a lot of measuring artefacts) but in other situations insensitivity to the outliers may be a problem (e.g. when outlier data represent a true effect). The arithmetic mean is the sum of all data, divided with the number of elements. However, it is not a good method to define the average in small or skewed datasets, because it is sensitive to outliers. Both the median and arithmetic mean require normally distributed data (i.e. bell-shaped). In skewed distributions of data (e.g. a lot of small values and few large values or vice versa), the geometric mean may be a better option as it is calculated with the product of all data and then divided with the n^{th} root (where n is the number of elements) or more often by calculating the arithmetic mean of log-transformed data and then converting the mean back to base 10 (figure 1). Also, because it is based on geometric sequences (and not arithmetic), it can be used to compare biomarkers with different units.

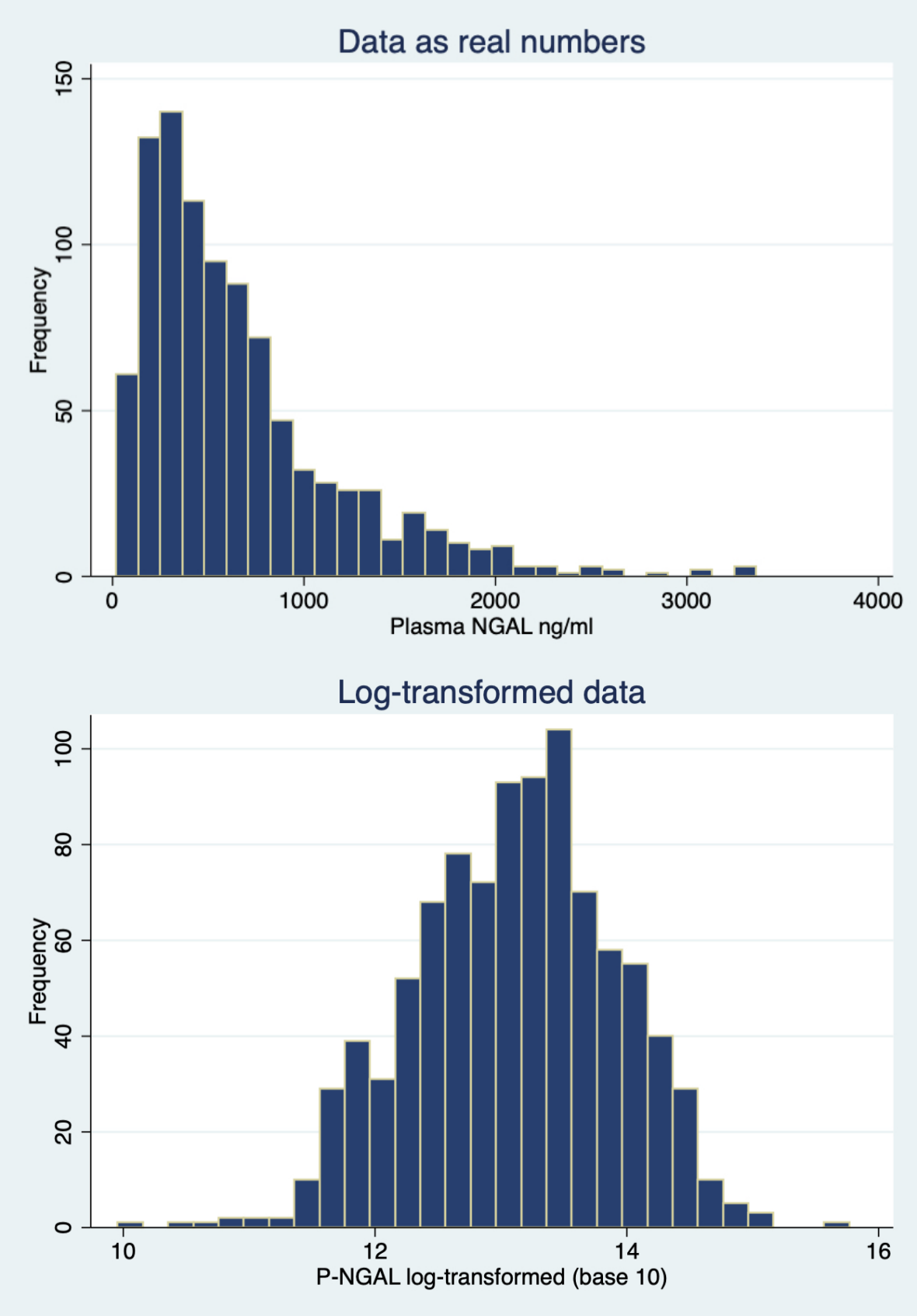

Figure 1. Example of skewed data (above) before and after log-transformation, base 10 (below).

Sensitivity and specificity

A diagnostic test classifies or predicts the presence (or absence) of a condition or disease. If there is a gold standard to diagnose the condition or disease, there can be four outcomes of the test: 1) true positive i.e. sick classified as sick, 2) true negative i.e. healthy classified as healthy, 3) false positive i.e. healthy classified as sick and 4) false negative i.e. sick classified as healthy. Sensitivity is the test's ability to classify or predict the disease when it is truly present (i.e. if sensitivity is 100 %, all patients with the condition we are looking for will have a positive test). Specificity is the ability of the test to exclude the condition or disease in patients when it is not present (i.e. if specificity is 100 %, all patients with a positive test will have the condition we are looking for). Depending on the cut-off value of the test, it may have a high specificity and a low sensitivity or vice versa. Increasing the cut-off value may increase the specificity but reduce the sensitivity (figure 2). Sensitivity and specificity of a test do not account for the prevalence of the condition or disease you are looking for (i.e. pre-test probability) and do not tell the probability of having the condition if the test is positive (i.e. positive predictive value). However, the positive likelihood ratio (i.e. how much more common is the condition after a positive test compared to a negative test?) is calculated by dividing the sensitivity by 1–specificity.

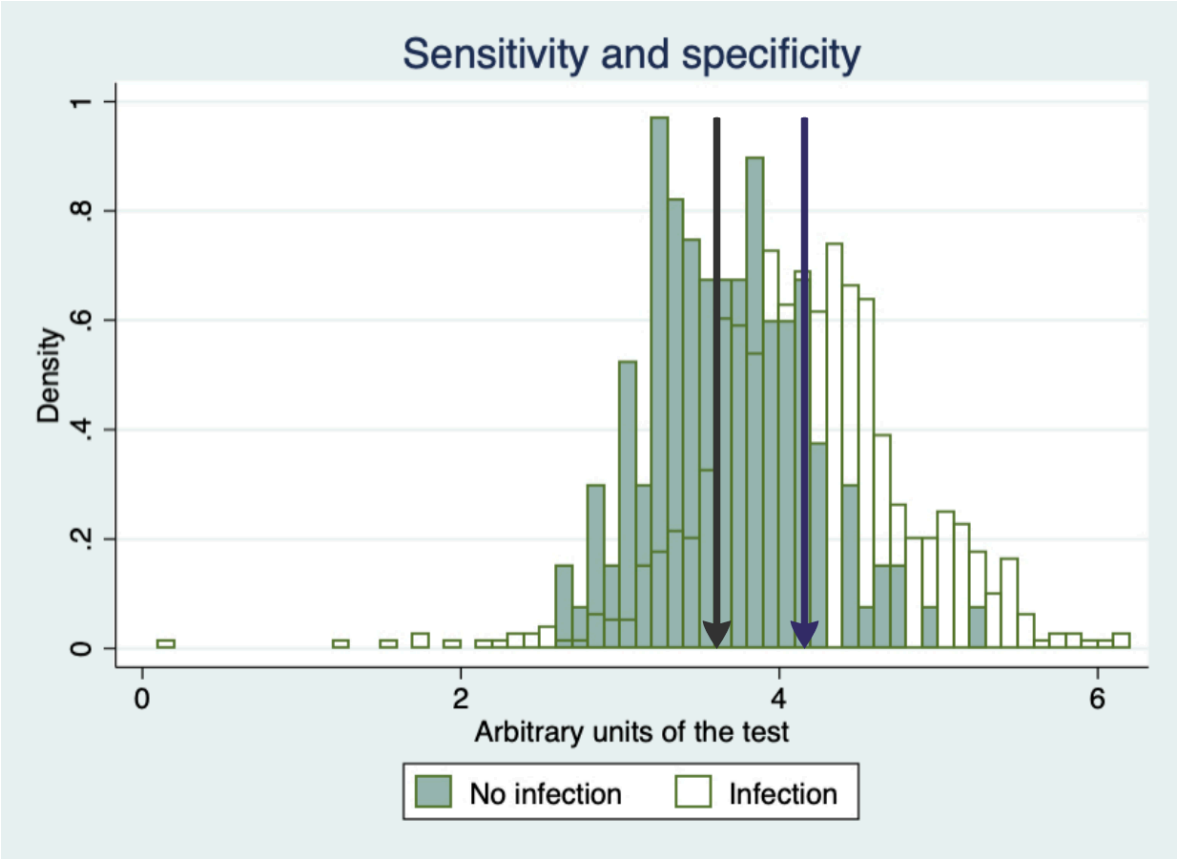

Figure 2. Histogram of biomarker values in a population, illustrating the result of an increased cut-off value: moving the cut-off to the right classifies less no-infection patients as having infection (i.e. increasing specificity) but also excludes more infection-patients from being classified as having infection (i.e. decreasing sensitivity).

Probability-value (p-value)

The null hypothesis (H_0) is a default position in statistical inference (inferring properties of a population from a sample of the population), claiming there is no difference or association between studied predictors or groups. H_0 is a claim we wish to nullify, to invalidate. The p-value is the probability of getting a result when the H_0 is true. The significance level must be set a priori (e.g. $p < 0.05$) and if the p-value is below the predefined limit we can reject the H_0 (i.e. the result is statistically significant). Consequently, the p-value can tell us if the data are incompatible with our statistical model - but it does not tell us anything about the importance of the result.

If the H_0 is true and we still reject it, we are making a type I error. With a significance level set to 0.05 we accept that there is a 5 % probability that the statistical model will show a difference when there is none (i.e. 5 % risk of type I error). Risk of type I error is increased with multiple testing since every test carries a risk of false discoveries. To deal with this the targeted p-value could be reduced if there are multiple tests.

If the H_0 is false and we don't reject it, we are making type II error. The risk of committing type II error is related to sample size (statistical power) and significance level - a lower significance level will make it harder to reject H_0, even when it is false, thus increasing the risk of type II error.

A confidence interval (CI) of a sample is another way to express statistical inference. A CI of 95 % equals the probability that the true mean of the population lies within the range. Hence, it can serve to reject or retain the H_0, but it may also provide information about the variability of the observed sample, i.e. it's precision.

Repeated-measure analysis of variance (RM-ANOVA), interaction effect

RM-ANOVA may be used to assess data from the same patients under repeated conditions, thus eliminating individual patient differences as a reason of variance. Using the variables of study I as a hypothetical example: To understand if variance of a dependent variable (e.g. endostatin) is affected by interaction of the independent factors (e.g. ICU day and group) it is possible to introduce a interaction variable into the model, constructed by the products of the independent variables (e.g. time x group). If, for sake of simplicity, endostatin levels in the two groups would follow two straight lines over time - the interaction effect would be the difference in slopes between the lines. No difference between groups (H0) would be demonstrated by two parallel lines. Rejection of H0 would be demonstrated as a significant difference in slopes (Figure 3).

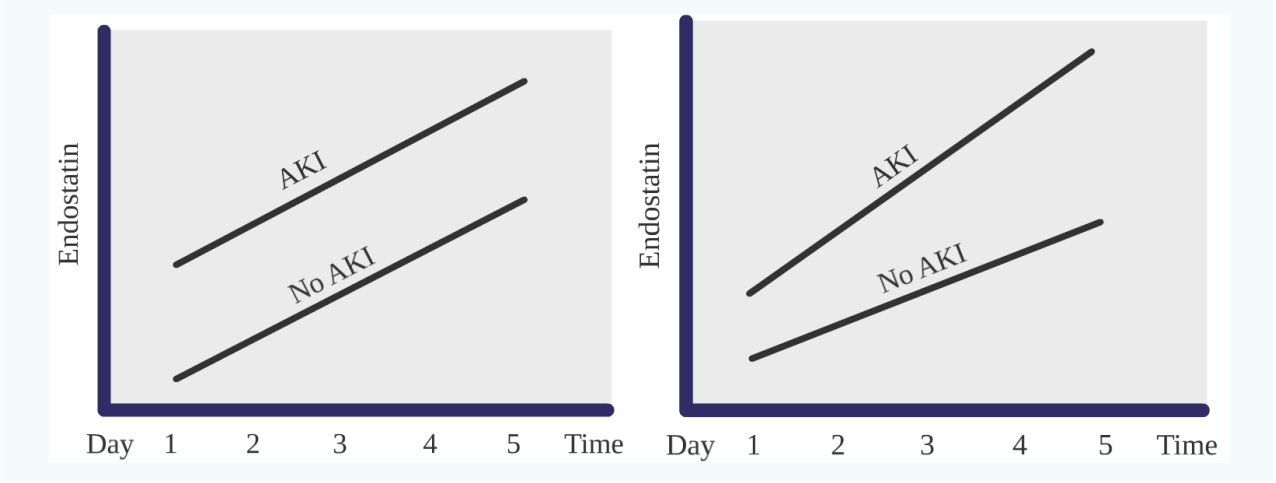

Figure 3. Hypothetical illustration of the interaction effect, based on variables in study I.

A prerequisite of the RM-ANOVA is that it requires all subjects to have complete data. If this is not the case, sample size will be reduced, as patients with missing data will be omitted from the analysis. This in turn introduces a risk of selection bias - patients with complete data may not represent a random sample.

Receiver Operating Characteristic curve (ROC-curve) analysis

ROC curves require one binary outcome and one or many ordinal and/or continuous predictor variables. ROC curves are made by plotting the true positive rate (sensitivity) for each value of the predictor variable(s) on the y-axis and the the false-positive rate (1 - specificity) on the x-axis. Subsequently, the plotted points are connected to form a curve. ROC curves that are plotted close to y = 1 and x = 0 (true positive rate = 1 and false-positive rate = 0) are highly predictive of the binary outcome. Curves plotted near the line of equality indicate a predictive value no better than random chance. The area under the ROC curve is a function of the predictor variables sensitivity and specificity and serves to quantify the overall accuracy of the predictor. The maximum area is 1 and the minimum area is in effect 0.5 (an area < 0.5 suggests a need to redefine the predictor from positive to negative or vice versa). An AUC ROC of 0.839, equals a 83.9 % probability that the risk score of a randomly picked AKI patient is higher than the score of a randomly picked non-AKI patient (Figure 4).

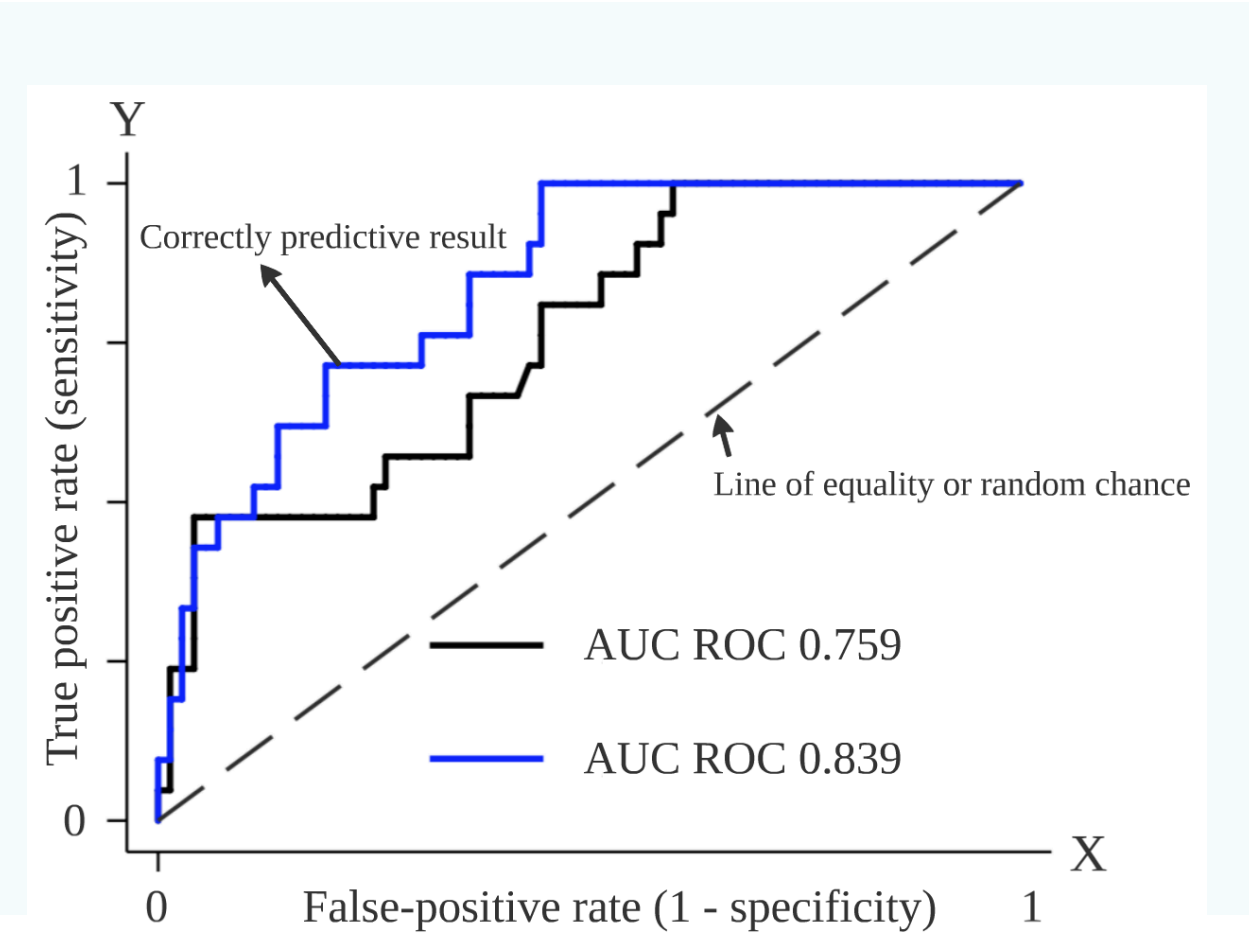

Figure 4. ROC curves of clinical prediction model alone (black line) and clinical prediction model together with endostatin (blue line).

4.6 STUDY I

Design and study population

A total of 138 patients were enrolled in the PEAK database between August 2007 and November 2010. We decided a priori to exclude patients having their first study sample obtained > 48 hours after ICU admission and patients with AKI on the day of their first study sample. Primary outcome was development of AKI within 72 h of the first study sample. We recorded AKI status until a maximum of 5 days following ICU admission. Altogether, we excluded 16 patients who were enrolled after > 48 h of ICU admission and another 29 patients with manifest AKI on the day of the first study sample. Among the 93 remaining patients, 21 (23 %) developed AKI within 72 hours.

Statistical analysis

We analysed data using Stata, version 11.2 (StataCorp, Texas, USA). To assess the change over time for plasma endostatin in patients not developing AKI and patients developing AKI, we used a RM-ANOVA, treating ICU day as the repeated-measures variable. Null hypothesis (H0) was that there was no difference in plasma endostatin-change over time between the groups, i.e., no interaction effect. To assess this, we introduced an interaction variable (time x group).

The following clinical predictor variables were considered for the clinical risk prediction model: age, sex, APACHE II score, baseline creatinine, delta creatinine, early oliguria (urine output < 0.5 mL/kg/h for > 2 hours), presence of SIRS, presence of sepsis and noradrenaline dose. We assessed the impact of each variable on the odds ratio of our outcome (AKI) with logistic regression analyses.

Because our sample size was small and the variables were many, we needed to reduce the number of variables to put in the multivariable model to avoid overfitting (overfitting risks too optimistic predictions about the models performance). To achieve this, we performed univariate analyses (one explanatory variable at a time) and kept only the variables with $p < 0.10$: age, APACHE II score and early oliguria.

Finally, the association of the remaining three clinical variables with AKI development was assessed by multivariable logistic regression analysis. To deal with skewed biomarker data they were log-transformed (base 10). Normal distribution of log-transformed data was verified graphically. To compare the goodness of fit between prediction of AKI with the clinical prediction model alone and prediction with the clinical prediction model together with the different biomarkers, we performed a likelihood-ratio test (LR test) for each biomarker. We also calculated the area under the receiver operating characteristic curve (AUC ROC) for the clinical prediction model with and without inclusion of the measured biomarkers. The equality of AUC ROCs was assessed by the method of DeLong et al, which utilizes random vectors in a non-parametric approach when comparing areas (56). To further assess the predictive contributions of the biomarkers, we employed the net reclassification

improvement method (NRI) and the integrated discrimination improvement (IDI) method. NRI is based on the change of probability of reclassification and IDI is based on differences in discrimination slopes (57).

4.7 STUDY II

Design and study population

A total of 188 patients were included in the PEAK database, between August 2007 and November 2013 and assessed for eligibility in study II. The aim of the study was to assess biomarkers of early infections. Hence, we excluded 42 patients who had initiated antibiotic therapy before ICU admission and 36 patients without a study sample before or on the day of antibiotic therapy initiation. Of the remaining 110 patients, 52 (47.3 %) did not have an infection during their ICU admission and 58 (52.7 %) had antibiotic therapy initiated due to infection, after a median of 2.6 days.

An infectious disease specialist, blinded to the study biomarker results, determined the likelihood of infection as: no infection, possible infection, probable infection or confirmed infection. The criteria for the likelihood classification (ISF criteria) is detailed in table 9.

We defined onset of infection as the time when the clinician prescribed antibiotic therapy. Perioperative or posttraumatic antibiotic prophylaxis initiation was not considered a marker of infection onset.

To compare biomarker levels in patients with possible, probable or confirmed infection with patients without infection, we did the following: 1) we took the median time from ICU admission to antibiotic therapy (2.6 days, equal to the third ICU day), 2) we identified all biomarker levels for the non-infected patients on the third ICU day and 3) we compared biomarker levels in patients with possible, probable or confirmed infection at the time they were prescribed antibiotic therapy with biomarker levels in the non-infected patients on their third ICU day. A similar approach was used to compare biomarker levels on the day before antibiotic therapy initiation.

Statistical analysis

We analysed data using Stata, version 12. Changes over time for biomarker levels were tested by RM-ANOVA, utilizing ICU day as the repeated-measures variable. An interaction variable (between group and time) was introduced in the RM-ANOVA model, to compare change over time between groups.

We used multivariable logistic regression analysis to assess the independent association between study biomarker levels and possible, probable or confirmed infection. Variables were included in the multivariable model if they were statistically significant at $P < 0.10$ in the univariate analyses.

We assessed predictive and diagnostic values by calculating the AUC ROCs. We tested equality of AUC ROCs by using the DeLong method. Optimal cut-off levels were determined using Youden index calculations together with the ROC curve analysis. The Youden index is a method to find the point where the minimum distance line crosses the ROC curve, i.e. the point with the predicting variable's optimum sensitivity and specificity.

Finally, a sensitivity analysis was performed to assess if infection misclassification had interfered with results (i.e. did patients with possible or probable infection really have no infection?). To reduce the risk of class overlapping in the sensitivity analysis, we compared patients with no infection only to patients with confirmed infection.

We also performed a second sensitivity analysis, matching patients with possible, probable or confirmed infection to patients without infection. In this, so-called greedy matching, we did the following: 1) for each patient with possible, probable or confirmed infection, we randomly selected one patient without infection with available biomarker data for the corresponding ICU day 2) the process was repeated until the list of patients for whom a matched control could be found was exhausted. The same approach was used to match patients on the day before initiation of antibiotic therapy.

4.8 STUDY III

Design and study population

Altogether, 198 patients were included in the PEAK database, between August 2007 and November 2014 and assessed for eligibility in study III. A total of 144 (72.7 %) patients had or developed infection and 54 (27.3 %) had no infection. Approximately one-third of the infection-group patients and two-thirds of the no infection-group patients were admitted following multi-trauma. Infection-group patients were older, had higher APACHE II score and had higher rate of AKI on admission. The comorbidities chronic obstructive pulmonary disease, asthma and malignancy were more frequent in the infection-group compared to the no infection-group.

We also included a control population of 145 healthy and non-infected volunteers, to establish assay specific reference values for the biomarker NGAL.

An infectious disease specialist (blinded to the study biomarker results) classified patients, according to ISF criteria, as having no infection (no infection-group) or possible infection, probable infection or confirmed infection (infection-group). We defined onset of infection as the time when the clinician prescribed antibiotic therapy. Empiric antibiotic therapy was considered appropriate if isolated pathogens were susceptible to the therapy administered, according to the susceptibility testing report.

We used the following method to compare biomarker levels on onset of infection for the infection-group to the levels of the no infection-group: firstly, we calculated the median ICU day for antibiotic therapy initiation; secondly, we identified all biomarker levels obtained on

the corresponding ICU day in the no infection-group patients; thirdly, we compared biomarker levels obtained on onset of infection with biomarker levels from the corresponding ICU day in no infection-group. We adopted the same approach to compare biomarker levels on the day before onset of infection.

Statistical analysis

We analyzed data using Stata, version 13.0. Biomarker levels were log-transformed (base 10) before analysis. We used a generalized linear mixed model (GLMM) with each patient treated as a random effect (i.e. uncorrelated with the fixed effects) to assess changes in biomarker concentrations over time. The interaction between group and time was introduced in the mixed model to compare the change over time between groups. Results were graphically presented as geometric means. GLMM is an extension of logistic regression that can handle both random and fixed effects (hence mixed model). GLMM can treat time as a continuous variable as opposed to categorical and can account for variability between patients regarding number of repeats.

Percent change in biomarker levels after appropriate antibiotic therapy initiation was assessed in 31 patients with confirmed, culture-verified infection and complete biomarker data from the time of antibiotic therapy initiation and the two following days. Equality between biomarker values on initiation and two days later was assessed with the Wilcoxon matched-pairs signed-ranks test of equality.

Independent associations between infection status, AKI and log-transformed biomarkers levels were assessed with multivariable linear regression analysis, adjusting for male sex and APACHE II score. The regression coefficients were expressed as 100 x (e^{coeff} – 1) and represent the geometric mean percent change in biomarker concentration associated with a one-unit change in the variable.

Predictive and diagnostic accuracy of the biomarkers were assessed with AUC ROC. We used Youden index calculations to assess the optimal cut-off value of the biomarkers.

4.9 STUDY IV

Design and study population

Altogether, 143 patients with AKI and requiring acute RRT were included in the EXCRETe database, between November 2008 and May 2016 and assessed for eligibility in study IV. Due to changes in the clinical course of 8 patients, they were never treated with RRT and therefore excluded from further analyses. The primary outcome was renal recovery, defined as alive and free from RRT on day 60 after ICU admission.

Plasma and urine samples were collected once daily (urine could not be collected when the patients were anuric). Analyses of study samples from plasma and urine samples were done late 2018. Plasma cystatin C was measured as a part of routine care.

Statistical analysis

We analysed data using Stata, version 12.1. Predictive accuracy of the biomarkers for renal recovery was assessed by calculating the AUC-ROC, using plasma and urine samples taken immediately before start of RRT and on each of the following 7 days.

To create a clinical prediction model, we assessed the association of clinical variables with renal recovery using multivariable logistic regression analysis. Clinical predictor variables were included in the multivariable models if they were statistically significant at $p < 0.20$ in the univariate analyses.

We calculated the AUC ROC for the clinical model with and without addition of the biomarker measurement to assess whether the addition of a biomarker improved the predictive power for renal recovery. As we did not know if the predictor variables would be negative or positive in relation to outcome, we performed bidirectional stepwise logistic regressions separately for each day. Biomarker levels were log-transformed (base 10) to deal with skewed data. Normal distribution of log-transformed data was verified visually (figure 1). We assessed the equality of AUC ROCs using the DeLong method.

5 RESULTS

5.1 STUDY I

AKI patients were older, had greater illness severity on presentation and had more comorbidities than non-AKI patients (table 11).

Table 11. Characteristics of non-AKI and AKI patients. Values are median (interquartile range) or n (%).

Variable	**No AKI (n = 72)**	**AKI (n = 21)**	**P-value**
Age (years)	50 (28, 65)	66 (57, 71)	0.002
APACHE II score	15 (11, 19)	19 (14, 24)	0.01
Diabetes	6 (8 %)	5 (24 %)	0.12
Cardiovascular disease	20 (28 %)	11 (52 %)	0.06
COPD/asthma	5 (7 %)	2 (10 %)	0.65
Gastrointestinal/liver disease	2 (3 %)	3 (14 %)	0.07
Any malignancy	11 (15 %)	3 (14 %)	1.0
Early oliguria*	6 (8.3 %)	9 (42.9 %)	0.01

*Early oliguria defined as urine output < 0.5 ml/kg/h during > 2 h. APACHE II Acute physiology and chronic health evaluation, COPD Chronic obstructive pulmonary disease.

Plasma endostatin levels were significantly higher in patients who developed AKI and remained higher during the first five study days (Figure 5 and table 12). This was also true for cystatin C, but not for NGAL. However, patients with sepsis had higher plasma NGAL at inclusion compared to patients without sepsis – whereas endostatin and cystatin C levels at inclusion were similar in patients with and without sepsis (figure 6).

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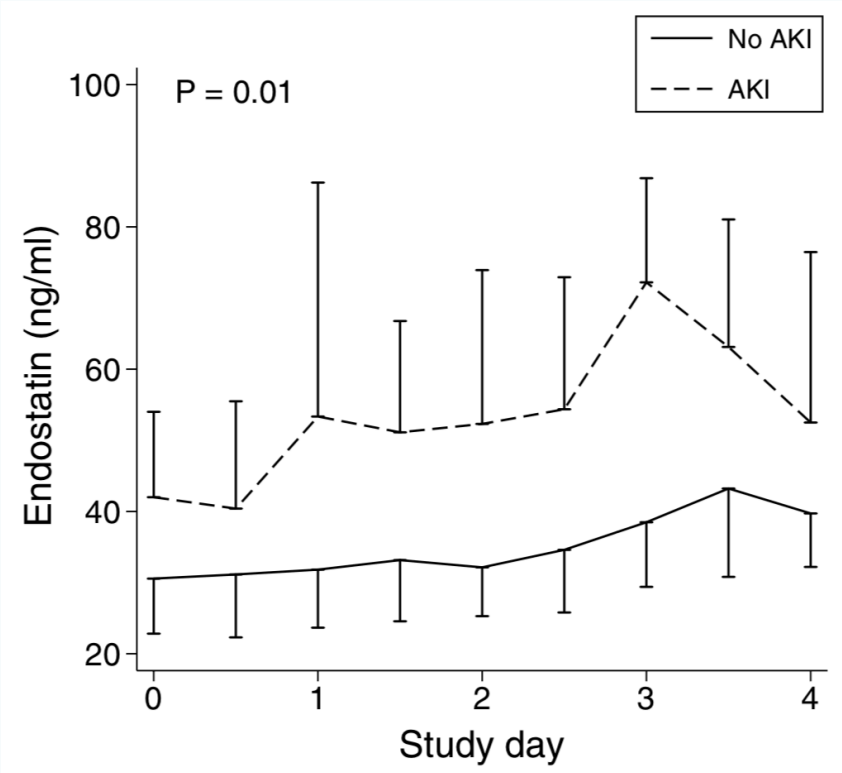

Figure 5. Plasma endostatin during the first five study days in patients with AKI and no AKI. Values are median and interquartile range. P-value is for the RM-ANOVA between groups

Table 12. Plasma biomarker levels at inclusion for patients without and with AKI. Values are median (interquartile range) or n (%).

Plasma biomarkers levels at inclusion	No AKI (n = 72)	AKI (n = 21)	P value
Endostatin ng/ml	31 (23, 40)	42 (35, 54)	0.002
Cystatin C mg/dl	0.75 (0.64, 1.00)	1.10 (0.82, 1.40)	0.02
NGAL ng/ml	97 (66, 149)	133 (67, 180)	0.29

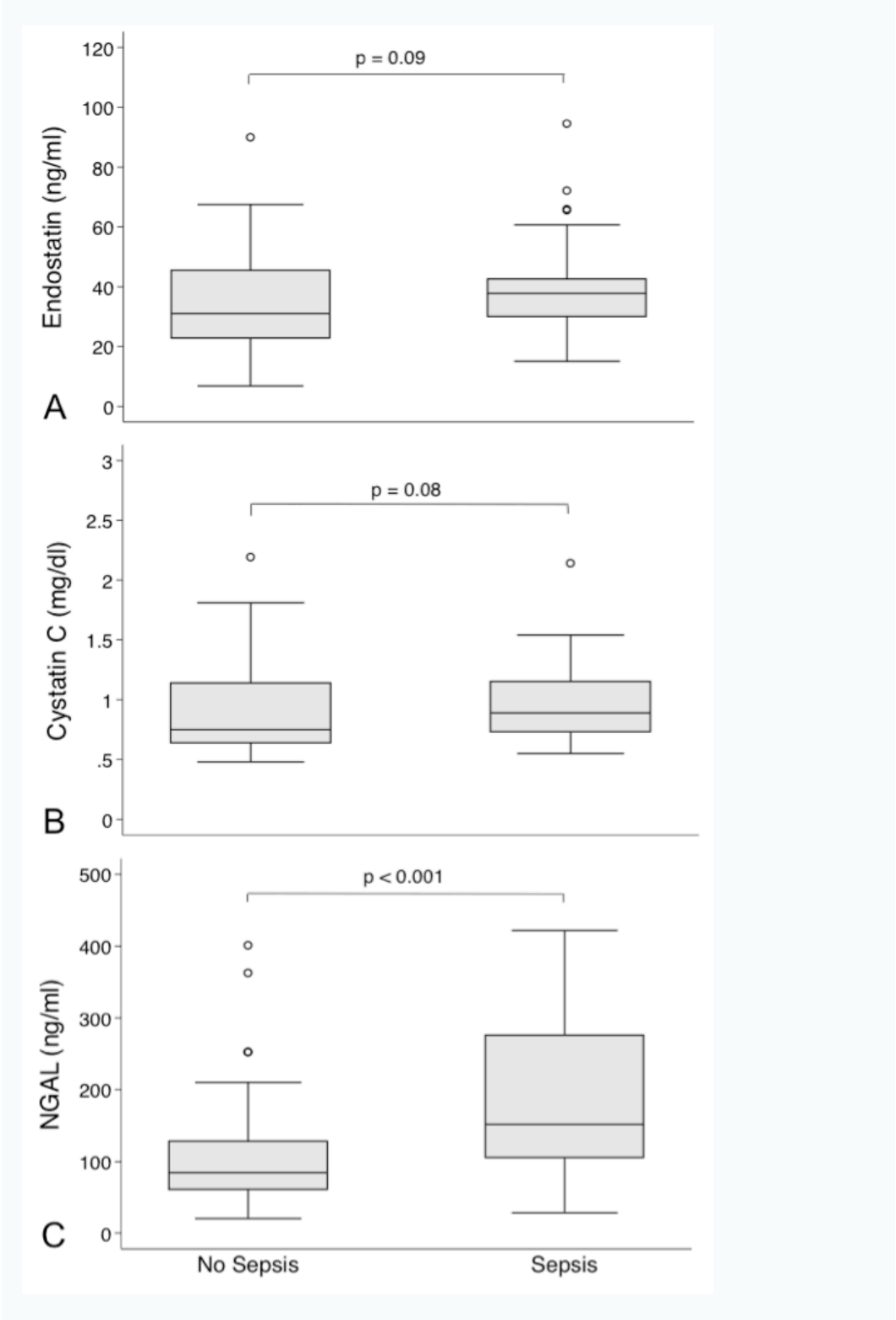

Figure 6. Plasma biomarker levels at inclusion for patients without and with sepsis. The Tukey boxplots show interquartile range (IQR) with the median highlighted as a horizontal line. The fences at the end of the whiskers are at 1.5 x the IQR from the median. The dots represent the outliers – the individual results above 1.5 x IQR from the median.

In the univariate analysis, age, APACHE II score and early oliguria were associated with development of AKI within 72 hours. We included these three variables to create a clinical risk prediction model. The model predicted AKI with a AUC ROC of 0.759 (95 % CI 0.646 – 0.872). Endostatin levels at admission predicted AKI with a AUC ROC of 0.726 (0.603 – 0.848). Adding endostatin to the prediction model, improved prediction further, demonstrated by an AUC ROC of 0.839 (0.752 – 0.925). Cystatin C and NGAL showed poor individual predictive values for AKI within 72 hours and adding Cystatin C or NGAL to the prediction model did not improve prediction (Figure 7). Since age is one of fourteen components of the APACHE II score, we performed a sensitivity analysis to exclude multicollinearity, removing age from the prediction model. In this analysis, AUC ROC for endostatin added to the prediction model, was 0.831 (0.741 – 0.922). Adding Cystatin C or NGAL to this prediction model did not improve prediction either.

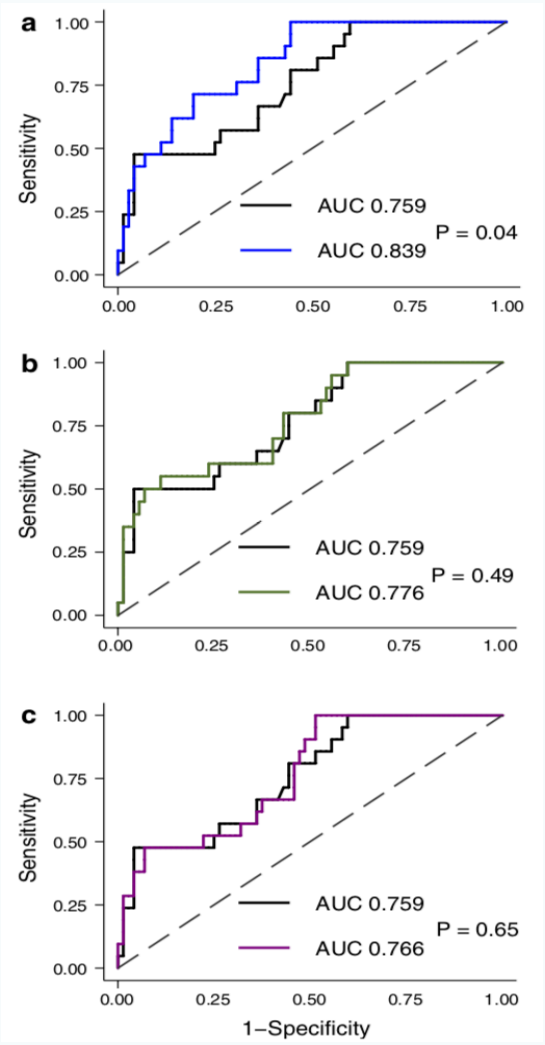

Figure 7. AUC ROCs for prediction of AKI within 72 h using the clinical model alone (black line in all graphs) and the clinical model together with a) endostatin (blue line); b) cystatin C (green line); c) NGAL (purple line) at study inclusion.

5.2 STUDY II

We enrolled 188 patients in the PEAK database. Altogether, 78 patients were excluded. Antibiotic therapy had been initiated before ICU admission for 42 patients and a study sample was missing before or on the day of antibiotic therapy initiation for 36 patients. Of the remaining 110 patients, 58 (52.7 %) had an infection during their ICU stay (infection group). Infection group were more likely to be male, had higher APACHE II score, higher SOFA score, were more likely to fulfill at least three SIRS criteria at admission, had higher baseline creatinine and stayed longer in the ICU. Patient characteristics are detailed in table 13.

Table 13. Characteristics of patients with no infection and possible, probable or confirmed infection. Values are median (interquartile range) or n (%).

Variable	No infection (n = 52)	Possible, probable or confirmed infection (n = 58)	P-value
Female	18 (35 %)	9 (16 %)	0.02
APACHE II score	13 (9, 19)	17 (13, 24)	0.001
SOFA score	5 (4, 8)	8 (6, 11)	< 0.0001
SIRS	36 (71 %)	51 (88 %)	0.02
Baseline creatinine*	78 (68, 88)	88 (76, 94)	0.01
Median ICU length of stay, days	3 (3, 5)	7 (4, 12)	< 0.0001

*When no preadmission creatinine was available, baseline creatinine was imputed based on the Modification of diet in renal disease (MDRD) equation and a GFR of 75 ml/min/1.73m^2.

In infection-group patients, antibiotic therapy was initiated after a median (IQR) of 2.6 (1, 4) days. Admission, peak and mean calprotectin levels were higher in the infection group than in the no-infection group and remained higher during the first week in the ICU (Figure 8). Peak calprotectin levels were higher in patients with SIRS than in patients without SIRS and increased significantly with increasing sepsis severity (Figure 9) Furthermore, calprotectin levels were higher at the time of antibiotic therapy initiation and on the day before, in the infection group, compared to no-infection group. Calprotectin levels are detailed in table 14.

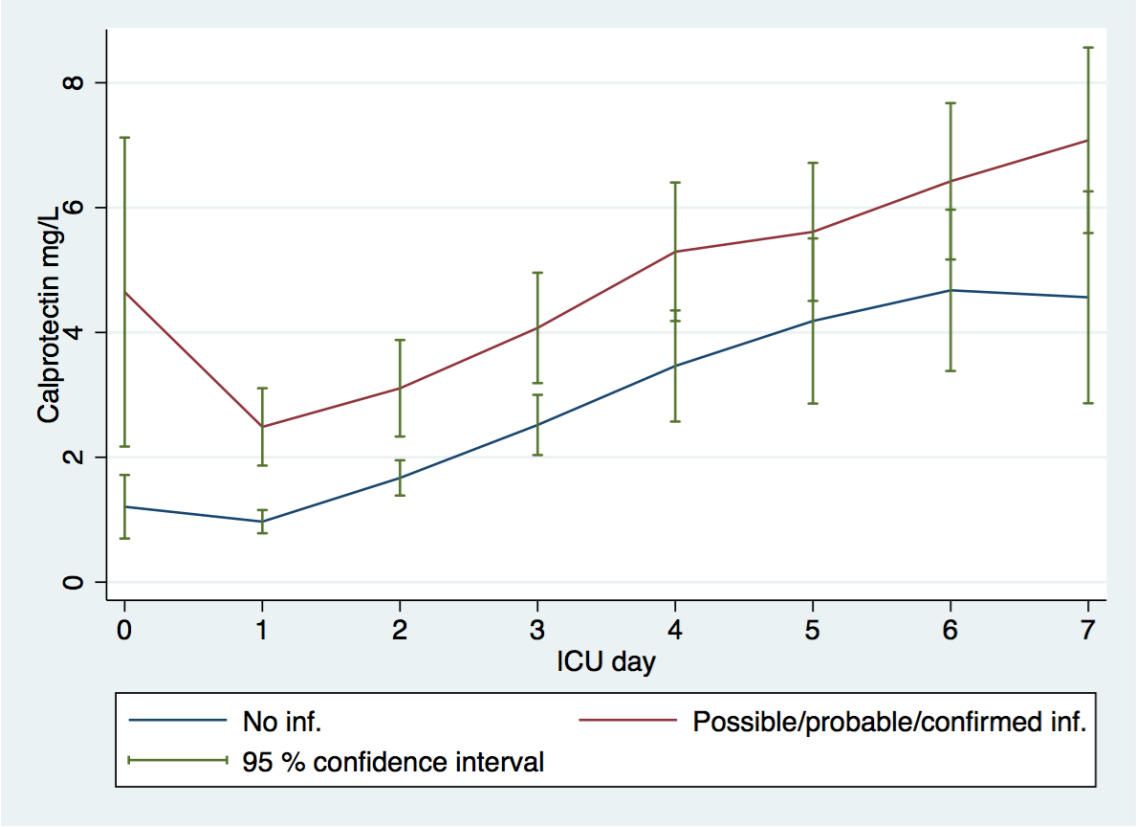

Figure 8. Daily mean calprotectin levels for infection group and no-infection group (CI 95 %).

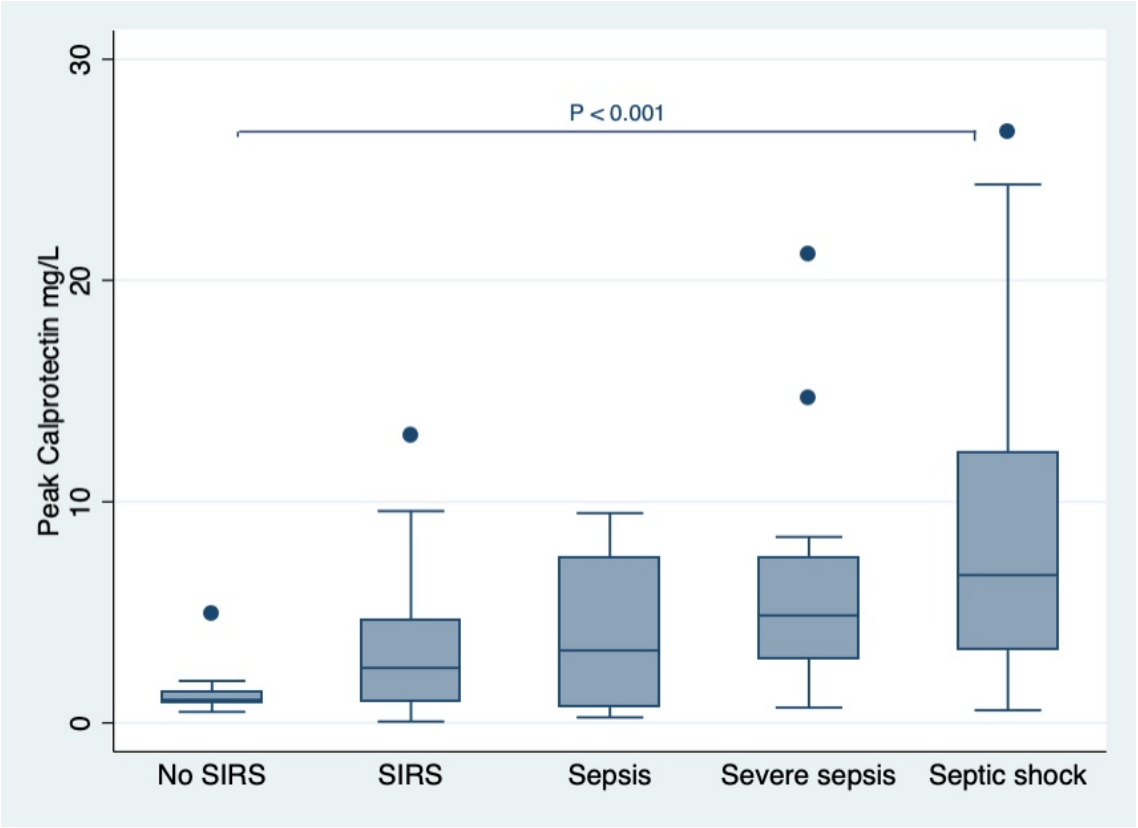

Figure 9. Peak calprotectin levels stratified with Sepsis-2 criteria.

Table 14. Calprotectin levels in infection group and no infection group.

Calprotectin, mg/L	**Infection**	**No infection**	**P-value**
Admission value	1.5 (0.69, 3.3)	0.78 (0.38, 1.6)	0.042
Peak value	5.0 (2.2, 8.4)	1.9 (1.0, 4.5)	< 0.001
Mean value	3.5 (1.5, 6.5)	1.6 (0.7, 3.6)	< 0.001
Day when antibiotic therapy was initiated	3.8 (1.4, 6.3)	1.3 (0.77, 2.3)	< 0.001
Day before initiation of antibiotic therapy	2.2 (0.82, 3.8)	1.1 (0.67, 2.0)	< 0.001

In the multivariable logistic regression analysis, calprotectin level on the day of antibiotic therapy was initiated was independently associated with infection, with an odds ratio of 2.0 (95 % CI, 1.32 – 3.14) for each mg/L increase. ROC-curve analysis of diagnostic accuracy of the biomarkers showed a higher AUC ROC for calprotectin than CRP, WBC and PCT. Predictive accuracy assessed with AUC ROC was also higher for calprotectin than the other biomarkers. AUC ROCs, optimal cut-offs, sensitivities and specificities are detailed in table 15.

Table 15. Diagnostic and predictive accuracies of studied biomarkers for infection.

Biomarker	AUC ROC (95 % CI)	Cut-off	Sensitivity	Specificity	P[a]
Diagnostic accuracy of biomarker (same day as antibiotic therapy started)					
Calprotectin	0.76 (0.65 – 0.86)	3.4 mg/L	56 %	92 %	-
CRP	0.69 (0.60 – 0.81)	133 mg/L	76 %	54 %	0.56
PCT	0.63 (0.49 – 0.77)	0.66 mg/L	70 %	58 %	0.30
WBC	0.54 (0.43 – 0.65)	10.7 x 10^9/L	43 %	74 %	0.01
Predictive accuracy of biomarker (One day before antibiotic therapy started)					
Calprotectin	0.78 (0.68 – 0.89)	1.80 mg/L	62 %	88 %	-
CRP	0.71 (0.68 – 0.89)	130 mg/L	62 %	94 %	0.41
PCT	0.50 (0.34 – 0.66)	0.78 mg/L	56 %	58 %	0.02
WBC	0.54 (0.43 – 0.65)	10.7 x 10^9/L	43 %	74 %	0.01

[a]P-value for the test of equality between the ROC area of each biomarker vs. calprotectin.

5.3 STUDY III

Of the 198 patients included in the study, 144 (72.7 %) were classified as being infected during their ICU stay (infection group). Infection-group patients were older, had higher APACHE II score, were more likely to have AKI the first ICU day and were more likely to have the comorbidities COPD/asthma and malignancy, compared to no infection-group patients. Infection-group patients stayed longer in the ICU, required more RRT and had worse AKI severity (KDIGO AKI stage). In the infection-group, 29 (20.1 %) patients had started antibiotic therapy before ICU admission. The remaining 115 (79.9 %) patients had antibiotic therapy initiated after a median of 2 days after admission to the ICU. According to the ISF criteria, 93 (64.6 %) patients had a confirmed infection, 30 (20.8 %) had a probable infection and 21 (14.6 %) had a possible infection. According to sepsis-2 criteria, 41 (28.5 %) patients developed severe sepsis and 82 (56.9 %) patients developed septic shock. By 30

days, 20 (14 %) patients in the infection group and 4 (7 %) patients in the no infection-group had died (P = 0.21). Patient characteristics are detailed in table 16.

Table 16. Characteristics of patients with and without infection. Values are median (interquartile range) or n (%).

Variable	Infection (n = 144)	No infection (n = 54)	P-value
Age	55 (36, 66)	38 (26, 64)	0.01
APACHE II score	17 (14, 23)	13 (9, 19)	0.001
AKI on first ICU day	34 (24 %)	6 (11 %)	0.05
Malignancy	31 (22 %)	3 (6 %)	0.008
COPD/asthma	24 (17 %)	2 (4 %)	0.016
ICU length of stay, days	7 (4.5, 12)	3 (2.8, 5)	< 0.0001
RRT	8 (6 %)	0	0.08
Worst AKI stage (KDIGO)			0.004
No AKI	77 (53 %)	43 (80 %)	
Stage 1	34 (24 %)	1 (2 %)	
Stage 2	21 (15 %)	3 (6 %)	
Stage 3	12 (8 %)	1 (2 %)	

ICU patients had higher dimeric NGAL and total NGAL than healthy controls (Figure 10). In healthy controls, the upper normal values (97.5th percentile) were 8.3 ng/ml for dimeric NGAL and 56 ng/ml for total NGAL.

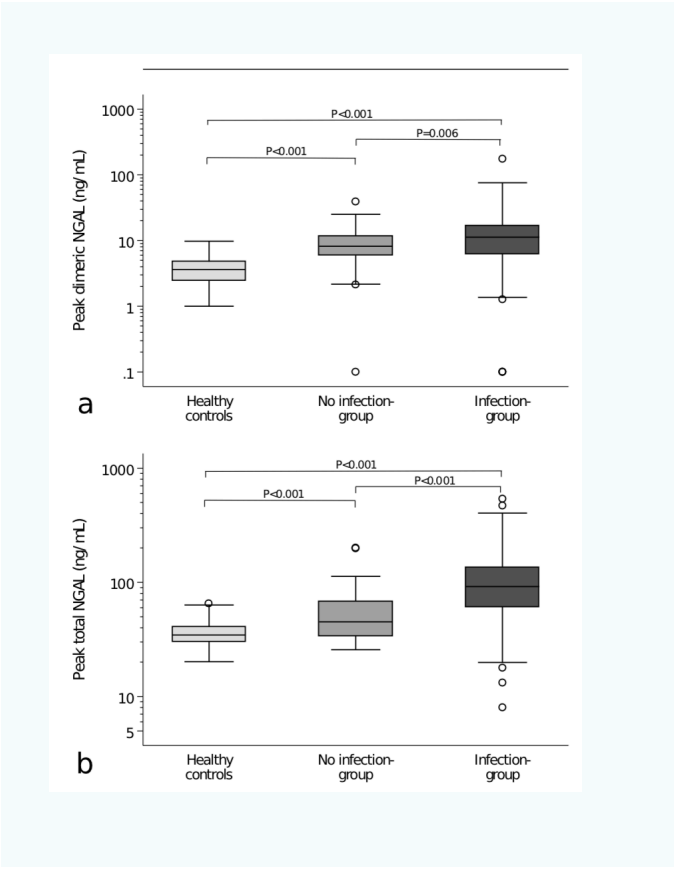

Figure 10. Peak levels of a) dimeric NGAL and b) total NGAL in healthy controls, no infection group-patients and infection-group patients.

Peak biomarker values for dimeric NGAL, total NGAL, CRP and PCT but not for WBC, were higher in infection-group patients compared to no infection-group patients. Likelihood of infection according to ISF criteria (no infection, possible, probable or confirmed infection) was also associated with higher dimeric NGAL, total NGAL, CRP and PCT but not WBC (Figure 11). Worse sepsis severity was consistently associated with higher values in all biomarkers (Figure 12).

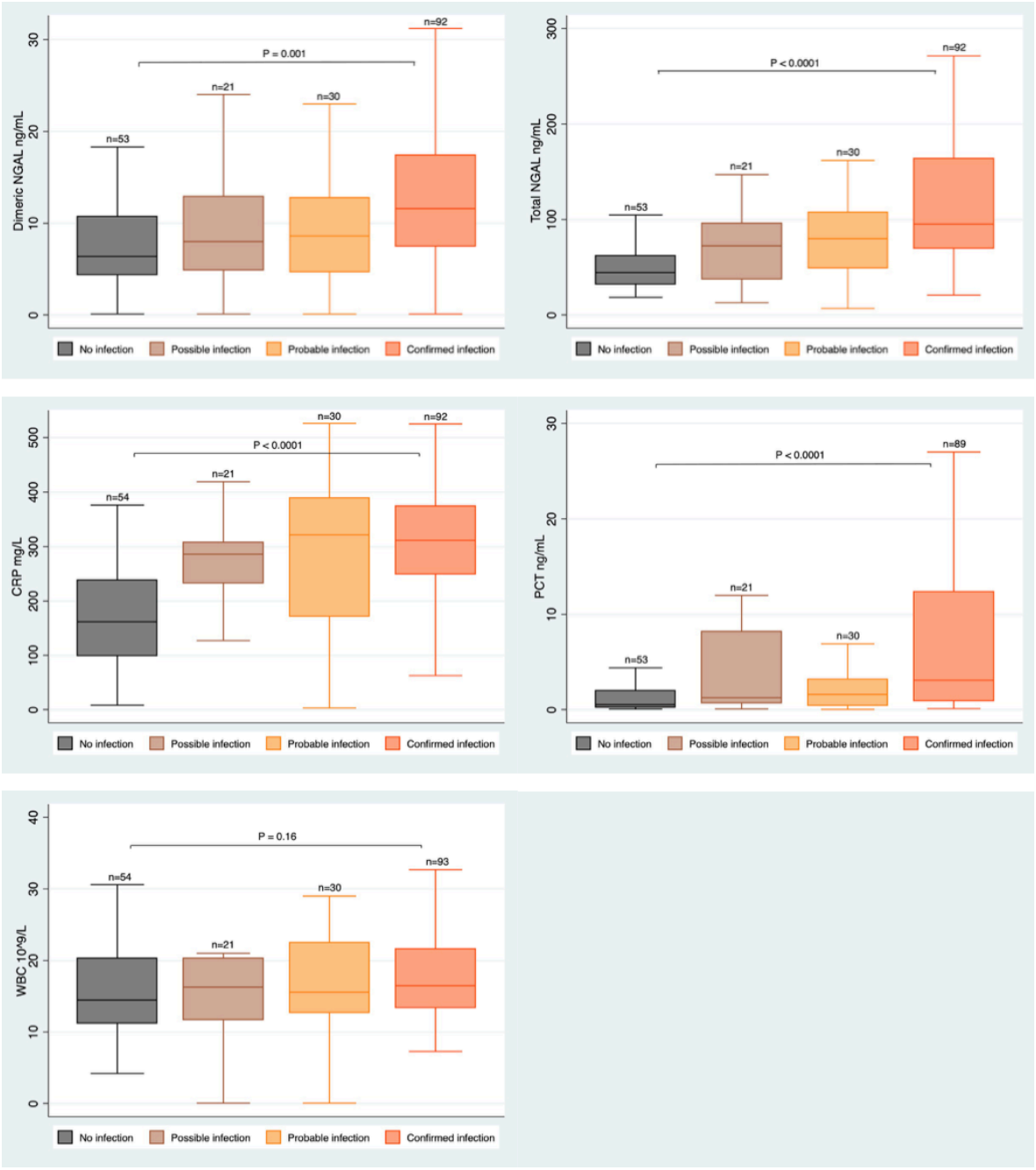

Figure 11. Biomarker values for patients depending on likelihood of infection according to ISF criteria.

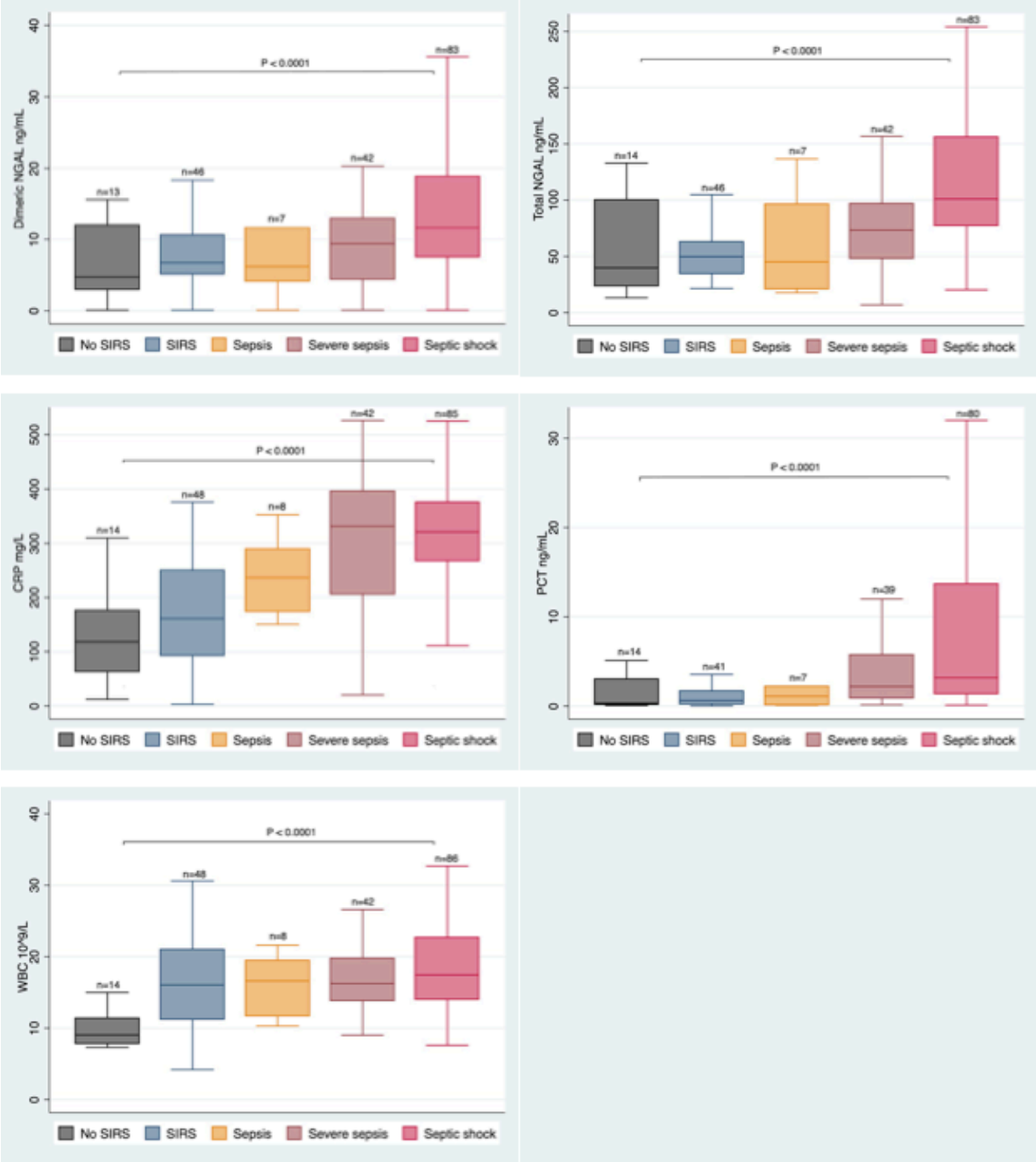

Figure 12. Biomarker values for patients depending on severity of infection according to modified sepsis-2 criteria (modifications are detailed in tables 1&2).

Biomarker kinetics on the day before appropriate antibiotic treatment was initiated (day -1 in figure 13) showed similar dimeric NGAL, PCT and WBC levels in infection-group and no infection group patients. Conversely, total NGAL and CRP levels were greater in infection group-patients and remained higher during the subsequent five days in ICU. Dimeric NGAL and PCT declined early in the no infection-group patients, whereas in infection-group patients, dimeric NGAL and PCT increased until or just after antibiotic therapy initiation and declined markedly thereafter (Figure 13). In a subgroup of patients with confirmed infection, the mean (95 % CI) change of dimeric NGAL in the first 2 days after antibiotic therapy initiation was -31 (-49, -13) %. Dimeric NGAL declined faster than PCT did (Figure 14).

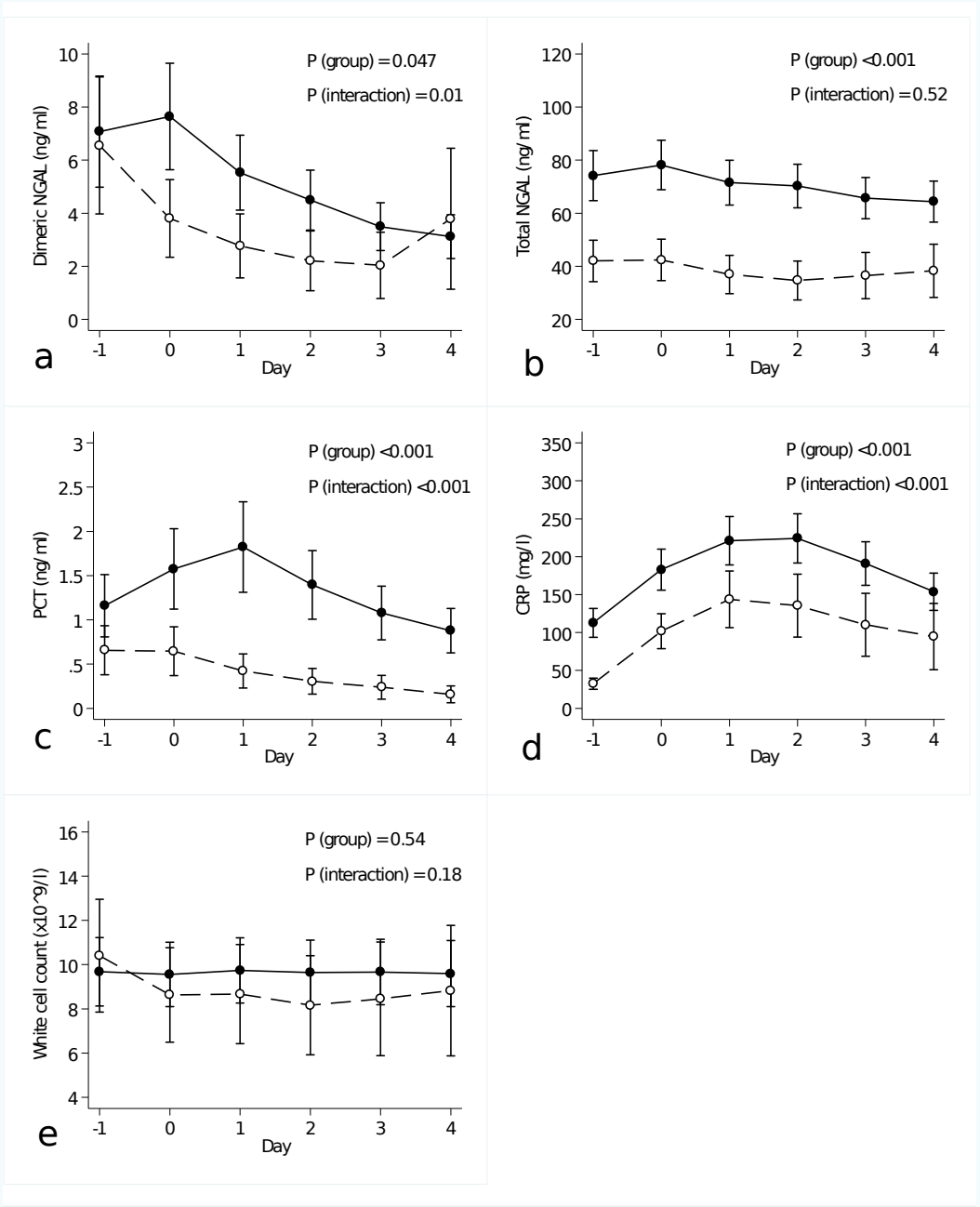

Figure 13. Biomarker kinetics relative to antibiotic treatment showed as geometric means with 95 % CI. Biomarker levels obtained on ICU day 3 represent day 0 in no infection group-patients. P-values for differences in biomarker levels between infection-group and no infection-group patients and for interaction between group and time, respectively.

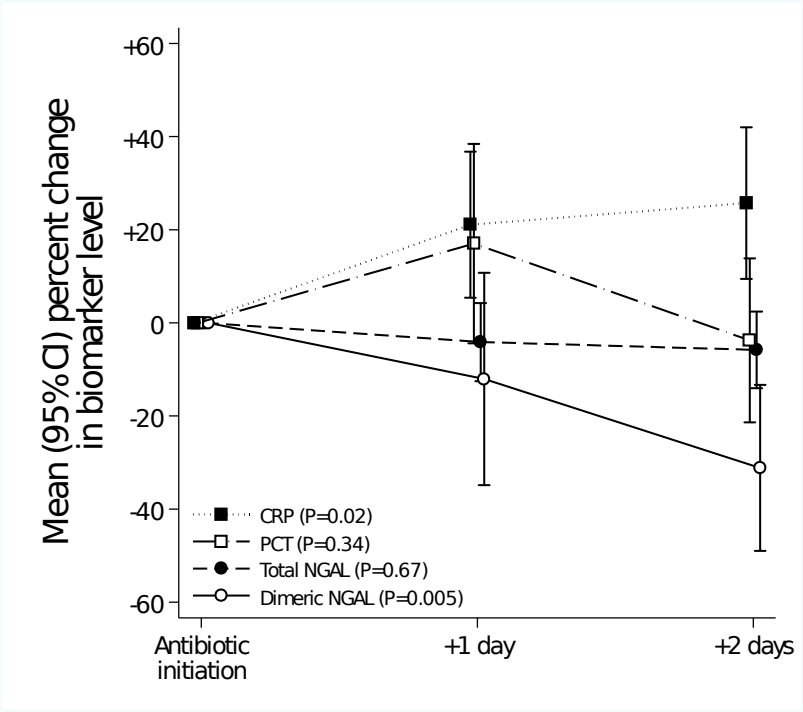

Figure 14. Percent change in biomarker levels after antibiotic therapy initiation in 31 patients with a confirmed bacterial infection. P-values were derived from the Wilcoxon matched-pairs signed-ranks test of equality between biomarker values on day of antibiotic initiation and 2 days later.

Multivariable linear regression analysis showed that infection was independently associated with 90% (95% CI 15-215%) higher dimeric NGAL and a 106 % (95 % CI 61 - 164 %) higher CRP than absence of infection. Manifest AKI was independently associated with a 35 % (95 % CI 0.6 - 81 %) higher total NGAL. We found no independent association between AKI and dimeric NGAL, PCT, CRP or WBC.

Diagnostic and predictive accuracies, assessed with ROC-curve analyses, were fair or poor for all the tested biomarkers. CRP showed the best combined diagnostic and predictive abilities of the studied biomarkers in this analysis (Table 17).

Table 17. Diagnostic and predictive accuracy for infection on the day of antibiotic therapy initiation and one day before antibiotic therapy initiation, respectively. Optimal cut-off, assessed with Youden index for the biomarkers together with corresponding sensitivity and specificity.

Biomarker	**AUC ROC (95 % CI)**	**Cut-off**	**Sensitivity (95% CI)**	**Specificity (95% CI)**
Diagnostic accuracy (value at antibiotic therapy initiation). n = 70 infection-group, 40 no infection-group.				
Dimeric NGAL	0.70 (0.60-0.79)	6.6 ng/ml	0.66 (0.53-0.77)	0.60 (0.43-0.75)
Total NGAL	0.77 (0.69-0.86)	47 ng/ml	0.76 (0.64-0.85)	0.68 (0.51-0.81)
PCT	0.63 (0.53-0.74)	0.85 ng/ml	0.63 (0.51-0.74)	0.65 (0.48-0.79)
CRP	0.74 (0.64-0.83)	170 mg/l	0.61 (0.49-0.73)	0.78 (0.62-0.89)
WBC	0.62 (0.51-0.73)	8.5 x10^9/l	0.67 (0.54-0.78)	0.58 (0.41-0.75)
Diagnostic accuracy (% increase from previous value). n = 43 infection-group, 24 no infection-group.				
Dimeric NGAL	0.59 (0.45-0.74)	4 %	0.42 (0.27-0.58)	0.75 (0.53-0.90)
Total NGAL	0.52 (0.38-0.66)	13 %	0.47 (0.31-0.62)	0.67 (0.45-0.84)
PCT	0.58 (0.42-0.74)	6 %	0.49 (0.33-0.65)	0.63 (0.41-0.81)
CRP	0.33 (0.18-0.47)	50 %	0.37 (0.23-0.53)	0.29(0.13-0.51)
WBC	0.45 (0.30-0.61)	2 %	0.44 (0.29-0.60)	0.55 (0.32-0.76)
Predictive accuracy (one day before antibiotic therapy initiation). n = 46 infection-group, 35 no infection-group.				
Dimeric NGAL	0.54 (0.41-0.66)	5.3 ng/ml	0.78 (0.64-0.89)	0.31 (0.17-0.49)
Total NGAL	0.64 (0.52-0.76)	43 ng/ml	0.78 (0.64-0.89)	0.51 (0.34-0.69)
PCT	0.52 (0.39-0.65)	0.75 ng/ml	0.54 (0.39-0.69)	0.60 (0.42-0.76)
CRP	0.79 (0.69-0.89)	105 mg/l	0.63 (0.48-0.77)	0.89 (0.73-0.97)
WBC	0.52 (0.38-0.65)	8.5 x10^9/l	0.68 (0.52-0.81)	0.50 (0.32-0.68)

5.4 STUDY IV

A total of 135 patients with AKI, who required acute RRT, were studied. Of these, 98 (73 %) were alive and free from RRT at 60 days (recovery group) and 37 patients did not recover (non-recovery group). In the non-recovery group, 16 patients (43 %) died in the ICU and 36 patients (97 %) died within 60 days. Hence, only one patient was alive and not free from RRT at 60 days. Median (IQR) time from ICU admission to death was 17 (6, 28) days. Recovery group patients were younger and were more likely to have diabetes and to be admitted due to sepsis and trauma, compared to the non-recovery group patients. In contrast, the proportion of admissions due to cardiovascular illness was greater in the non-recovery group. Non-recovery group patients had a greater incidence of at least one episode of anuria the during the ICU stay, compared to recovery-group patients. Recovery group patients stayed longer in the ICU, compared to non-recovery patients. Patient characteristics are detailed in table 18.

Table 18. Characteristics of patients with and without renal recovery at 60 days. Values are median (interquartile range) or n (%).

Characteristics	**Recovery (n=98)**	**Non-recovery (n=37)**	**P-value**
Age (years)	63 (53, 70)	70 (64, 78)	0.0008
Malignancy	15 (15)	10 (27)	0.12
Diabetes	25 (26)	2 (5)	0.009
Days in ICU before RRT	1.6 (1.0, 2.4)	1.2 (0.9, 2.1)	0.20
APACHE II score	23 (18, 27)	25 (20, 31)	0.15
Daily urine output (ml)	1111 (220, 2556)	305 (50, 1605)	0.16
Anuria ≥ 1 day	23 (23)	16 (42)	0.02
Length of ICU stay (days)	11 (6, 17)	8 (4, 14)	0.08

Analyses of urinary biomarkers immediately before start of RRT showed significantly higher endostatin levels in the recovery group, than in the non-recovery group. On RRT day three, urine creatinine and urea were significantly higher in the recovery group. On RRT days six and seven, plasma urea was significantly higher in recovery group patients. No other significant differences were seen in urine or plasma biomarker levels.

However, we did observe a trend towards higher biomarker levels, starting around RRT day two and onwards, in renal recovery patients compared to non-recovery patients. Biomarker levels on RRT day 7 are shown in figure 15). Only urine and plasma NGAL were excepted from this (not significant) trend. Trend of endostatin levels are shown in figure 16.

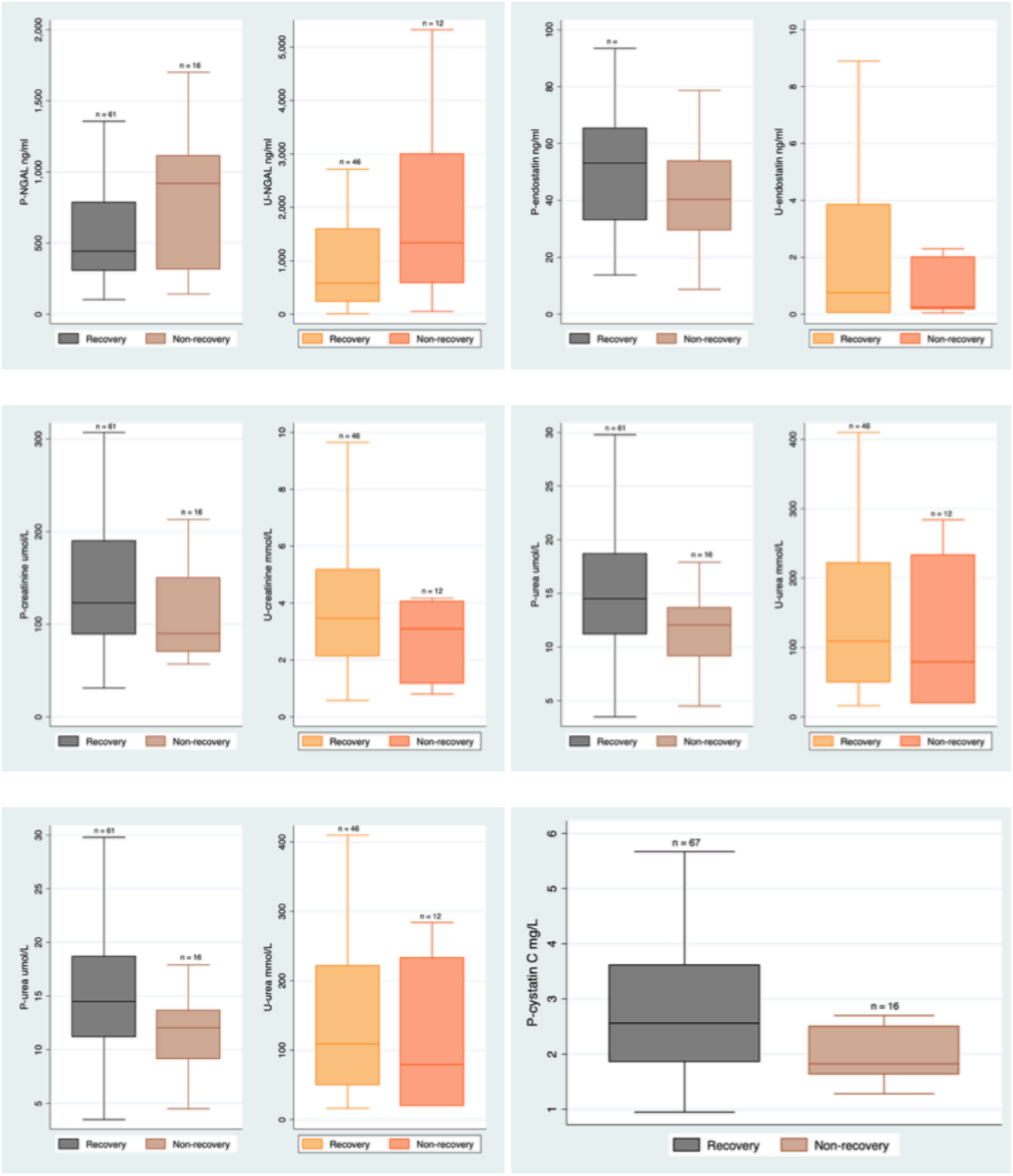

Figure 15. Boxplots with biomarker levels on RRT day 7 for patients with and without renal recovery. Interquartile range (IQR 25-75) with the median highlighted as a horizontal line. The fences at the end of the whiskers are at 1.5 x the IQR from the median.

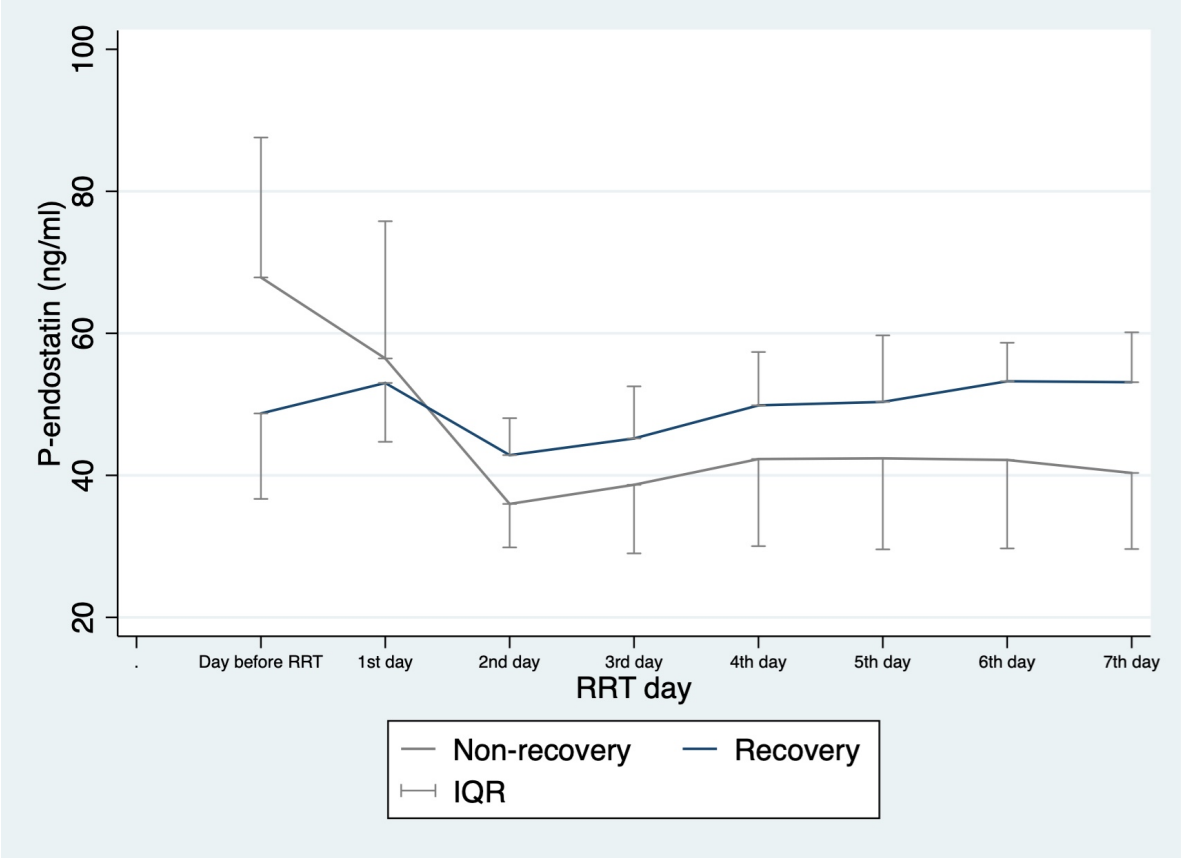

Figure 16. Endostatin expression in patients with and without renal recovery.

Accuracy of prediction of renal recovery with individual plasma and urine biomarkers, assessed with ROC-curve analyses immediately before RRT and on days one to seven of RRT, were poor. Best predictive accuracy was observed with plasma urea on day 7 of RRT (AUC ROC 0.69, 95 % CI 0.55, 0.83).

Daily urine output was significantly higher on all observed days in the recovery group, except on the day before start of RRT (Figure 17).

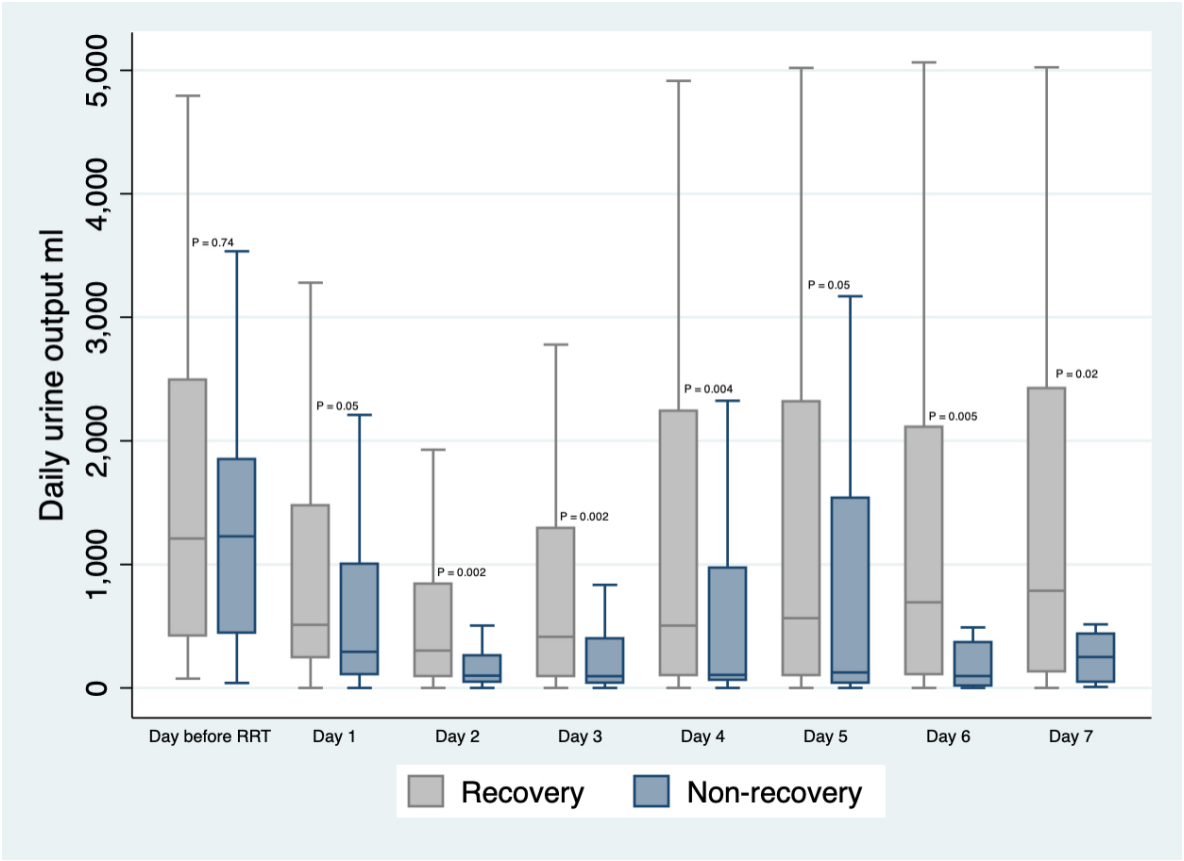

Figure 17. Daily urine output (ml) for patients with and without renal recovery. Interquartile range and IQR with the median highlighted as a horizontal line. The fences at the end of the whiskers are at 1.5 x the IQR from the median. P-values were derived from the Wilcoxon matched-pairs signed-ranks test of equality between biomarker values between recovery and non-recovery group.

However, the predictive accuracy of daily urine output, assessed with ROC-curve analysis was moderate. The best predictive accuracy was observed on day 6 of RRT (AUC ROC 0.72, 95 % CI 0.58, 0.86). The predictive value of combining clinical variables was best using the patients age together with urine output on day 7 of RRT (AUC ROC 0.84, 95 % CI 0.73, 0.96). Combining clinical variables and biomarkers to predict renal recovery did not improve prediction significantly (table 19, 20).

Table 19. Prediction of renal recovery before start of RRT.

	n	Variable alone AUC ROC (95 % CI)	Clinical model alone AUC ROC (95 % CI)	Variable & clinical model AUC ROC (95 % CI)	p
P-NGAL	75	0.63 (0.45, 0.80)	0.56 (0.42, 0.71)	0.64 (0.47, 0.81)	0.35
P-endostatin	75	0.44 (0.29, 0.59)	0.56 (0.42, 0.71)	0.59 (0.44, 0.73)	0.48
P-creatinine	75	0.57 (0.42, 0.72)	0.56 (0.42, 0.71)	0.60 (0.45, 0.76)	0.43
P-urea	75	0.53 (0.37, 0.70)	0.56 (0.42, 0.71)	0.62 (0.46, 0.78)	0.21
P-Cystatin C	72	0.43 (0.26, 0.60)	0.54 (0.40, 0.70)	0.59 (0.44, 0.74)	0.44
U-NGAL	60	0.50 (0.32, 0.68)	0.54 (0.39, 0.69)	0.55 (0.39, 0.71)	0.81
U-endostatin	60	0.54 (0.39, 0.70)	0.54 (0.39, 0.69)	0.69 (0.55, 0.84)	0.13
U-creatinine	60	0.42 (0.26, 0.59)	0.54 (0.39, 0.69)	0.60 (0.45, 0.75)	0.47
U-urea	60	0.47 (0.30, 0.64)	0.54 (0.39, 0.69)	0.58 (0.41, 0.75)	0.60

Table 20. Prediction of renal recovery from the seventh day of RRT.

Biomarker	n	Variable alone AUC ROC (95 % CI)	Clinical model alone AUC ROC (95 % CI)	Variable & clinical model AUC ROC (95 % CI)	p
P-NGAL	77	0.39 (0.18, 0.59)	0.84 (0.73, 0.96)	0.84 (0.73, 0.96)	0.88
P-endostatin	77	0.57 (0.40, 0.75)	0.84 (0.73, 0.96)	0.85 (0.74, 0.96)	0.31
P-creatinine	77	0.68 (0.52, 0.83)	0.84 (0.73, 0.96)	0.86 (0.75, 0.96)	0.44
P-urea	77	0.69 (0.55, 0.83)	0.84 (0.73, 0.96)	0.86 (0.74, 0.97)	0.31
P-Cystatin C	83	0.65 (0.49, 0.80)	0.84 (0.73, 0.95)	0.84 (0.73, 0.95)	0.88
U-NGAL	58	0.35 (0.16, 0.52)	0.85 (0.72, 0.99)	0.86 (0.72, 0.99)	0.53
U-endostatin	58	0.54 (0.36, 0.71)	0.85 (0.72, 0.99)	0.87 (0.75, 0.99)	0.07
U-creatinine	58	0.63 (0.46, 0.79)	0.85 (0.72, 0.99)	0.86 (0.75, 0.98)	0.53
U-urea	58	0.57 (0.35, 0.80)	0.85 (0.72, 0.99)	0.86 (0.73, 0.99)	0.57

6 DISCUSSION

6.1 METHODOLOGICAL CONSIDERATIONS

Generalizability

All four studies were single center studies, enrolling patients from an ICU with a relatively high rate of admissions following multi-trauma. The trauma-patients admitted to the Karolinska ICU are likely to be younger and have less comorbidities than other ICU populations reported in the critical care literature. In addition, studies I-III excluded patients with renal dysfunction on admission, excluding some of the sickest patients (this was done to be able to study the progress of de novo AKI in the ICU). Altogether, this reduces the generalizability of our results to other ICU populations (who may be older, have more comorbidities and have AKI before ICU admission).

In studies I and IV, we developed prediction models using data from the study populations. After developing a prediction model in one population, external validation in another population is needed to confirm the model's generalizability. None of the models have been externally validated - hence the results should be looked upon as hypothesis generating. In study III (PEAK database) the mean age of the study population was 52 years and in study IV (EXCRETe database) the mean age of the study population was 62 years. The mean age for all patients admitted to the Karolinska ICU during the same time period was 53 years. The mean age for patients with AKI (not necessarily treated with RRT) in all of Sweden's ICUs during the same period was 70 years (49). Because patients in both the PEAK and EXCRETe database were younger than the typical ICU population the results and conclusions should primarily be looked upon as hypothesis generating and only with due caution inferred to other populations.

Misclassification of infection

The perfect classification of a disease or condition without a gold standard test is not possible - which is the case with infection in the ICU. We know from previous studies of blood cultures in patients with high pre-test probability of infection, that as many as four blood culture sets over a 24-h period may be needed for a 99 % test sensitivity (58, 59). We believed that the closest thing to a gold standard would be a combination of clinical criteria and microbiological cultures. An infectious disease specialist (blinded to the study biomarker results) classified patients retrospectively, according to ISF. This method was employed to reduce the risk of misclassification. The sensitivity analyses performed in study II (after removing patients with a lower likelihood of infection) could suggest that the risk of misclassification having was low (i.e. if misclassification had occurred, omitting the patients with lower likelihood of infection should affect the result). The definition of sepsis has changed over the last decade but we did not change the way of classification in studies I-IV: patients were classified as having sepsis, severe sepsis or septic shock according to Sepsis-2 criteria if three or more SIRS criteria were fulfilled (together with suspected or confirmed

infection). The use of three or more SIRS criteria (instead of two or more) was proposed by the Protein C Worldwide Evaluation in Severe Sepsis (PROWESS) study group (54) to increase the specificity of the sepsis definition and prevent patients with tachypnea and tachycardia from being identified as having sepsis. The rationale of doing the same in studies I-IV was to increase the specificity for a systemic response to infection, as opposed to risking inclusion of patients with physiological responses to pain, hypovolemia, and other stressors not necessarily related to infection. The definition of onset of infection may also introduce misclassification. Due to the study design (and the nature of the sepsis syndrome) there was no way of deducing the true onset of infection. For some patients, infection onset could be attributed to a traumatic perforation of the colon, but for most patients we could not know the true onset of infection. To deal with this, we chose a pragmatic approach - onset of infection was set to the time when the clinician (at her own discretion) started antibiotic treatment. By reading the patients electronic health record we could identify when antibiotic prophylaxis was initiated and avoid that such initiation was labeled infection onset.

Misclassification of AKI

When we started to enroll patients and collect data to the PEAK database in 2007, AKI was defined according to the RIFLE criteria. The AKIN (Acute Kidney Injury Network) criteria were introduced in 2008 and finally, the KDIGO criteria were introduced in 2012. The transition from RIFLE to AKIN to KDIGO has been shown to affect incidence, timing and outcome of AKI (60). The transition was not a significant methodological problem in studies I-IV as we, during all the years, had gathered sufficient data to reclassify patients according to the KDIGO AKI criteria in the analysis phase of each study. All three definitions employ changes is plasma creatinine and/or urine output as markers of kidney function (i.e. GFR). Increased creatinine or decreased urine output can be physiologically adequate in patients with hypovolemia/hypotension. Contrariwise, damage to the kidney may pass undetected by changes in creatinine and urine output - it may not confer any signs or symtoms at all.

The PEAK database only enrolled patients with eGFR > 60 ml/min/1.73 m^2 in order to exclude patients with known kidney disease. However, when baseline creatinine was unavailable it was estimated with the MDRD formula. MDRD estimation of baseline creatinine in patients with undiagnosed CKD may cause a misclassification of them as having AKI, when they in fact do not. The MDRD formula has a known tendency to overestimate the baseline creatinine level, hence risking a misclassification of patients with AKI as not having AKI. Even patients with normal creatinine may have reduced GFR. One study found that 25 % of patients admitted to the ICU with normal creatinine levels, had urinary creatinine clearance < 60 ml/min/1.73 m^2 (61). Furthermore, the KDIGO AKI criteria classify patients as having AKI or not based on kidney *function* (i.e. urine output and need for RRT) and creatinine - a biomarker of kidney *function*. One might speculate that there could be patients in the cohort that had suffered kidney parenchymal injury that did not affect kidney function enough to classify as AKI - but that the kidney injury per se was a negative predictor of outcome (52). Such patients (if they existed in our cohorts) would have been classified as

not having AKI and subsequently reduced the difference in outcome between patients classified as non-AKI and AKI (62). Another limitation of the current AKI classification is timing - it does not tell us when the AKI starts. Elevated creatinine levels appear only after significant loss of GFR, lagging behind significantly (63).

Confounding and bias

Confounders can be defined as factors related - but without causal relation - to both the exposure and the outcome. They can be dealt with in study design by randomization, restriction or matching.

Studies I-IV were designed as prospective cohort studies. Therefore, confounding was dealt with in the analysis of data (stratification of data and multivariable adjustments in regression analyses). Cohort studies are susceptible to selection bias. Inclusions for PEAK and EXCRETe were ongoing from 2007 - 2015 and 2008 - 2016, respectively and Karolinska ICU treats around 900 patients per year. The low number of inclusions may have introduced a selection bias. Inclusion of new patients and collection of blood and urine samples were at times restricted to week-days, which may have introduced a selection of certain patients and exclusion of others - as well as a surveillance bias during week-ends i.e. samples not collected properly or not at all during week-ends due to staffing issues. However, missing biomarker data (from week-ends and altogether) was similar for all patients, independent of outcome in the particular study - hence making surveillance bias less likely. Misclassification bias is covered in previous sections.

Random error

With a predefined significance level set to 0.05 we have chosen to accept a 5 % risk of type I error - seeing a difference when there is no difference. The "one in ten" rule of thumb in regression analysis is not to use more than one explaining variables per ten patients with the outcome, as it may cause overfitting (and increase the risk of type I error). To avoid overfitting we pre-selected explaining variables in univariate analyses before running the multivariable analyses. Since sample size in all the studies was relatively small all had an inherent risk of type II error - not seeing a difference even when there was one.

6.2 INTERPRETATION OF FINDINGS

Infection in the ICU

As the phenotype of a sterile inflammatory process may be so close to the inflammatory processes in infection, the decision to prescribe or not to prescribe antibiotic therapy in the ICU is demanding (7). The similarity may be understood at a molecular level, as the same innate pattern recognition receptors and pathways are employed in both conditions (64, 65). Furthermore, recent studies have described the resemblance in gene transcription patterns between various conditions that we encounter in the ICU (e.g. severe trauma, burns, bacterial endotoxemia) (26, 27). There seems to be a one-size-fits-all basic human response to severe inflammatory stress - and it is in this mix we are trying to differentiate the various conditions from one another. Consequently, if the studied biomarker is expressed during the course of systemic inflammation and not only in relation to infection, we may not get a useful diagnostic or predictive accuracy for infection.

The results from study II, assessing calprotectin as an early biomarker of infection in the ICU, suggest that it is useful to predict and diagnose infection. It predicted infection with the same accuracy as CRP and better than PCT and WBC and it had equal diagnostic accuracy to CRP and PCT and better than WBC. The study was limited by the inherent risk of misclassification - the outcome (infection) does not have a gold standard test. A total of 81 % of patients classified as the infection-group had a positive microbial culture. Similarly, in EPIC II, the rate of culture confirmed infection in Western Europe was 83 % (21). According to the ISF criteria, 65 % of patients in the infection-group in study II had a "confirmed infection", i.e. not only a positive uncontaminated culture but also typical clinical, radiological, surgical or laboratory findings. Sensitivity analysis - excluding patients who had a suspected infection but no confirmed culture - did not change the results, indicating that our classification was reasonably good. Whether or not calprotectin guided diagnosis improves patient outcome remains to be studied.

In study III, we compared ICU patients with and without infection, measuring both total NGAL and, for the first time in this setting dimeric NGAL - a protein released mainly from activated neutrophils. We also compared dimeric NGAL levels in healthy controls to levels in ICU patients. In the studied cohorts, we found that ICU patients had higher plasma dimeric NGAL levels than healthy controls.

Further, infection was independently associated with increased levels of dimeric NGAL and total NGAL. AKI was with independently associated with higher levels of total NGAL, but not with dimeric NGAL. This finding supports the hypothesis that monomeric NGAL (and not dimeric NGAL) is expressed in AKI. This is also supported by previous studies (36, 66-68). The catching and detecting antibodies (p763/p764) in the ELISA used to quantify total NGAL bind both monomeric and dimeric NGAL and the catching and detecting antibodies (p763/p765) in the dimeric NGAL ELISA bind mainly to the dimeric form (36). Unfortunately, it is not possible to calculate the amount of monomeric NGAL using results

from the two ELISAs together. This is because the antibody combination p763/p764 binds to monomeric and dimeric NGAL in a relation of ~3:1 and the p763/p765 combination binds to monomeric and dimeric NGAL in a relation of ~1:240 (36).

Despite appearing to be unique for neutrophils, the ability of dimeric NGAL to diagnose and predict infection, was poor. The reason for this may have several reasons: 1) the assay is not showing the true concentration, 2) NGAL is eliminated fast from the plasma and our tests miss the peak concentrations, 3) patient comorbidities also affect the expression of dimeric NGAL and dilute the differences between infection group patients and non-infection group patients, 4) systemic inflammation also affects the expression of dimeric NGAL and dilutes the differences between groups.

We also studied plasma levels of dimeric NGAL and total NGAL following appropriate antibiotic therapy initiation and found that dimeric NGAL decreased more rapidly than any of the other biomarkers, including PCT. The most prominent difference between dimeric NGAL and PCT, following appropriate antibiotic therapy initiation in study III, was that peak dimeric NGAL levels were observed on the same day as antibiotic therapy was started - whereas PCT levels peaked on the day after antibiotic therapy was started. Similar PCT kinetics have been described in similar studies (69, 70), and may suggest an advantage for dimeric NGAL over PCT. PCT has been thoroughly studied in this regard (71-73), thus supporting further assessment of dimeric NGAL-guided antibiotic therapy de-escalation.

Looking back on the studies focusing on infection (studies II, III) we regret not having collected serum instead of plasma. Serum may have some advantages over plasma - this requires an short background: Centrifugation of anti-coagulated whole blood isolates the plasma from the blood cells. Biomarker concentration in the supernatant plasma is believed to represent the concentration of biomarkers in vivo. Serum is the liquid fraction of whole blood that can be collected after coagulation. The temperature and time a sample of whole blood is allowed to coagulate will affect the continued (ex vivo) release of a cytosolic or granulae protein from the neutrophil. A neutrophil that has been activated in vivo will release proteins more than a neutrophil that has not (i.e. an activated neutrophil continues it's mission ex vivo). Whole blood with non-activated neutrophils will therefore produce serum with the same biomarker concentration as plasma from the same blood sample. To conclude: Serum-measurement of cytosolic or granulae proteins can amplify concentrations (compared to a plasma measurement from the same patient), because activated neutrophils continue releasing proteins ex vivo). Traditionally, collecting serum requires the test tubes of whole blood to sit in room temperature for 15-30 minutes to allow clotting. However, recent studies have demonstrated a method to reduce the separation of serum to 10 minutes (74, 75).

AKI in the ICU and renal recovery

The pathophysiology of AKI is not well understood. In the prevailing paradigm of organ dysfunction it is assumed that kidney dysfunction is preceded by kidney damage of some kind. It is likely that AKI of different aetiology have unique pathophysiology and potential treatments (76). There is evidence that the renal microcirculation has a central role in AKI-development, independent of aetiology (43). It is therefore interesting that we have been able to show that a biomarker that is closely associated with the renal microcirculation - endostatin - indeed increased prediction of AKI, when used together with a clinical prediction model. A study similar to study I - predicting AKI with endostatin and a clinical risk model - was performed on the FinnAKI cohort (1112 patients), showing no benefit of adding endostatin levels to the clinical prediction model (which included SAPS II, urine output and age) (77). The difference may be caused by differences in onset of AKI in relation timing of the study sample or the difference in predictor variables of the clinical prediction model or in differences in the populations - a majority of patients in study I were trauma-patients. Another explanation may lie in the lack of gold standard test for AKI. Endostatin is assumed to reflect kidney matrix damage and the AKI definition is based on kidney function. Of interest in the FinnAKI endostatin study is that endostatin showed better AUC ROC for RRT than for AKI. The similarity between the studies and the fact that the FinnAKI cohort was > 10 times larger than the study I cohort, suggests a type I error in study I. Another endostatin-study employed the same study design, using plasma endostatin and a clinical risk model - this time to predict renal recovery within 7 days (defined as creatinine > 150 % above baseline creatinine or RRT dependence). Plasma endostatin together with a clinical prediction model predicted renal recovery within 7 days with AUC ROC of 0.89.

In study IV we showed a trend of higher plasma and urine endostatin levels in patients who would subsequently survive, compared to those who did not survive or survived with remaining need for RRT (only one patient). One might speculate that endostatin in a later phase of AKI has a function as a reparative protein or that the increased expression is a result of ongoing rebuilding of damaged endothelial matrix. Endostatin has been shown to regulate interactions between endothelial cells and the underlying basement membrane (78).

Study IV also showed a trend with higher plasma and urinary creatinine, urea and cystatin C values in patients with subsequent renal recovery compared to non-recovery patients, i.e. all the biomarkers except plasma and urinary NGAL. A known and common cause of decreased plasma creatinine is volume overload. Volume overload has been associated with increased mortality in previous AKI-studies (79-81). There is also speculation that volume overload and increased central venous pressures could be a cause of renal congestion, resulting in decreased perfusion pressure in the kidneys (82-84). Low plasma creatinine has been associated with muscle wasting and increased mortality (85, 86). A previous study of urinary biomarkers to predict renal recovery showed a similar (non-significant) trend with higher biomarker levels in recovery patients, compared to non-recovery (87). The study also found that the decline of urinary NGAL during the first two weeks after RRT initiation was

associated with renal recovery by day 60. None of the studied biomarkers in study IV showed any significant individual ability to predict renal recovery. The insufficient knowledge of timing of AKI onset may have reduced the value of comparing biomarker levels on different days of RRT, i.e. the biomarker value on the day before RRT may have coincided with recovery of AKI for some patients and the injury phase for others. The studied biomarkers may reflect different pathophysiological events during renal injury and recovery. The timing of such events may vary depending on the aetiology and underlying AKI mechanism. The studied cohort was composed of ICU patients exposed to a diversity of AKI triggers. Timing may partly explain the biomarkers' low predictive values in our setting. A study with known timing of AKI onset (e.g. AKI after cardiac surgery) would likely be subject to less error in this aspect.

7 CONCLUSIONS

Plasma endostatin levels added to a clinical risk prediction model, increased the accuracy of AKI prediction in our cohort of ICU patients. This finding is interesting because increased levels of circulating endostatin may reflect early damage to the renal epithelium and endothelium, suggesting that such damage may play a role during the early AKI phase.

Plasma calprotectin predicted infection in the ICU with better accuracy than PCT and WBC and on par with CRP. Calprotectin is released from activated neutrophils, suggesting a neutrophil response to infection that differs from the response to sterile inflammation.

Infection but not the presence of AKI was associated with greater plasma dimeric NGAL levels. The method to measure dimeric NGAL had not been tested in this setting before and the results indicate thatthis method likely measures neutrophil-specific dimeric NGAL rather than monomeric NGAL predominantly released from kidney epithelial cells. However, dimeric NGAL had limited value as a predictor of infection. We did, however, observe a rapid decrease following initiation of antibiotic therapy.

None of the studied biomarkers (endostatin, NGAL, creatinine, urea, cystatin C) predicted renal recovery within 60 days in patients with AKI treated with RRT. We did, however, identify clinical predictors that did: A clinical prediction model based on patient age and daily urine output predicted renal recovery with reasonable accuracy. The finding does not exclude biomarkers from being used in this context in the future, but the timing of biomarker measurement in relation to AKI debut should be considered carefully in future studies.

8 POPULÄRVETENSKAPLIG SAMMANFATTNING

Det kan vara svårt att med säkerhet identifiera förekomsten av infektion hos patienter som vårdas på intensivvårdsavdelning (IVA) eftersom de vanliga symptomen (feber, hög puls, låg urinproduktion etc) är mycket vanligt förekommande hos dessa patienter, oavsett orsak. Detta är ett problem eftersom obehandlad infektion riskerar att leda till allvarlig och livshotande organsvikt. Ett exempel är akut njursvikt, som drabbar cirka 40 procent av IVA-patienterna (andelen varierar dock mellan olika studier). Idag behandlas njursvikt med dialys, i ett skede när funktionsnedsättningen redan är omfattande. Förhoppningen är att det i framtiden ska gå att upptäcka och behandla såväl infektion som akut njursvikt hos IVA-patienter på ett tidigare stadium än vad som i regel är möjligt idag. En potentiell metod är att identifiera mätbara indikatorer i patientens blod eller urin, så kallade biomarkörer. Mot denna bakgrund är det övergripande syftet med denna avhandling att undersöka ett antal potentiella biomarkörer för njursvikt (delstudie I och IV) respektive infektion (delstudie II och III) hos patienter som vårdas på IVA. Nedan presenteras en kort sammanfattning av respektive delstudie som ingår i avhandlingen.

I delstudie I studerades huruvida endostatin – ett protein som frisätts vid sönderfall av stödjevävnaden i njurens kärlväggar och slemhinnor – kunde förutsäga vilka patienter som skulle utveckla akut njursvikt inom tre dygn efter inläggning på IVA. Av patienterna som ingick i studien utvecklade 23 procent akut njursvikt inom tre dygn. Resultaten visade att nivån av endostatin i blodet vid inskrivning kunde prediktera utfallet med relativt hög sannolikhet. Högst risk att utveckla akut njursvikt hade patienter som i kombination med en högre nivå av endostatin i blodet hade högre ålder, lägre urinproduktion samt högre poäng på riskjusteringsmodellen APACHE II.

I delstudie II undersöktes om daglig mätning av proteinet calprotectin i blodet från IVA-patienter kunde bidra till tidig upptäckt av infektion. Calprotectin lagras i vanliga fall inuti specifika vita blodkroppar (neutrofiler) och frisätts när cellerna får signaler om fara för kroppen. Calprotectin har också en bakteriedödande effekt. Av patienter som ingick i studien utvecklade 53 procent infektion under tiden de var inskrivna på IVA. Sammantaget visade studien att calprotectin var lika bra markör för tidig infektion som det traditionella infektions-provet CRP och bättre än LPK (antalet vita blodkroppar) och procalcitonin.

I delstudie III ville vi bedöma värdet av proteinet dimeric neutrophil-gelatinase associated lipocalin (dNGAL) som en tidig markör för infektion hos IVA-patienter, samt studera hur det påverkades vid antibiotikainsättning. Tidigare studier visar att dNGAL huvudsakligen lagras inuti neutrofiler och har en bakteriedödande effekt när det frisätts. dNGAL mättes dagligen hos de patienter som ingick i studien. Av dem bedömdes 73 procent ha en infektion. Vi mätte även dNGAL hos en kontrollgrupp med friska personer för att etablera normalvärden.
Resultaten visade att IVA-patienterna hade högre dNGAL-värden än de friska kontrollpersonerna, samt att IVA-patienter med infektion hade högre dNGAL-värden än IVA-patienter utan infektion. Vi använde en statistisk metod för att verifiera att de högre nivåerna hos infekterade patienter inte berodde på andra faktorer - såsom akut njursvikt eller hög ålder (det vill säga det fanns en oberoende association mellan högre dNGAL-värden och infektion). Värdet av att använda dNGAL för att tidigt diagnostisera infektion på IVA var däremot inte bättre än de traditionella infektionsmarkörerna CRP, LPK och procalcitonin. Efter antibiotikainsättning sjönk dNGAL avsevärt snabbare än de traditionella infektionsmarkörerna, vilket talar för att värdet av dNGAL-styrd antibiotika-utsättning bör undersökas i framtida studier.

I delstudie IV studerades IVA-patienter som drabbats av akut njursvikt som krävde dialysbehandling. Flera biomarkörer mättes dagligen i både plasma och urin, före och under dialys, för att bedöma om biomarkörerna kunde förutsäga vilka som skulle överleva 60 dagar efter IVA-inläggningen, och dessutom klara sig utan fortsatt dialys. Vi utvecklade även en egen riskjusteringsmodell baserad på patientens ålder och daglig urinproduktion. Av de studerade patienterna levde 73 procent 60 dagar efter inläggning, utan behov av dialys. Resultaten gav inget stöd för att de studerade biomarkörerna kunde användas som utfallsindikatorer. Däremot fann vi att den statistiska modellen kunde prediktera utfallet med relativt god träffsäkerhet.

Sammantaget pekar denna avhandling på att det finns ett flertal biomarkörer som skulle kunna användas som indikatorer på infektion respektive akut njursvikt hos patienter som vårdas på IVA. Fynden kan också bidra till att öka förståelsen för den bakomliggande patologin. Det krävs dock uppföljande studier innan de biomarkörer som studerats i denna avhandling kan användas i den kliniska vardagen.

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