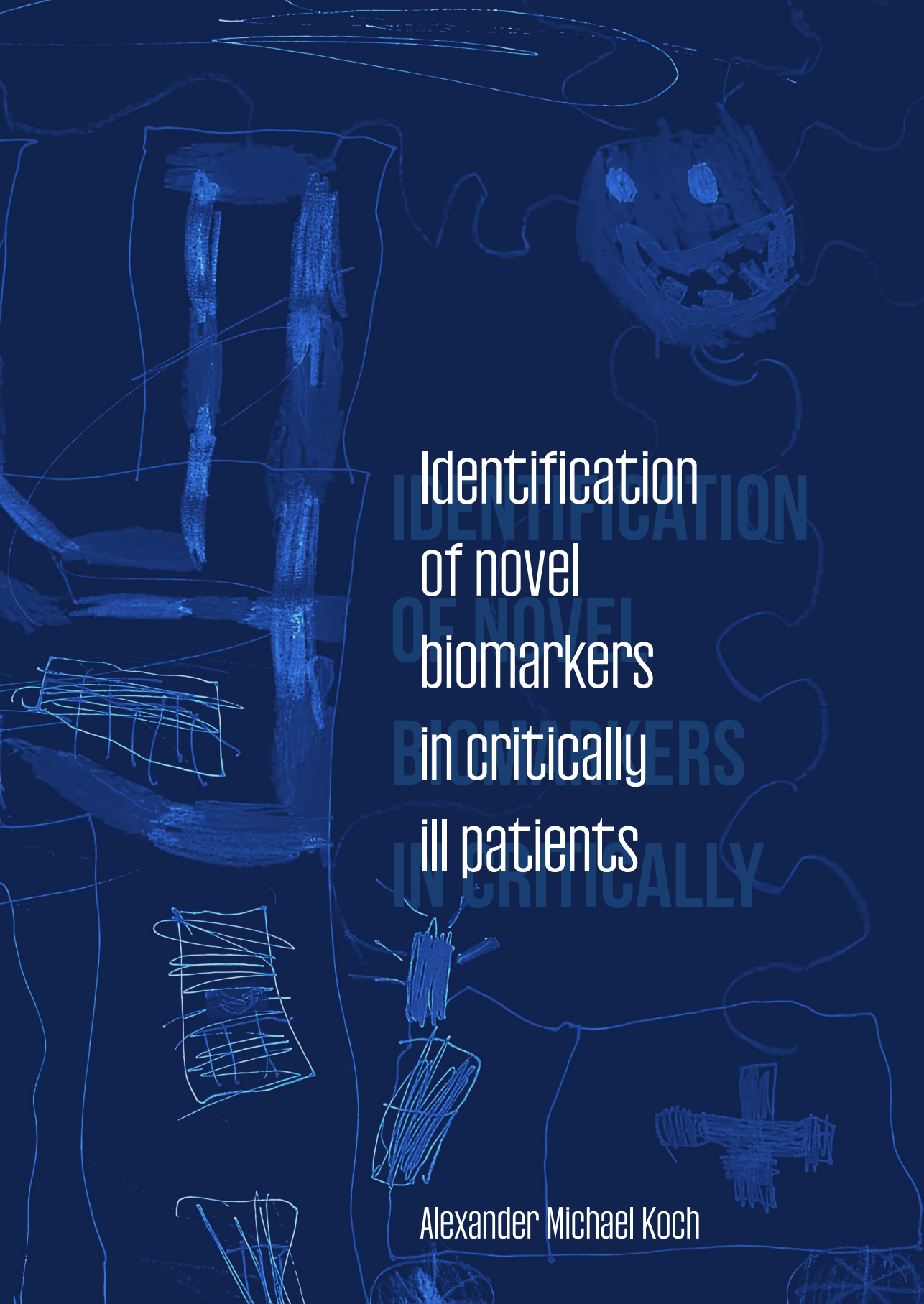
Identification of novel biomarkers in critically ill patients



Identification of novel biomarkers in critically ill patients

Alexander Michael Koch

Identification of novel biomarkers in critically ill patients

Cover design: Jörg Stanzick
Cover image: University Hospital Aachen painted by Peter Koch (at the age of 6 years)
Layout: Tiny Wouters
Production: Proefschriftmaken.nl

ISBN: 978-3-00-075171-4

Identification of novel biomarkers in critically ill patients

DISSERTATION

To obtain the degree of Doctor at the Maastricht University,
on the authority of the Rector Magnificus,
Prof. dr. Pamela Habibović,
in accordance with the decision of the Board of Deans,
to be defended in public on
Tuesday 13th of June 2023 at 13.00 hours

by

Alexander Michael Koch
Born on 8 October 1967 in Nuremberg, Germany

Supervisors

Dr. G.H. Koek
Prof. dr. F. Tacke (Charité Berlin, Germany)

Assessment Committee

Prof. dr. J.C.C. van der Horst, CHAIR
Dr. D.C.J.J. Bergmans
Prof. dr. C. Bollheimer (RWTH Aachen University, Germany)
Dr. T.J.G. Gevers
Prof. dr. P. Strnad (RWTH Aachen University, Germany)

Table of contents

Chapter 1

General Introduction and Outline of this Thesis

General introduction

Multiple organ dysfunction, the key characteristic of critically ill patients, is a result of a broad spectrum of severe medical conditions, such as acute and chronic cardiac, respiratory and hepatic disorders, infections, bleedings or trauma. An individualized rational decision making in the intensive care setting is crucial for an optimal prognosis in these patients. Biomarkers play an important key role in this context.

The World Health Organization (WHO) has defined a biomarker as "any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease".[1] Moreover, biomarkers have a wide range of application in detecting diseases and monitoring the health status of a patient. They may act as diagnostic tools to identify diseases or abnormal conditions, predict the stage of a disease and its severity and can be used as an indicator of disease prognosis or a predictor of clinical response to an intervention.[2]

For intensivists biomarkers are indispensable tools for diagnosing, therapy monitoring and prognostication in daily routine. Individualized or personalized medicine is based on stratification of patients in different subgroups, for example in terms of diagnostication and prognostication, which can be achieved by the availability of specific biomarkers.[3] Thus, the identification and validation of novel biomarkers contribute substantially to the medical innovation and personalized medicine in intensive care treatment and can directly improve the prognosis of critically ill patients.

In this PhD thesis, novel biomarkers are investigated in a large, prospectively collected, cohort of critically ill patients who were admitted to the medical intensive care unit (ICU) at the University Hospital Aachen, Germany.

Thematically, this thesis is divided into three parts. The first part deals with "Biomarkers of Systemic Inflammation in Critical Disease", in the second part the "Role of Specific Adipokines in Critical Ill Patients" is examined and in the third part "Biomarkers of Biological Stress in Critical Illness" are discussed.

Part I: Biomarkers of systemic inflammation in critical disease

Sepsis, non-infectious systemic inflammation and biomarkers

According to the current Sepsis-3-definition sepsis is defined as "life threatening organ dysfunction caused by a dysregulated host response to infection".[4] Up to two percent of hospital admissions refer to sepsis and intensive care treatment is required in half of

these patients.[5] Sepsis remains the leading cause of death for critically ill patients in medical, non-cardiac ICU.[1] Even with optimal therapy, the mortality rates of severe sepsis and septic shock are about 40-50% worldwide, with multi-organ failure (MOF) as the most important cause of death.[6]

But also, ICU patients who survive sepsis often suffer from prolonged late sequelae of sepsis such as physical, neurological and psychological limitations. The extent of these sepsis-related restrictions directly correlates with the disease severity and by this emphasizes the absolute necessity of early diagnosis and consequent treatment of sepsis.[7] Current treatment strategies of sepsis and septic shock comprise detecting sepsis as early as possible, identification and control, respectively eradication of the septic focus, appropriate anti-infective treatment, fluid resuscitation and use of (organ) supportive therapies.[8]

Easily accessible biomarkers from patients' serum are important tools for the early detection of sepsis, for risk stratification and the monitoring of disease progression. Microbiological cultures are the gold standard for the diagnosis of infection in systemic inflammation and thereby sepsis, but their results are usually available days after sample collection. The identification of early available biomarkers that are a surrogate for the presence of sepsis are a matter of ongoing research.[9] A biomarker intended to substitute for a clinical endpoint, such as sepsis, has been defined as a surrogate endpoint.[10]

The best known and widely used surrogate endpoint for inflammation and infection is the C-reactive protein (CRP).[11] CRP is an acute phase protein, that is secreted by hepatocytes in response to inflammation, infection and tissue damages. The name 'C-reactive protein' is derived from its ability to bind the C-polysaccharide of streptomyces pneumoniae. However, CRP has limited specificity in the differentiation of sepsis and states of non-infectious systemic inflammation such as cancer, thromboembolic or cardiovascular and autoimmune diseases. Beyond that, due to the hepatic origin of CRP, CRP levels in severe acute or chronic liver dysfunction rather reflects hepatic synthesis capacity than the actual present inflammatory status.[12]

Besides CRP, procalcitonin (PCT) has been introduced in clinical use in 2005 for the diagnosis and surveillance of severe bacterial infections. Procalcitonin is a precursor of the hormone calcitonin and is synthesized under normal conditions in the thyroid gland and there metabolized by intracellular proteolytic cleavage to calcitonin. PCT is not detectable in serum in healthy states. In conditions of bacterial infection and sepsis PCT is secreted by cytokine-activated macrophages and by the parenchyma of most organs, resulting in rapidly increased serum concentrations of intact PCT.[13] Theoretically, PCT should be the ideal biomarker to allow early diagnosis of sepsis and prompt initiation of

anti-infective therapy. However, a meta-analysis of three large clinical trials comparing PCT-guided protocols with usual care for initiation of antimicrobial therapy found no difference in mortality, length of ICU stays or hospitalization.[8]

Based on this, the current International Guidelines for Management of Sepsis and Septic Shock 2021 suggest against using PCT plus clinical evaluation to decide when to start antimicrobials, as compared to clinical evaluation alone.[8] However, the guideline suggests the use of PCT in combination with clinical evaluation to decide on duration of anti-infective therapy. It should be noted that both statements are made with a weak level of recommendation strength and a very low quality of evidence. Taken together, PCT is at present the only biomarker clinically used to guide antimicrobial treatment based on at least weak evidence from randomized clinical trials.[14]

Interleukin-6 (IL-6) has been proposed as another clinical biomarker in the context of sepsis, systemic inflammation and critical disease. IL-6 is the most potent inducer of acute phase protein secretion in the liver. In sepsis, IL-6 serum concentrations are highly increased and associated with disease severity and prognosis.[15] However, it applies to IL-6 as for CRP and PCT, that elevated serum concentrations are also found in non-infectious systemic inflammation, limiting its specificity for detection of sepsis in critically ill patients.[16] At the present time, there is no diagnostic or prognostic biomarker for critical illness, sepsis or non-infectious systemic inflammation with sufficient specificity and sensitivity and real comprehensive clinical benefit.

The research in the present work aims at the identification of innovative biomarkers reflecting novel pathophysiological concepts comprising systemic inflammation, adipocytokines and circulation to improve the outcome of critically ill patients.

As potential novel biomarkers for systemic inflammation **calprotectin**, **caspase-cleaved keratin 18 fragments (M30)**, **high-mobility group box 1 (HMGB1)** has been evaluated.

Calprotectin

Calprotectin was identified and characterized in the 1980s and is a validated and routinely used biomarker for the evaluation of gut inflammation. Calprotectin is a member of the family of calcium-binding S100 leucocyte proteins and represents a heterodimer of mammalian protein S100A8 and S100A9.[17] The name 'calprotectin' derives from its ability to bind Ca^{2+} and its antimycotic activity against *candida albicans.*[18] Calprotectin is abundant in the cytosol of neutrophil granulocytes and is released upon neutrophil activation or endothelial adhesion of monocytes, specifically during inflammation. Extracellular functions of calprotectin are mainly mediated by toll-like receptor 4 (TLR4) and receptor for advanced glycation end products (RAGE).[19] Calprotectin promotes the expression of pro- and anti-inflammatory mediators such as

IL-1ß, IL-6, TNF-α and IL-10 during oxidative stress. Besides that, calprotectin modulates cell proliferation, differentiation and apoptosis through RAGE ligation and NF-κB activation.[20]

As an acute phase reactant its expression levels are increased in infection, trauma, and inflammatory diseases. Assessment of faecal calprotectin can reliably discriminate between inflammatory and non-inflammatory bowel diseases and correlates with the severity of intestinal inflammation.[21]

Beyond that, calprotectin has been intensively investigated as a serum-based biomarker in acute and chronic inflammatory diseases such as rheumatoid arthritis, systemic lupus erythematosus, type 2 diabetes, atherosclerosis, myocardial infarction, stroke and infections.

Infection induced inflammation is one of the major sources for S100A8 and S100A9 secretion and by this increased calprotectin serum concentrations.[22,23] Calprotectin has been demonstrated as a diagnostic biomarker for sepsis in critically ill patients, which has prognostic capabilities for short-term mortality.[24]

Most recently, calprotectin has been suggested as a diagnostic and prognostic biomarker in COVID-19 infection. High calprotectin serum concentrations have been demonstrated to discriminate severe from non-severe clinical courses of COVID-19 infections, to predict outcome and to assess concomitant gut inflammation.[25]

Interestingly, the presence of gut symptoms in COVID-19 infection, such as nausea, vomiting and diarrhoea is associated with COVID-19 disease severity as expressed by a higher rate of ICU admission and need for ventilator support.[26]

Moreover, calprotectin might serve as a potential therapeutic target in COVID-19 infection and other inflammatory diseases, as the binding of calprotectin to TLR4 and RAGE could be blocked by molecule such as tasquinimod (quinoline-3-carboxamide) and by this attenuate systemic inflammation. This is currently investigated in a clinical trial, evaluating the efficacy of tasquinimod in reducing severe respiratory distress in COVID-19 (trial registration ACTRN12621000016831p).

The important role of calprotectin in systemic inflammation, its potential as a novel therapeutic target and its properties as diagnostic and prognostic biomarker for short-term mortality prompted us to investigate calprotectin serum concentrations in critically ill patients with regard to long-term mortality during a follow-up period of one year (**chapter 2**).

Caspase-cleaved keratin 18 fragments (M30)

Excessive systemic inflammation is a key characteristic of critical illness, multiorgan failure and sepsis. It is a complex process which involves activation of phagocytotic cells and the secretion of pro- and anti-inflammatory mediators. Important triggers are infection related molecules, termed pathogen-associated molecular patterns (PAMPs) and endogenous, immunological signals termed danger-associated molecular patterns (DAMPs).

In infection, specific pattern recognition receptors (PRRs) on cell surface and cytosol recognize PAMPs and trigger signalling pathways promoting the production and release of pro- and anti-inflammatory mediators such as acute-phase proteins, cytokines, chemokines, as well as antimicrobial peptides, in order to localize and control bacterial invasion.[27] PRRs comprise several molecule families, as TLRs, nucleotide-binding oligomerization domain-like receptors (NOD)-like receptors (NLRs), retinoic acid-inducible gene I (RIGI)-like receptors (RLRs) and C-type lectin receptors (CLRs), and intracellular DNA-sensing molecules. PAMPs can also be recognized non-PRRs, which include receptors for advanced glycation end products (RAGE), triggering receptors expressed on myeloid cells (TREM), and G-protein-coupled receptors (GPCRs).

The host immune response and the PAMPs contribute to inflammatory cell stress, cell injury and finally cell death. This results in the release of host-derived non-microbial stimuli (DAMPs), e.g., nuclear, cytoplasmatic, or mitochondrial structures, which further exacerbate the inflammatory response. Under physiologic conditions, these factors are subject to intracellular degradation and are therefore not recognized by the immune system. The release of DAMPs into extracellular environment by dying cells can trigger inflammation under sterile conditions or respectively, aggravate inflammation in infections, as they are also sensed by PRRs.[28]

A variety of DAMPs have been identified and some of them, such as the high mobility group box 1 (HMGB1, see also **chapter 4**), heat shock proteins (HSPs) and members of the S100 family (e.g., calprotectin, see also **chapter 2**) have been investigated as biomarkers reflecting systemic inflammation.[29]

As a result of excessive perpetuating systemic inflammation, cellular death signalling cascades in immune and parenchymal cells are widely initiated. During sepsis various cell death pathways, such as necrosis, apoptosis, necroptosis, pyroptosis, and autophagy-induced cell death can be activated.

Apoptosis (programmed cell death) comprises several physiologic and morphologic cellular changes, finally resulting in cell death as the principal mechanism for eliminating senescent or defect cells.[30] In apoptotic cell death, caspase-cleaved

fragments of keratin 18 (K18), a major type I intermediate filament protein of the cytoskeleton, are released. K18 exposes a circulating neoepitope, that is recognized by the specific monoclonal antibody M30.

M30 reflects the extent of apoptosis and has been suggested as a biomarker in the setting of acute and chronic liver diseases.[31,32] For example, in patients with acute liver failure (ALF) increased M30 serum concentrations have been reported and modification of the model of end stage liver disease (MELD) with inclusion of M30 improved prediction of ALF outcome. In line, M30 levels were predictive for outcome in acetaminophen-induced ALF, but not superior to King's College Criteria.[31,32] M30 has been as well investigated as a diagnostic and prognostic biomarker in critical illness. In clinical studies increased serum concentrations have been reported in sepsis and connected to an adverse outcome. However, it has been unclear whether M30 could be uses as a valid biomarker in heterogenous cohorts of critical ill patients with and without sepsis, in which not only infection (via PAMPs) but also sterile inflammation (via DAMPs) trigger cell death mechanisms.[33] For this reason, we performed a study, in which we analysed M30 serum concentrations in a large cohort of 243 critically ill medical patients and without infection patients and assessed its capability as a diagnostic and prognostic biomarker (**chapter 3**).

High-mobility group box 1 (HMGB1)

High-mobility group (HMG) proteins were initially discovered in 1973 as a group of nuclear, ubiquitously expressed, chromosomal non-histone proteins with high electrophoretic mobility, categorized into three superfamilies, HMGB, HMGN and HMGA. HMGB1, the most abundant member of HMG proteins, participates in maintenance of nucleosome integrity and plays an important role in DNA architecture and transcriptional regulation.[34] Besides its nuclear functions, HMBG1 has significant extracellular activity and functions as DAMP (see also **chapter 3***)*.

It exerts key functions in cellular crosstalk, including production of proinflammatory cytokines, cell proliferation, cell differentiation and cell death, as well as antimicrobial defence and tissue regeneration and by this orchestrates inflammatory and immune response.[35] HMGB1 mediates its cytokine, chemokine and growth factor activities as a ligand for TLR 2 and 4, G protein-coupled CXCR4 receptor, and for RAGE.[36,37] In infection and inflammation HBGB1 is actively secreted for chemotactic and cytokine functions by immune cells and passively released by damaged or necrotic cells, which immediately initiates inflammatory response via proinflammatory cytokines such as TNF-α.[38]

HMGB1 has been demonstrated as a late mediator of sepsis and in mouse models the administration of HBGM1 antibodies significantly improved mortality. Following that, further animal studies targeted HMGB1 with different molecules such ethyl pyruvate or

nicotine. This led to an attenuated increase of serum HMGB1 elevation and interference with the activation of the NF-κB pathway, resulting in substantially decreased cytokine release in models of sepsis.[39,40] These characteristics of HMGB1 indicate that it might be a valuable diagnostic tool in critically ill patients in terms of sepsis, systemic inflammation, disease severity and mortality.

In fact, clinical studies have reported elevated HMGB1 levels in critically ill patients with infectious and non-infectious systemic inflammation and demonstrated association to outcome parameters.[41-45] Most recently, the activation of HMGB1-RAGE cascade has been suggested in the pathogenesis of hyperinflammation in severe COVID-19, as HMGB1 levels reflect inflammatory, oxidative, as well as neurological alterations associated with the development of critical respiratory failure in patients with SARS-CoV-2 infection.[46-48]

Undoubtedly, increased HMGB1 serum concentrations are a typical feature of critical illness. Nevertheless, the association between HMGB1 and clinical outcome is controversial and it remained unclear whether severe infection or critical illness itself is the main determinant of increased HMGB1 levels. We therefore revisited the role of HMGB1 in large heterogenous cohort of medical patients with and without sepsis (**chapter 4**).

PART 2: Role of Specific Adipokines in Critical Ill Patients

Hyperglycemia and insulin resistance in critically ill patients

Hyperglycemia, impaired glucose tolerance and insulin resistance are frequent findings in critically ill patients with sepsis and non-infectious systemic inflammation, e.g., after heart failure, stroke or surgery. This phenomenon has been also summarised under the term "diabetes of injury" and is associated with an impaired prognosis in these patients.

This hyperglycaemic state is suggested to be related to a variety of pathophysiological stress regulating mechanisms. On the one hand, the release of major stress and steroid hormones and a catecholamine overload, and on the other hand glucagon are contributing to a state of insulin resistance with increased hepatic glucose output and emptying glycogen storage.

Stress hyperglycemia results in mitochondrial impairment, oxidative stress-related cell injury, promotion of systemic inflammatory reaction, impaired immune function,

endothelial damage, dysfunction of several cellular membrane integrity, and disturbances of fluid and electrolytes balance (Table 1.1).[49]

Table 1.1 Adverse effects of acute hyperglycemia in intensive care medicine (modified from[49]).

Adverse effects of acute hyperglycemia in critically ill patients
Impairment of capillary blood flow
Endothelial damage
Increase in vascular permeability
Electrolyte imbalances
Volume shifts
Disturbances in the acid-base balance
Expression of proinflammatory genes
Dysfunction of the immune system
Disorders of the complement system
Obstruction to opsonization
Catabolism

Several large prospective clinical studies have proved that control of blood glucose fluctuations improve the patients' survival in sepsis, acute myocardial infarction and stroke.[50-52]

Surprisingly, although patients with pre-existing diabetes displayed similar metabolic alternations during acute illness, hyperglycemia is associated with a worse prognosis in patients without diabetes and an acute ST-elevation myocardial infarction (STEMI) than in patients with diabetes, previously treated with insulin.[53] Insulin exerts positive cardiovascular effects, such as counter-regulation of oxidative stress-mediated cellular injury by suppression of peroxynitrite (ONOO−) production and inhibition of cardiomyocyte apoptosis.[54]

Recently, the concept of 'glucotoxicity' has been introduced, providing an explanation, for the observations that patients with diabetes seem to have survival benefits during acute hyperglycemia in comparison to patients without diabetes.[55] It is still unclear to which extent stress hyperglycemia represents a phenomenon of pathophysiological regulating mechanisms or to what extent the hyperglycemia itself promotes unbeneficial effects.

Adipokines in critical illness

Adipokines may represent an important causal link between hyperglycemia, insulin resistance and excessive systemic inflammatory reaction in sepsis and critical illness. Historically, adipose tissue was considered as an inactive energy store.

However, novel studies have demonstrated that adipose tissue also performs functions of a hormonally active organ system and directly influences metabolic and

inflammatory responses.[56] Proteins, that are regulating these functions and are mainly secreted by adipocytes are collectively referred to as adipokines.[57,58] The disturbed balance in the secretion of pro- and anti-inflammatory adipokines is contributing to chronic systemic inflammation and finally results in metabolic disorders and cardiovascular diseases.[56]

As adipokines are secreted in response to acute injury and are regulators of body homoeostasis their pathophysiological relevance in critical disease and sepsis has been extensively studied during the last years. Because the serum concentrations of adipokines can be easily measured in the clinical routine, adipokines have been evaluated as novel biomarkers for the early diagnostication and prognostication in critically ill patients. Moreover, owing to the strong pathophysiological involvement of adipokines in hyperglycemia, insulin resistance and inflammation, some of them have been suggested as potential future therapeutic options.

In the present work the adipokines visfatin, members of the C1q/TNF-related (glycol-)proteins family CTRP1, CTRP3 and perilipin 2 (PLIN2) have been evaluated as potential novel biomarkers in critically ill patients.

Visfatin

Visfatin, is an adipokine with proinflammatory and immunomodulating properties. It was initially identified in lymphocytes and is therefore also referred as pre-B-cell colony-enhancing factor (PBEF). It is the extracellular molecule of nicotinamide phosphoribosyltransferase (eNampt), a 52 kDa pleiotroptic molecule, whose intracellular molecule (iNampt) is the rate-limiting enzyme in the nicotinamide adenine dinucleotide (NAD+) salvage pathway that converts nicotinamide to nicotinamide mononucleotide (NMN) which is responsible for NAD+ formation.

Visfatin is mainly expressed by macrophages, neutrophils, and activated lymphocytes and is strongly involved in inflammatory processes. It activates B cells, T cells and monocytes, stimulates the release of inflammatory cytokines such as IL-6, IL-8 and TNF-α, and inhibits macrophage and neutrophil apoptosis.[59-61]

Smaller clinical studies have reported increased visfatin serum concentrations in various chronic and acute inflammatory diseases, such as inflammatory bowel disease, rheumatoid arthritis and psoriasis, as well as acute diseases like sepsis, pneumonia, severe trauma, critical neurological illnesses.[62,63] The limited data available on visfatin regulation in sepsis prompted us to evaluate visfatin serum concentrations in a large cohort of 229 critical ill medical patients. We performed extensive statistical correlation analyses with a large number of novel and established biomarkers and clinical

measures such as fluid management, ventilation and renal replacement therapy (RRT), as well as short- and long-term outcome analyses (**chapter 5**).

C1q/TNF-related (glyco-)proteins (CTRP1 and CTRP3)

C1q/TNF-related (glyco-)proteins are discussed to exert numerous physiological and pathophysiological functions, comprising immunity, systemic inflammation, cell differentiation and apoptosis, autoimmunity and metabolism.

The close association of inflammation and metabolic derangements in critically ill patients, seem to make the members of the CTRP family promising biomarkers for the intensive care setting. In the present work the clinical relevance of CTRP1 and CTRP3 in critically ill patients have been extensively studied.

CTRP1

CTRP1 is a member of the C1q/TNF-related protein adipokine family, which consist of 15 proteins and is mainly secreted by adipocytes. CTRP1 performs key functions in the regulation of systemic energy homeostasis and insulin sensitivity and most recently, CTRP1 has been related to inflammation.

It has been demonstrated in animal models, that circulating CTRP1 is distinctively decreased in diet induced obesity and otherwise increased in adiponectin knock-out mice or mice treated with anti-diabetic drugs.[54,64] In line with this, injection of CTRP1 reduce blood glucose levels and overexpression of CTRP1 enhanced insulin sensitivity and decreased fat mass in transgenic mice fed with high-fat diet.[64,65] Physiologically, this seems to be an effect of enhanced energy expenditure from increased fat oxidation in skeletal muscle, mediated by the highly conserved AMP-activated protein kinase (AMPK).[64] Therefore, it has been suggested that circulating CTRP1, as a secreted regulator of skeletal muscle fat oxidation, have positive metabolic effects in humans.

CTRP1 has been also proposed as a key modulator in inflammatory disease. A recent study revealed that the stimulation of human vascular smooth muscle cells by CTRP1 results in an upregulated expression of pro-inflammatory cytokines such as IL-6, monocyte chemoattractant protein 1 (MCP1) and intracellular adhesion molecule 1 (ICAM1).[66]

The clinical relevance of CTRP1 in systemic inflammation and sepsis in critically ill patients is currently insufficiently understood. Therefore, CTRP1 serum concentrations were assessed in 218 medical patients at admission to the ICU prior to therapeutic intervention. As controls, blood samples of 66 healthy blood donors were used. Patients were categorized in sepsis and non-sepsis according to the Third International

Consensus Definitions for Sepsis (Sepsis-3)[4] and treated according to the current International Guidelines for Management of Sepsis and Septic Shock (Surviving Sepsis Campaign)[8,67] (**chapter 6**).

CTRP3

In line with CTRP1, CTRP3, also known as CORS26/carductin, is a recently recognized adipokine which is secreted mainly by adipocytes and adipose tissue stroma cells. It is involved in numerous biological processes such as inflammation, metabolism and food intake, cardiovascular disorders and responses to ischemic injury, as well as tumour metastasis, apoptosis and angiogenesis.[68,69] CTRP3 is considered to mainly exert beneficial effects by contributing to metabolic homeostasis, increasing angiogenesis and anti-inflammatory properties.[70,71]

In terms of metabolism, CTRP3 levels are negatively correlated to waist circumference, blood pressure, fasting glucose, triglyceride and cholesterol in patients with and without metabolic syndrome.[72] An anti-inflammatory and protective role of CTRP3 has been suggested in lipopolysaccharide (LPS)-induced animal model of sepsis, where intramyocardial overexpression of CTRP3 resulted in an attenuated myocardial dysfunction.[73]

CTRP3 is an antagonist of adipose tissue inflammation through TLR4 and thereby inhibits metabolic inflammation mediated by free fatty acids and TLR4 in vitro and in vivo.[74,75] It inhibits thee LPS-TLR4-mediated release of the chemoattractant protein C-C motif chemokine ligand 2 (CCL2) from adipocytes and of the pro-inflammatory cytokines IL-6 and TNF-α from monocytes.[74] Therefore, CTRP3 has been recently proposed as a key link of adipocyte-monocyte/macrophage crosstalk in systemic inflammation and bacterial infection.[76]

In this work the clinical and prognostic relevance of CTRP3 concentrations has been investigated in 218 critically ill septic and non-septic medical patients (**chapter 7**).

Perilipin 2 (PLIN2)

Perilipins are proteins residing on the surface of lipid droplets. Perilipin 2 (PLIN2), also known as adipose differentiation-related protein (ADRP) or adipophilin is a member of the PAT family of lipid droplet proteins, which name refers to the first three proteins that were attributed to this group, namely Perilipin (PLIN), ADRP/PLIN2 and TIP47 (tail-interacting protein of 47kDa/PLIN3).

Lipid droplets (LD) are ubiquitous in nature and have long been regarded as functionally inert fat drops. Lately, LD have been identified as multi-functional organelles exerting

key functions in cellular metabolism and homeostasis.[77] Their structure consists of a core of neutral lipids, primarily triacylglycerols, and sterolesters which is coated by a stabilizing monolayer of phospholipids and specific proteins (perilipins). Depending on the energy demand of the cell, such as inflammation and infection, triacylglycerols are degraded to fatty acids and glycerol, which are utilized in mitochondrial energy production.

Although, storage of energy is the most prominent function of LD, the members of the PAT family of specific LD proteins are substantially involved in lipid metabolism. Perilipins, especially Perilipin 1 control lipolysis by reducing the access of cytosolic lipases to triacylglycerol stored in lipid droplets.[78] Perilipin 2 is ubiquitously expressed and in contrast to perilipin 1 just exerts minimal control over lipolysis and most likely attenuates lipolysis.

Experimental and clinical data suggest that PLIN2 is involved in the pathophysiology of insulin resistance, type 2 diabetes mellitus, dyslipidaemia and fatty liver disease.[79,80] Additionally, PLIN2, as a regulator of intracellular lipid accumulation, has been connected to atherosclerosis and chronic heart disease.[81]

Beyond metabolic and cardiovascular implications, PLIN2 has been described as a potential biomarker in colorectal and lung adenocarcinoma.[82,83] PLIN2 also has been attributed a pathophysiological role in muscle weakness and sarcopenia caused by excessive intramuscular triglyceride accumulation.[84-86]

Disturbances in lipid metabolism are main drivers of chronic inflammation, finally resulting in metabolic diseases, such as such as type 2 diabetes, hypertension, cardiovascular diseases, dyslipidemia, non-alcoholic fatty liver disease (NAFLD), chronic kidney disease, obstructive sleep apnea and physical restrictions.[56] These metabolic diseases, as comorbidities in critically ill patients substantially increase morbidity and mortality.[87]

PLIN2 might be a promising biomarker that may reflect on the one hand acute metabolic disturbances and on the other hand chronic metabolic diseases which are both contributing to outcome in critical illness. This and the fact, that up to this point, PLIN2 serum concentrations have to not been assessed in critically ill patients, prompted us to analyse the usefulness of PLIN2 in a large cohort of well-characterized patients at the timepoint of admission to a medical ICU (**chapter 8**).

PART 3: Biomarkers of biological stress in critical illness

The condition of critical illness comprises a large variety of causative insults such as infections, different forms of shock (e.g., septic, cardiogenic and hypovolaemic shock), metabolic disturbances, trauma, and burns. The severity of critical illness is closely related to the degree of systemic inflammation, the extent of resulting organ failures, subsequent hemodynamic changes, finally summarized in excessive biological stress. As potential biomarkers reflecting biological stress, we investigated **copeptin**, **mid-regional pro atrial natriuretic peptide (MR-proANP)** and **clusterin** in critically patients.

Copeptin

Biological stress activates neuroendocrine pathways, most typically the hypothalamic-pituitary-adrenal axis, with excessive release of corticotropin-releasing hormone (CRH), adrenocorticotropic hormone (ACTH) and cortisol, as well as vasopressin. Vasopressin or anti-diuretic hormone (ADH) is synthesized together with copeptin (also C-terminal proAVP [AVP, arginine vasopressin], CT-proAVP) and neurophysin II as a prohormone (preprovasopressin) in the hypothalamus. Stepwise proteolytic cleavage of the prohormone results in the release of the peptides vasopressin, neurophysin II, and copeptin in equimolar amounts.

Vasopressin serves the organism in the control of fluid homeostasis and is released in response to hypovolemia, hypoxia, acidosis and changes in plasma osmolality.[88] The name vasopressin refers to its vasoconstrictive effect at higher doses (derived from Latin vas '(blood) vessel' and pressus 'pressure').

In contrast to vasopressin, which has a short serum half-life, is tightly bound to platelets and is biochemically instable, copeptin is a stable protein and has been found to reliably reflect biologically functional vasopressin in healthy as well as acutely ill patients.[88,89] Due to its biochemical properties copeptin has been investigated in various acute and chronic diseases. In relation to the biological functions of vasopressin which comprise fluid homeostasis, regulation of osmolality, vasoconstriction and central nervous effect, increased copeptin serum concentrations have been described in diabetes insipidus, metabolic diseases, chronic kidney disease, as well as cardiovascular disorders.[90-92]

In critically ill patients elevated copeptin serum concentrations have been found in shock, sepsis, acute and chronic heart failure and pulmonary failure.[89,92-94] Besides its role in fluid and electrolyte balance and vasoconstriction, copeptin, respectively vasopressin has been described as a stress hormone and has been proposed as a biomarker for short-term mortality.[95]

Based on the favourable biochemical properties of copeptin, its implication in pathways of biological stress as a general common feature of critical disease and its potential association to clinical outcome we conducted a clinical study in 218 critical ill patients in a medical ICU. We measured copeptin serum concentration at admission to the ICU prior to medical interventions and assessed mortality during a 2-year observational follow-up period (**chapter 9**).

Mid-regional pro atrial natriuretic peptide (MR-proANP)

The natriuretic peptides (ANP, atrial natriuretic peptide, BNP, brain natriuretic peptide and CNP, C-type natriuretic peptide) are members of a family of hormones which are structurally related but functionally diverse hormones. ANP and BNP are predominately derived from the heart, whereas CNP as a paracrine messenger is notably expressed in bone, brain and vessels.

Natriuretic peptides exert diuretic, natriuretic and vasoactive actions and play a key role in maintenance of cardiovascular and fluid homeostasis, as wells as blood pressure regulation[96-98] (Table 1.2).

Table 1.2 Physiological actions of natriuretic peptides (modified from[98]). GFR: glomerular filtration rate.

Target organ system	Physiological functions
Kidney	- Increase of GFR (by vasodilatation of afferent arterioles and vasoconstriction of afferent arterioles) - Induction of natriuresis - Induction of diuresis
Heart	- Decrease of cardiac output (by reduction of cardiac preload) - Inhibition of cardiac remodeling
Hemodynamic System	- Vasodilatation - Reduction of cardiac preload and afterload
Endocrine system	- Suppression of the renin-angiotensin-aldosterone axis, sympathetic outflow, arginine vasopressin, and endothelin

ANP is the best studied natriuretic peptide and represents more than 95% of natriuretic peptides in blood circulation.[99] Atrial wall stress is the main driver for the secretion of ANP, which is cleaved from the precursor hormone into amino-terminal (NT-proANP) and the active hormone. Because active ANP has a short serum half-life of less than 5 min and NT-proANP is released in equimolar amounts and is biochemically stable, NT-proANP is has been considered as the more reliable biomarker.

However, since NT-proANP can be further degraded into smaller fragments in vivo, the assessment of mid-regional proANP (MR-proANP) is the current biochemical detection

method of choice and reliably mirrors ANP serum concentrations.[100,101] The name 'MR-proANP' derives from a new sandwich immunoassay that recognizes a mid-regional sequence of pro-ANP. ANP increases capillary permeability and decreases vascular contractility, sympathicotonus, renin and aldosterone secretion, and renal tubular sodium transport.[97]

In summary, under physiological conditions, ANP is mainly involved in body fluid regulation. In critical illness, excessive serum concentrations of natriuretic peptides have been observed in sepsis and septic shock, as wells multiple organ failure, with highest levels in lethal conditions.[102]

Interestingly, in critical disease, besides effects on fluid homeostasis, ANP seems to be involved in regulation of systemic inflammation exerted by macrophage activation, priming of neutrophils and expression of pro-inflammatory biomarkers.[103] Moreover, ANP has been related to metabolism. ANP induced intracellular cyclic guanosine monophosphate (cGMP) activation results in lipolysis and mobilization of free fatty acids, ANP increases secretion of adiponectin (an adipocytokine with insulin-sensitizing properties) and ANP stimulates insulin secretion the pancreas.[104]

In the last years, knowledge on the physiological functions of ANP has been expanded, beyond its functions as a regulator of fluid homeostasis and now as well comprising roles in inflammation and metabolism.

As systemic inflammation, alterations in fluid homeostasis and metabolism are key characteristics of critical diseases, ANP have been previously investigated in critically ill patients. High MR-proANP levels have been associated with disease severity and unfavorable outcome.[100,105] In our study, we investigated the clinical and prognostic relevance of MR-proANP in critically ill patients and focused on systemic inflammation, organ dysfunction and metabolic alterations (**chapter 10**).

Clusterin

Clusterin was first isolated from rat testis in 1979 and was named clusterin for its ability to aggregate blood cells in vitro.[106] Clusterin comprises three isoforms, one localized to the nucleus, one localized to the cytoplasm and the secreted form of clusterin. It is a multifunctional protein and is expressed in a broad range of tissues and present in almost all body fluids.[107]

Secreted clusterin owns chaperone activities and was first associated with Alzheimer's disease. It was attributed a neuroprotective role as it is involved in clearance of misfolded proteins, such as ß-amyloid, and interstitial cellular debris.

Besides its neuroprotective properties, clusterin has been identified as protective against cancer related pain, chronic metabolic conditions such as obesity and cardiac disease.[106,108] In recent years it has become more and more apparent that clusterin exerts highly diversified functions including immunity regulation, stress response, apoptosis and cellular energy metabolism.

By this, clusterin has been related to conditions caused by oxidative stress including acute and chronic inflammatory diseases, neurodegenerative diseases, metabolic disorders, cancer and aging.

Both, inflammatory and metabolic alterations a frequent in critical illness, therefore we investigated the regulation of secreted clusterin in a large cohort of intensive care patients with particular reference to sepsis and metabolic parameters (**chapter 11**).

References are given in the References section of the Addendum

Part I

Biomarkers of Systemic Inflammation in Critical Disease

Chapter 2

Association of Serum Calprotectin Concentrations with Mortality in Critically Ill and Septic Patients

Theresa H Wirtz
Lukas Buendgens
Ralf Weiskirchen
Sven H Loosen
Nina Haehnsen
Tobias Puengel
Samira Abu Jhaisha
Jonathan F Brozat
Philipp Hohlstein
Ger Koek, Albrecht Eisert
Raphael Mohr
Christoph Roderburg
Tom Luedde
Christian Trautwein
Frank Tacke
Alexander Koch

Diagnostics (Basel). 2020:10(11):990

Abstract

Background
Calprotectin is present in the cytosol of neutrophil granulocytes and released upon activation. Fecal calprotectin is applied in the clinical management of inflammatory bowel disease whereas serum calprotectin has been discussed as a biomarker in inflammatory disorders. However, its long-term prognostic relevance in critical illness remains unclear. Our aim was to investigate serum calprotectin concentrations as a prognostic biomarker in critically ill and septic patients.

Methods
Serum calprotectin concentrations were analysed in 165 critically ill patients (108 with sepsis, 57 without sepsis) included in our observational study. Patients were enrolled upon admission to the medical intensive care unit (ICU) of the RWTH Aachen University Hospital. Calprotectin concentrations were compared to 24 healthy controls and correlated with clinical parameters, therapeutic interventions, and survival.

Results
Serum calprotectin concentrations were significantly increased in ICU patients as well as in septic patients compared to respective controls ($p<0.001$ for ICU patients and $p=0.001$ for septic patients). Lower calprotectin concentrations were measured in patients with comorbidities i.e., coronary artery disease. Calprotectin concentrations strongly correlated with the C-reactive protein ($p<0.001$) and were closely associated to parameters of mechanical ventilation (i.e., inspiratory oxygen fraction, FiO2; $p<0.001$). The overall survival was significantly impaired in septic patients with high baseline calprotectin concentrations ($p=0.036$). However, patients with increasing calprotectin serum concentrations within the first week of ICU admission showed an improved overall survival ($p=0.009$).

Conclusions
In summary, serum calprotectin concentrations are significantly increased in critically ill patients with sepsis. High calprotectin concentrations at ICU admission predict long-term mortality risk, whereas increasing calprotectin concentrations are associated with a favourable long-term outcome.

Introduction

Calprotectin represents a heterodimer of the mammalian proteins S100A8 and S100A9. It accounts for 60% of the soluble protein in the cytosol of neutrophil granulocytes and is released by neutrophils upon their activation and turnover[1,2]. Fecal calprotectin is an established marker for neutrophil mediated inflammation in the gut mucosa as it correlates with the numbers of infiltrating neutrophils and the severity of intestinal inflammation[3,4]. Calprotectin was furthermore characterized as a serum-based biomarker in several inflammatory diseases, i.e., juvenile rheumatoid arthritis[5] and systemic lupus erythematosus[6].

Since one of the main sources for S100A8/S100A9 secretion is infection-induced inflammation, calprotectin concentrations in serum were found to be elevated during bacterial infections[7–10] and moreover indicated complicated courses of disease[11]. Importantly, both S100 proteins were identified to participate in innate immunity in a proinflammatory manner as they mediate inflammatory responses by triggering cytokine release and neutrophil recruitment—both essential mechanisms in inflammatory responses and immune defence[12,13].

Bacterial infections may cause life-threatening conditions referred to as sepsis or septic shock. Here, a dysregulated immune response progresses to an organ dysfunction of varying degree, representing a substantial cause of death among critically ill patients[14]. An evidence-based risk evaluation of the patient's individual clinical characteristics could increase the prognosis substantially[15].

Moreover, during the course of sepsis, clinical markers are needed to improve diagnosis and risk-adaptive treatment in critically ill patients. Besides clinical assessment and scores to evaluate organ failure[16], biomarkers may indicate patients at risk for unfavorable courses as well as impaired short- and long-term survival.

Due to its role in bacterial induced inflammation, we hypothesized that calprotectin might play a relevant role in the course of critically ill patients with and without sepsis. As it is already known that calprotectin predicts short-term mortality in septic patients[17], we here investigate the association between circulating calprotectin and severity of critical illness as well as sepsis in a long-term perspective. Moreover, we ask whether calprotectin might function as a long-term prognostic biomarker in critically ill patients treated on a medical intensive care unit (ICU).

Materials and methods

Study design and patients' characteristics

This observational cohort study was performed to investigate the role of circulating calprotectin in critically ill patients treated on a medical ICU. A total of n=165 patients who were admitted to the Department of Gastroenterology, Digestive Diseases, and Intensive Care medicine of the RWTH Aachen University Hospital between 2006 and 2012 were included in the study. Written informed consent was obtained from the patient, her/his spouse or the appointed legal guardian. As patients´ recruitment took place on a medical intensive care unit for adults, patients with an age below 18 years could not be included in the study.

Moreover, patients with an expected short-term ICU stay of less than 72 h, e.g., due to post-operative observation, were excluded. As a control population, n=24 healthy blood donors without severe acute or chronic disease and who are medically examined on a regular basis were included. The Third International Consensus Definition for sepsis was used to retrospectively discriminate sepsis and non-septic patients[14]. The study protocol was approved by the local ethics committee (ethics committee of the University Hospital RWTH Aachen, RWTH Aachen University, Aachen, Germany, reference number EK 150/06, 02 November 2006) and performed according to the ethical standards laid down in the 1964 Declaration of Helsinki.

Calprotectin measurements

After admission to the ICU, blood samples were collected at day 1 and day 7. Samples were centrifuged at 4°C for 10 min and serum aliquots of 1 mL were frozen immediately at −80°C until use. Calprotectin serum concentrations were measured using a commercially available ELISA according to the manufacturer's instructions (PhiCal Calprotectin ELISA Kit, Immundiagnostik AG, Bensheim, Germany). Calprotectin measurements were performed fully blinded to any clinical or other laboratory data of the patients or controls.

Statistical analysis

Data are given as median and range due to the skewed distribution of most of the parameters. Box plot graphics are used to illustrate differences between subgroups. All values have been included for statistical analyses. Differences between two groups were assessed by Mann–Whitney U test. Correlations between variables were analyzed by linear regression analyses.

To investigate prognostic value of the variables, univariate analysis using the Cox regression model was performed. Bootstrapping was added as an internal validation for the predictive value of calprotectin. Variables correlating with overall survival with a p-value of <0.200 in univariate testing were included in a multivariate Cox regression analysis using a stepwise backward procedure with calprotectin as the dependent variable to find independent predictors of patients´ outcome.

In order to illustrate differences in survival, Kaplan–Meier curves were plotted. The log-rank test was used to test the level of significance. The ideal cut-off value for the identification of patients with an impaired OS was calculated by fitting Cox proportional hazard models to the dichotomized survival status as well as survival time and defines the optimal cut-off as the point with the most significant split in the log-rank test[18]. All statistical analyses were performed with SPSS Version 23 (SPSS, Chicago, IL, USA).

Results

Patients´ characteristics

In our analysis, n=165 patients were included who were admitted to our medical ICU due to critical illness. The median age of the study cohort was 64 years with a range from 18 to 90 years. In total, 59.4% of ICU patients were male and 42.4% were female. In addition, 31.6% of included patients had previously been diagnosed with diabetes mellitus type 2; 10% suffered from malignant disease at the time point of ICU admission.

The underlying cause of ICU admission was distributed as follows: 65.5% sepsis, 13.9% cardiopulmonary disease, 5.5% acute pancreatitis, 4.2% decompensated liver cirrhosis, 2.4% gastrointestinal bleeding, 1.2% acute liver failure, 7.3% others. The main site of infection in patients admitted due to sepsis was pulmonary (55.6%). Further detailed patient characteristics are summarized in Table 2.1.

For quantitative variables, median and range (in parenthesis) are given. Abbreviations are: BMI, body mass index; COPD, chronic obstructive pulmonary disease; SOFA, sequential organ failure assessment; APACHE, acute physiology and chronic health evaluation; ICU, intensive care unit.

Table 2.1 Baseline patient characteristics at the time point of admission and calprotectin serum concentrations at day 1 and day 7.

Parameter	Patients
Number	165
Gender	
Female (%)	40.6
Male (%)	59.4
Age, median in years (range)	64 (18–90)
BMI, median (range)	25.8 (15.9–86.5)
Diabetes mellitus type 2 (%)	31.6
Coronary artery disease (%)	22.3
COPD (%)	29.8
Malignant disease (%)	10
Solid tumour	5
Hematological malignancy	4
Main diagnosis/reason for admission (%)	
Sepsis	65.5
Infectious focus of sepsis (%)	
Pulmonary	55.6
Abdominal	19.4
Urinary tract	2.8
Other	22.2
Liver cirrhosis	4.2
Cardiopulmonary disease	13.9
Acute liver failure	1.2
Acute pancreatitis	5.5
Gastrointestinal bleeding	2.4
Other	7.3
APACHE-II score at day 1	17 (3–43)
<17 (%)	50.7
>17 (%)	49.3
SOFA score at day 1	9 (0–17)
<9 (%)	56.6
>9 (%)	43.4
Mechanical ventilation demand (%) at day 1	41.8
Vasopressor demand (%) at day 1	62.4
Death on ICU (%)	19.4
30 d mortality (%)	23.7
90 d mortality (%)	32.3
180 d mortality (%)	40.4
365 d mortality (%)	57.0
Long-term mortality (%)	40.0
Calprotectin [µg/mL]	
Day 1	2.482 (0.004–12.5)
Day 7	4.073 (0.544–3.023)

Calprotectin serum concentrations are increased in critically ill and septic patients

We compared serum calprotectin concentrations between patients at the time-point of ICU admission and healthy control samples. In these analyses, we observed significantly

higher calprotectin serum concentrations in the patients´ group (3.97 µg/mL vs. 2.48 µg/mL in controls, p<0.001; Figure 2.1A).

Subsequently, we aimed at evaluating whether calprotectin serum concentrations are altered regarding different demographic subgroups. However, we did not observe a significant alteration of calprotectin serum concentrations between patients younger or older than 64 years (median of study cohort, Figure 2.1B), male and female patients (Figure 2.1C), as well as between patients with a body mass index (BMI) below or above 30 kg/m^2 (Figure 2.1D).

In a next step, we analyzed if chronic comorbidities of ICU patients influence circulating calprotectin concentrations. Patients with coronary artery disease (CAD) showed significantly lower concentrations of calprotectin compared to patients without CAD (p=0.007, Figure 2.1E). In contrast, calprotectin serum concentrations were comparable between patients with and without diabetes mellitus (p=0.498, Figure 2.1F). We observed a significant difference of calprotectin serum concentrations between patients with or without liver cirrhosis (p=0.001, Figure 2.1G) and patients suffering from chronic obstructive pulmonary disease (COPD) compared to patients without COPD (p=0.047, Figure 2.1H).

Interestingly, patients with sepsis had significantly higher calprotectin serum concentrations (p=0.001, Figure 2.1I). Moreover, calprotectin concentrations were independent of the site of infection since patients with different sepsis focus had comparable calprotectin serum concentrations (Figure 2.1J).

Calprotectin serum concentrations in ICU patients positively correlate with markers of systemic inflammation and parameters of mechanical ventilation

To further investigate potential drivers of increased calprotectin concentrations in ICU patients, we performed extensive linear regression analyses between calprotectin and clinical parameters of critically ill and septic patients including markers of organ dysfunction. Here, calprotectin concentrations did not correlate with duration of ICU or hospital stay neither in all critically ill patients included in the analysis nor in septic patients. Correlation of calprotectin serum concentrations with markers of systemic inflammation at ICU admission revealed a significant correlation between calprotectin and CRP serum concentrations both in critically ill (regression coefficient, r: 17.63 with 95% confidence interval, CI, 10.64–24.62; p<0.001) as well as septic patients (r: 17.67, 95% CI 7.01–28.33; p=0.001).

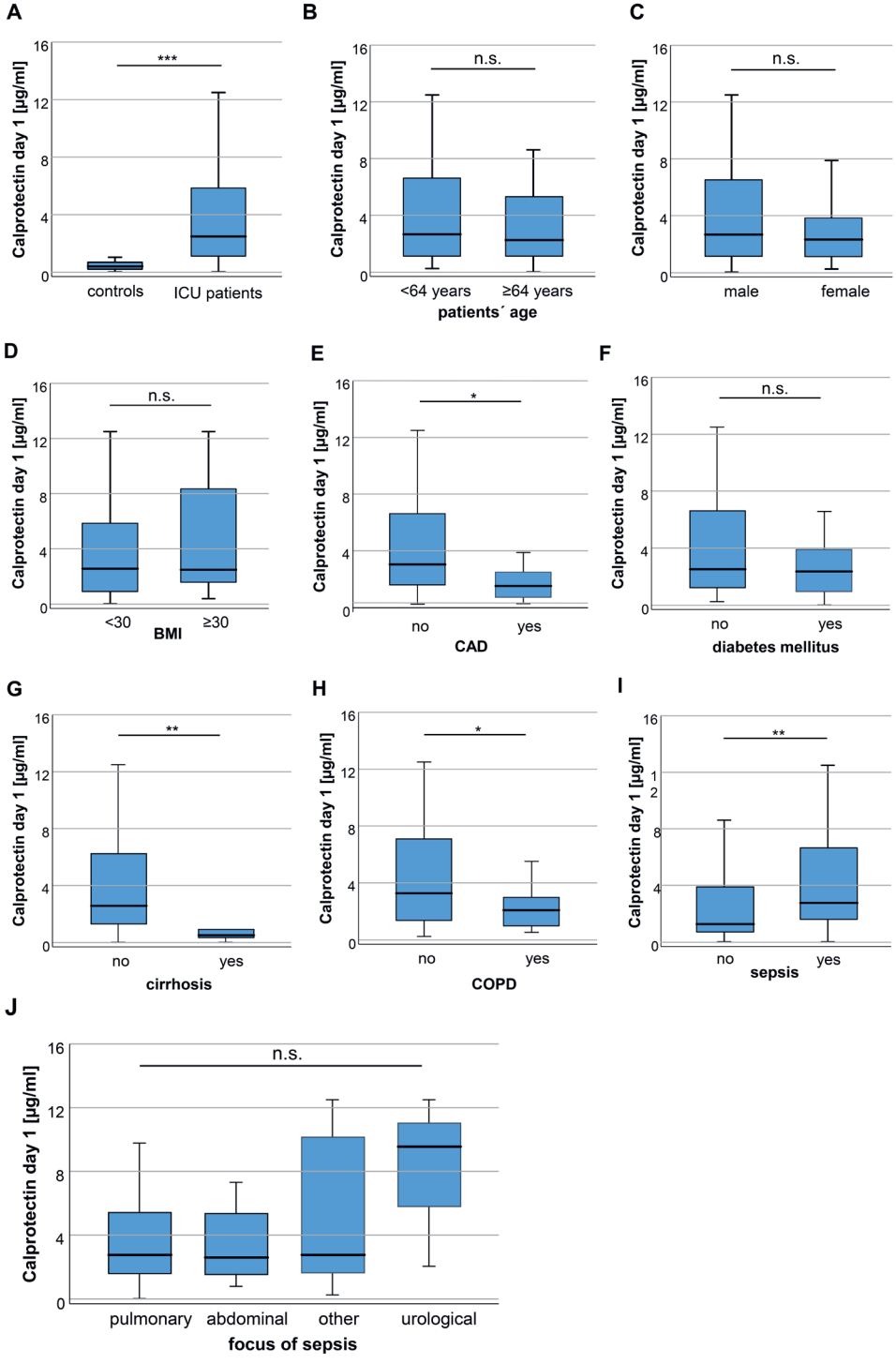

A
Calprotectin day 1 [µg/ml]
***
controls
ICU patients
B
n.s.
<64 years
≥64 years
patients´ age
C
n.s.
male
female
D
n.s.
<30
≥30
BMI
E
*
no
yes
CAD
F
n.s.
no
yes
diabetes mellitus
G
**
no
yes
cirrhosis
H
*
no
yes
COPD
I
**
no
yes
sepsis
J
n.s.
pulmonary
abdominal
other
urological
focus of sepsis

Figure 2.1 Calprotectin serum concentrations are significantly higher in critically ill and sepsis patients. (**A**) Critically ill patients at admission to the ICU have significantly lower calprotectin serum concentrations compared to healthy controls. (**B**) Calprotectin serum concentrations did neither differ between patients younger or older than 64 years nor between male and female patients (**C**) or patients with a body mass index (BMI) below or above 30 (**D**). (**E**) Patients with coronary artery disease (CAD) but not diabetes mellitus (**F**) showed significantly lower calprotectin concentrations. (**G**) Patients with liver cirrhosis or chronic obstructive pulmonary disease (COPD) (**H**) also had significantly lower calprotectin serum concentrations at day 1 of ICU admission. (**I**) Calprotectin concentrations are significantly increased in patients admitted due to sepsis compared to patients without. (**J**) Different sites of infections in patients with sepsis did not result in significantly different serum concentrations of calprotectin. * p<0.05; ** p<0.005, n.s.: not significant.

However, no correlation was found for calprotectin concentrations and leukocytes, procalcitonin or interleukin 6 neither in critically ill nor septic patients. Regarding laboratory markers of organ function, we dissected a correlation of calprotectin concentrations with duration of renal replacement therapy (RRT, r: 186.97, 95% CI 59.41–314.53; p=0.004) and urea (r: 12.74, 95% CI 1.22–24.26; p=0.030) in critically ill patients but not septic patients, even if there was a trend regarding the correlation of RRT duration with calprotectin concentrations in this subgroup (r: 130.45, 95% CI 10.99–272.0; p=0.070). Creatinine concentrations or the glomerular filtration rate (GFR) did not show a significant correlation. Additionally, we did not observe a correlation between calprotectin concentrations and markers of an impaired liver or cardiac function (AST, bilirubin; NT-proBNP; Table 2.2).

Linear regression analysis was performed to test significance; the regression coefficient is depicted as "r" with 95% confidence interval (CI) with * p<0.05; ** p<0.005, *** p<0.001. Abbreviations: BMI, body mass index; CRP, C-reactive protein; IL-6, interleukin 6; GFR, glomerular filtration rate; RRT, renal replacement therapy; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; BNP, brain natriuretic peptide; APACHE, acute physiology and chronic health evaluation score; SOFA, sepsis-related organ failure assessment score; SAPS2, simplified acute physiology score; FiO_2, inspired oxygen fraction; P_{max}, maximum ventilation pressure; PEEP, positive endexpiratory pressure.

Calprotectin concentrations did not significantly correlate with scores of disease severity such as acute physiology and chronic health evaluation (APACHE II) score, sequential organ failure assessment (SOFA) score and simplified acute physiology (SAPS II) score.

Nevertheless, calprotectin concentrations were found to be associated with severity of respiratory failure, as investigation of parameters of mechanical ventilation revealed: Here, the fraction of inspired oxygen (FiO_2), the maximum airway pressure (P_{max}), and the level of positive end-expiratory pressure (PEEP) at first day of admission

significantly correlated with calprotectin concentrations both in critically ill and septic patients (Table 2.2).
However, calprotectin concentrations did not differ between patients with and without dependence of mechanical ventilation (data not shown); also, duration of mechanical ventilation was not associated with higher calprotectin concentrations (Table 2.2).

Table 2.2 Linear regression analysis of calprotectin with baseline characteristics, markers of inflammation, markers of organ dysfunction, other laboratory markers, and clinical scores at day 1 of ICU admission.

	All patients			Sepsis		
	r	**95% CI**	***p***	**r**	**95% CI**	***p***
Baseline Characteristics						
Age	−28.25	−68.75–12.25	0.170	-40.14	−91.61–11.33	0.125
BMI	51.24	−8.66–111.14	0.093	40.29	−25.54–106.13	0.227
ICU Days	4.95	−29.50–39.39	0.777	−22.81	−62.51–16.88	0.257
Hospital days	15.180	−12.25–42.61	0.275	−2.30	−35.45–30.86	0.890
Markers of Inflammation						
Leukocytes	39.37	−17.32–96.06	0.172	25.53	−39.0–90.03	0.434
CRP	17.63	10.64–24.62	<0.001 ***	17.67	7.01–28.33	0.001 **
Procalcitonin	18.38	−4.0–40.73	0.106	15.16	−9.35–39.67	0.221
IL-6	-0.12	−0.37–0.13	0.340	−0.151	−0.413–0.11	0.255
Markers of Organ Dysfunction						
Creatinine	287.92	−7.14–582.98	0.056	221.73	−129.37–572.84	0.213
GFR	−27.21	−66.69–12.27	0.175	−20.08	−69.79–29.62	0.423
Duration of RRT in Days	186.97	59.41–314.53	0.004 **	130.45	−10.99–272.0	0.070
Urea	12.74	1.22–24.26	0.030 *	10.79	−4.09–25.66	0.153
Sodium	−43.85	−148.60–60.91	0.409	−38.57	−163.89–86.76	0.543
Kalium	−560.22	−1465.68–345.23	0.223	−556.72	−1764.11–650.67	0.362
AST	−0.190	−1.02–0.64	0.650	0.045	−2.52–2.62	0.972
Bilirubin Total	−26.53	−335.50–282.44	0.865	579.00	−116.06–1274.06	0.101
LDH	0.90	-0.37–2.17	0.165	3.77	−0.171–7.72	0.061
Lactate	114.17	−158.44–386.78	0.409	185.17	−180.55–550.89	0.317
NT-proBNP	0.032	−0.06–0.13	0.503	0.024	−0.09–0.13	0.671
Clinical Scores						
APACHE II	-25.23	−110.75–60.29	0.560	−89.70	−202.80–23.40	0.118
SOFA	-30.19	−229.89–169.51	0.764	−151.36	−481.0–162.07	0.323
SAPS2	-43.06	−124.12–38.00	0.291	−18.56	−119.84–82.73	0.711
Mechanical Ventilation Parameters						
FiO_2 at Day 1	64.23	33.13–95.33	<0.001 ***	52.61	10.0–95.22	0.017 *
P_{max} at Day 1	106.58	23.98–189.19	0.012 *	114.37	15.37–213.37	0.025*
PEEP at Day 1	315.12	145.40–484.83	<0.001 ***	358.23	159.59–556.86	0.001 **
Duration of Mechanical Ventilation in Days	−0.23	−1.95–1.50	0.796	−1.38	−3.33–0.58	0.166

Baseline calprotectin serum concentrations predict long-term survival in septic patients

As it is known that calprotectin serum concentrations predict short-term mortality in critically ill patients[17], we next hypothesized that the increased concentrations of

circulating calprotectin in critically ill and septic patients could indicate the individual patient's long-term outcome.

First, the 180- and 365-days mortality after ICU admission was investigated. We compared serum calprotectin concentrations in critically ill patients who survived the first 180 or 365 days respectively after ICU admission to patients who deceased during this time period. Here, we revealed a trend towards higher calprotectin concentrations in patients who did not survive the respective time-point (p=0.104, Figure 2.2A and p=0.076, Figure 2.2B), even if these results did not reach statistical significance.

Next, we investigated the impact of calprotectin serum concentrations on the long-term mortality in patients fulfilling sepsis criteria. In this subgroup, we observed significantly higher calprotectin concentrations in sepsis non-survivors after 180 and 365 days (p=0.019, Figure 2C; p=0.013, Figure 2.2D).

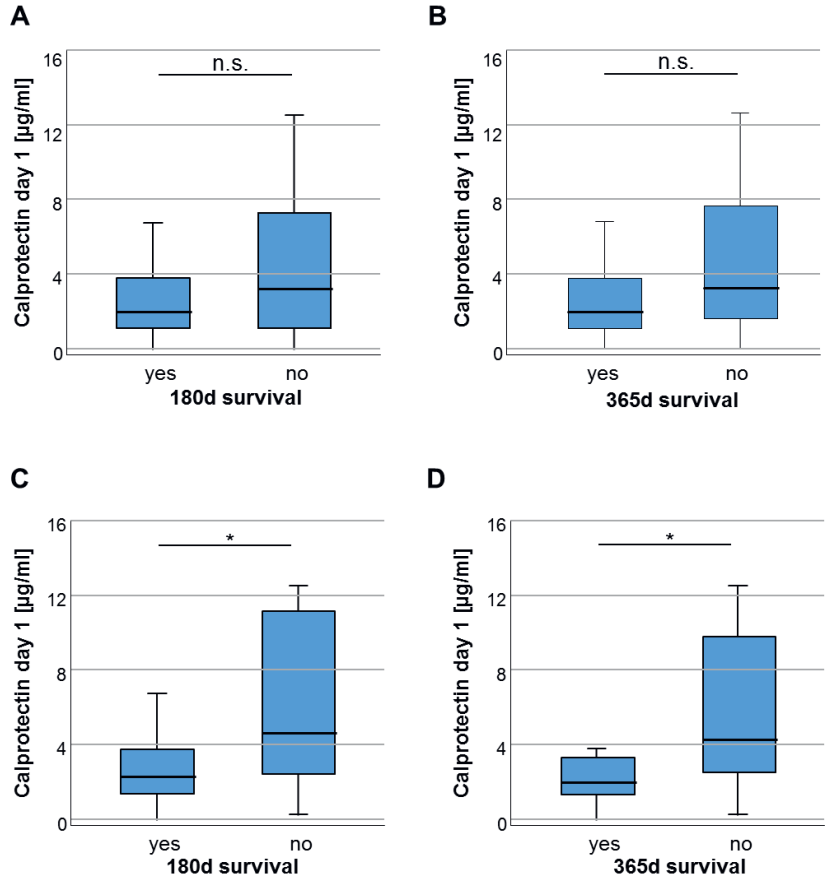


Figure 2.2 High baseline calprotectin concentrations predict poor 180 and 365 days outcome in septic patients. The subfigures (**A**,**B**) include the whole cohort of critically ill patients whereas

subfigures C,D are based on subcohort data of patients admitted to the ICU due to sepsis. (**A**) Critically ill patients who died 180 days or 365 days after ICU admission show a trend towards higher calprotectin serum concentrations (p=0.104 for 180 d; A; p=0.076 for 365 d, B). (**C**, **D**) Septic patients who deceased during the first 180- or 365-days following ICU admission show significantly higher calprotectin concentrations compared to survivors (p=0.019 for 180 d; E; p=0.013 for 365 d, F). * p<0.05; n.s.: not significant.

Increasing calprotectin serum concentrations during the course of critical illness indicate an improved overall survival

Based on these results, we assumed that higher calprotectin concentrations might affect overall survival (OS) in septic patients. Therefore, we compared the OS in the subgroup of septic patients with high or low calprotectin serum concentrations. Using the median calprotectin serum level (2.763 µg/mL) as cut-off value, Kaplan–Meier curve analysis revealed a trend towards an impaired OS in septic patients with calprotectin serum concentrations at ICU admission (p=0.057, Figure 2.3A).

We subsequently established an ideal prognostic cut-off value (see Materials and Methods for details). When applying this ideal cut-off value, septic patients with a baseline calprotectin serum level above 2.001 µg/mL had a significantly impaired OS compared to patients with calprotectin concentrations below this cut-off value (p=0.036, Figure 2.3B). The median OS in the calprotectin-low group was 342 days but was not reached in the calprotectin-high group.

To further underline the prognostic value of circulating calprotectin, we next performed univariate Cox-regression analysis. Here, a calprotectin serum concentration above the ideal cut-off value at the first day of ICU admission turned out as a significant predictive factor for overall mortality (HR: 4.002, 95% CI: 1.190-13.459, p=0.025). Bootstrapping analysis for internal validation was complemented, which also revealed a significant result (p=0.025).

To exclude overestimation of effects, Cox regression was repeated including calprotectin baseline concentrations as a continuous variable; here, calprotectin concentrations at day 1 of ICU admission did not significantly predict overall mortality but showed a trend (p=0.154).

For a subset of critically patients (n=67), serum samples at day 7 following ICU admission were available. Interestingly, calprotectin serum concentrations were significantly higher at day 7 when compared to the respective values at ICU admission (p=0.030, Figure 2.3C). To evaluate whether the prognostic role of circulating calprotectin also existed during the course of ICU treatment, we again compared the overall survival between patients with high or low calprotectin values at day 7. Applying the 50th percentile of calprotectin serum concentrations at day 7 (4.188 µg/mL, Figure

3D), no difference regarding the overall survival could be shown for critically ill patients above compared to patients below this cut-off.

Finally, we analyzed if the individual course of calprotectin serum concentrations might have an impact on the critically ill patients' OS. Therefore, we calculated the delta of calprotectin serum concentrations between day 7 and admission (day 1). Here, patients with an increase in calprotectin concentrations between day 1 and day 7 of ICU admission had a significantly improved overall survival compared to patients who showed decreasing calprotectin concentrations during the first week of critical illness (p=0.009, Figure 2.3E). Correspondingly, septic patients with increasing values of calprotectin serum concentrations within the first week of ICU admission displayed a significantly better overall survival (p=0.033, Figure 2.3F).

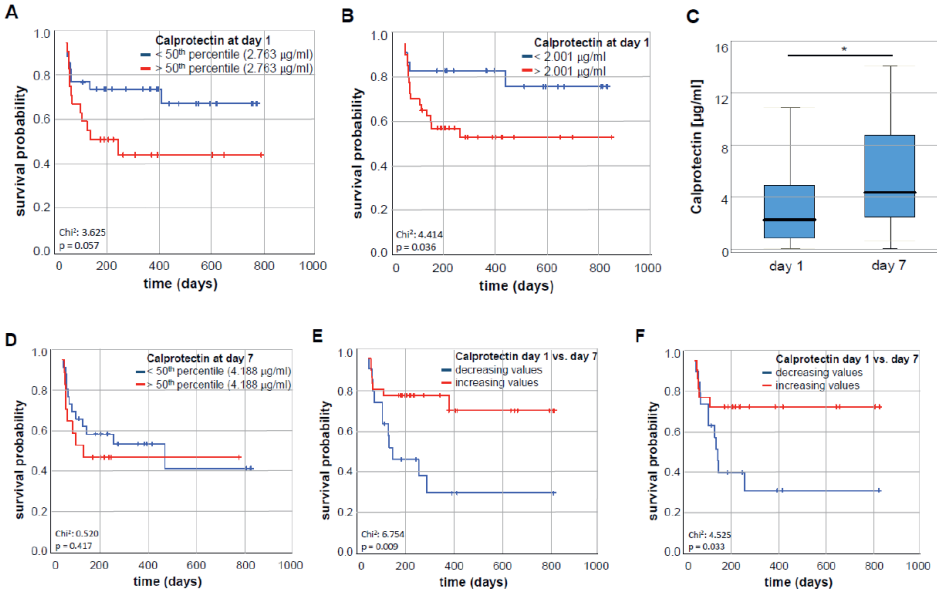


Figure 2.3 Calprotectin serum concentrations during the course of critical illness. (**A**) Using the median calprotectin serum level (2.763 µg/mL) as cut-off value, Kaplan–Meier curve analysis reveals a trend towards an impaired overall survival (OS) in septic patients with high calprotectin serum concentrations at ICU admission (p=0.057). (**B**) When the ideal cut-off value was applied, septic patients with a baseline calprotectin serum level above 2.001 µg/mL have a significantly impaired OS compared to patient with calprotectin concentrations below the cut-off value (p=0.036). (**C**) Calprotectin serum concentrations are significantly higher in critically ill patients at day 7 following ICU admission compared to the respective concentrations at day 1. (**D**) Patients with calprotectin serum concentrations below the 50th percentile (4.188 µg/mL) at day 7 following ICU admission do not show significant differences regarding overall survival compared to patients above the 50th percentile (p=0.417). (**E**) ICU patients with increasing calprotectin concentrations between day 1 and 7 have a significantly better OS compared to patients with decreasing calprotectin concentrations. (**F**) Septic patients with increasing calprotectin concentrations between day 1 and 7 have a significantly better OS compared to

patients with decreasing calprotectin concentrations. * p<0.05.

Both in the study group of all patients as well as in the subgroup of septic patients, the categorized variable of increasing calprotectin serum concentrations (delta between day 1 and day 7 of >1) remained a significant predictor for reduced overall mortality according to the univariate Cox regression analysis (HR 0.304; 95% CI 0.117–0.789, p=0.014 for all critically ill patients; HR 0.335; 95% CI 0.116–0.968, p=0.043). Statistical significance was also reached for the calprotectin delta as a continuous variable (HR 0.850; 95% CI: 0.761–0.948, p=0.004 for all patients; HR 0.861; 95% CI: 0.761–0.974, p=0.018 for septic patients). Both results were confirmed by bootstrapping as an internal validation (p=0.003 for all patients and p=0.010 for septic patients).

Next, we aimed at investigation whether dynamics of calprotectin serum concentrations represent an independent predictor of critically ill patients´ outcome.
We therefore evaluated a wide range of clinicopathological parameters (age, sex, BMI) as well as various laboratory parameters of organ dysfunction including markers of systemic inflammation (CRP, PCT) and an impaired liver (bilirubin) or renal (creatinine) function in a univariate Cox-regression analysis.

In multivariate Cox-regression analysis with a stepwise backward selection of variables procedure including parameters with a potential prognostic relevance in univariate testing (p<0.200), the delta of calprotectin serum concentrations remained an independent prognostic marker for OS (HR: 0.815, 95% CI: 0.720–0.922, p=0.001; Table 2.3).

Table 2.3 Univariate and multivariate Cox regression analysis for the delta of calprotectin serum concentrations between day 1 and day 7 in critically ill patients.

Parameter	Univariate Cox Regression		Multivariate Stepwise Backward Cox Regression	
	p-Value	Hazard Ratio (95% CI)	*p*-Value	Hazard Ratio (95% CI)
Calprotectin delta #	0.004**	0.850 (0.761–0.948)	0.001 **	0.815 (0.720–0.922)
Age	0.002**	1.041 (1.014–1.068)	0.001 **	1.043 (1.018–1.070)
Sex	0.518	0.811 (0.429–1.532)		
BMI	0.119	0.955 (0.901–1.012)	0.016 *	0.921 (0.860–0.985)
CRP	0.113	1.003 (0.999–1.007)	0.140	1.003 (0.999–1.007)
Procalcitonin	0.851	1.001 (0.990–1.012)		
Creatinine	0.498	1.039 (0.931–1.159)		
Bilirubin	0.339	1.167 (0.850–1.601)		
Lactate	0.295	1.081 (0.935–1.249)		

Multivariate Cox regression was performed applying a stepwise backward variable selection procedure. # Calprotectin delta was calculated by subtraction of calprotectin serum concentrations at day 1 of ICU admission from serum concentrations at day 7 of ICU admission. * p < 0.05; ** p < 0. 005. Abbreviations: BMI, body mass index; CRP, C-reactive protein.

Discussion

In this study, we demonstrate that serum concentrations of calprotectin are increased in critically ill patients as well as septic patients at ICU admission compared to controls. High calprotectin concentrations were indicative for an unfavorable clinical outcome since septic patients with high baseline calprotectin concentrations had a more severe clinical course and displayed an impaired overall survival.
Importantly, changes in calprotectin serum concentrations during the first week of admission were prognostically relevant, since patients with increasing calprotectin concentrations were observed to have an improved overall survival. These results are in line with previous data demonstrating serum-based calprotectin to be increased in critically ill patients[19–22].

The patients submitted to our ICU showed a broad spectrum of specific diseases and comorbidities that might influence systemic calprotectin concentrations as well as overall survival. For instance, 10% of the included patients suffered from hematological malignancy or solid tumor. Moreover, calprotectin concentrations were significantly lower in patients with CAD compared to healthy controls.

However, one would have expected increased calprotectin serum concentrations in CAD patients as CAD is referred to as a chronic inflammatory disease and patients with myocardial infarction show increased calprotectin concentrations[23]. We argue here, that due to a selection bias, CAD patients are underrepresented in our cohort of critically ill patients. Therefore, we do not claim to adequately characterize calprotectin serum concentrations in critically ill patients. However, our data point at further triggers of increased calprotectin serum levels in critically ill patients other than an underlying cardiovascular disease.

Moreover, and also due to selection bias, only 7 patients included in our analysis had a previous diagnosis of liver cirrhosis. These patients had significantly lower calprotectin concentrations compared to non-affected patients. Previous data revealed differential calprotectin serum concentrations in patients with different etiologies of liver cirrhosis and it was discussed that elevated calprotectin concentrations indicate bacterial infections in cirrhotic patients[24]. One possible explanation of lower calprotectin concentrations in our included cirrhosis patients compared to non-cirrhotic patients next to a selection bias is that only 3 out of 7 (43%) fulfilled the diagnostic criteria of sepsis compared to a prevalence of sepsis of 68% in the non-cirrhotic cohort.

We describe significantly lower calprotectin serum concentrations in COPD compared to non-COPD patients.

However, calprotectin concentrations in the included COPD patients were still increased compared to serum concentrations in patients with exacerbated COPD described in the literature (median of calprotectin serum concentrations in COPD patients included in our cohort 2079.7 ng/mL compared to a median of 176 ng/mL in exacerbated COPD[25]) pointing at further triggers of circulating calprotectin expression in the included critically ill patients of our cohort with coincidental COPD.

In both critically ill as well as septic patients, calprotectin concentrations correlated with serum concentrations of CRP but not leukocyte count or procalcitonin serum concentration. Calprotectin expression is linked to systemic inflammation as calprotectin is derived from neutrophils and macrophages participating in inflammatory processes. Molecular pathways resulting in upregulated calprotectin concentrations have been established in different models of bacterial infections[7,8,26].

Here, the protein complex of S100A8 and S100A9 magnifies the inflammatory response by accelerating the cytokine release of immunocytes and neutrophil recruitment[26]. Our data describing calprotectin as an early biomarker of bacterial infections in critically ill patients are in line with previous studies[20]. However, calprotectin did not indicate the site of infection, since patients with different septic foci did not show significantly different calprotectin serum concentrations.

Correlation analyses furthermore revealed a trend towards higher creatinine concentrations and a significant correlation with duration of RRT in critically ill patients with higher calprotectin concentrations. These data complete previous studies describing higher calprotectin serum concentrations in critically ill patients fulfilling the criteria of acute kidney injury[27].

Calprotectin as a potential biomarker in sepsis-related organ failure is furthermore supported by the strong correlation of calprotectin concentrations with mechanical ventilation parameters: In both critically ill as well as septic patients with respiratory failure, calprotectin indicated respiratory deterioration. In line with that, calprotectin has previously been shown to be elevated in pulmonary fluid of patients suffering from lung injury and to contribute to amplification of ventilator-induced lung injury in mice[28,29]. Interestingly, recent investigations on COVID-19 identified rising calprotectin serum concentrations in COVID-19 patients requiring higher oxygen fractions and furthermore discovered elevated calprotectin concentrations to distinguish severe from mild COVID-19[30]. Exogenous application of the S100A8/A9 complex has resulted in further amplification of pulmonary inflammation in murine models of lung injury[29]. Our human and recent experimental data should encourage further studies to investigate potential therapeutic effects of calprotectin extraction or neutralization in respiratory failure in critically ill patients.

Larsson et al., among others, revealed a predictive ability of calprotectin serum concentrations regarding short-term mortality in ICU patients with sepsis[17,31]. Our study is the first to investigate the prognostic value of calprotectin serum concentrations in long-term and overall survival (OS) of critically ill patients with and without sepsis. We show that non-survivors after 180 and 365 days show a trend towards higher calprotectin concentrations.
Especially, septic patients who died within 180 and 365 days after ICU admission have significantly higher calprotectin concentrations at ICU admission. These results are further reflected by analysis of OS of septic patients: At the respective optimal cut-off value that was established using a recently described biometric software[18], high calprotectin concentrations (>2.001 µg/mL) turned out as a powerful predictor of OS in septic patients and its prognostic impact was furthermore confirmed by bootstrapping as an internal validation.

Our data therefore appropriately follow up on decreased short-term survival in patients with increased calprotectin concentrations. However, when we included calprotectin serum concentrations at day 1 as a continuous parameter to avoid overestimation of effects, Cox regression analysis only revealed a trend towards an impaired overall survival. Nevertheless, our data demonstrate, that increased calprotectin serum concentrations reflect severity of systemic inflammation in critically ill patients as shown for other acute as well as chronic inflammatory diseases. Experimental approaches blocking the S100A8/A9 activity exerted beneficial effects on disease activity in autoimmune and inflammatory bowel disease[32].

Interestingly, both critically ill patients as well as septic patients who had increasing calprotectin concentrations between day 1 and day 7 of ICU admission showed favorable outcome as described by improved overall survival. We therefore argue that excessive calprotectin concentrations during the first phase of systemic inflammation reflects a severe inflammatory response accompanied by impaired long-term survival. However, calprotectin appears to display a double-edged function in critical illness as well as sepsis, as based on our data after the acute phase of critical disease, ICU patients could benefit from a further increase of calprotectin secretion during systemic inflammation.

In this context, calprotectin has been described in previous studies to regulate inflammatory responses, thereby preventing hyper-inflammation[33]. Beyond that, calprotectin was identified to exert anti-inflammatory, antimicrobial, e.g., beneficial effects during latter stages of inflammation as it was shown to modulate production of pro-inflammatory mediators and also trap pro-inflammatory cytokines in vitro[34,35]. It was moreover even described to exert bacteriostatic activity[36]. S100A8/A9 binds and controls the concentrations of metals such as Zn^{2+} and Mn^{2+} required for bacterial

growth[37] and enhances the antimicrobial efficiency of neutrophil phagocytosis, thereby accelerating clearance of pathogens[38,39].

In line with that, in a murine model of *Staphylococcus aureus* infection, S100A8/A9 deficient mice showed progression of pneumonia in contrast to wild type mice[40]. These experimental studies, supported by our human data, should encourage further exploration of potential therapeutic effects of calprotectin in systemic inflammatory response syndromes, as well as in sepsis.

There are some limitations of our study. First, all data analyses were performed in an exploratory single centre cohort of adult patients leading to a potential lack of generalizability of results. This includes that our data are not comparable to further studies on calprotectin in pediatric critical illness patients, since patients below 18 years were not included in our study. Secondly, we included critically ill patients with a broad spectrum of underlying disease etiology. We assume that this approach provides a certain amount of transferability of results.

However, it might result in disease-specific confounders. Furthermore, our study lacks a validation cohort. This limitation could partly be compensated by additional bootstrapping analysis for internal validation. Although we were able to evaluate calprotectin serum concentrations in a small subset of patients at day 7 following ICU admission in addition to day 1, our study lacks calprotectin measurements at further time-points during ICU treatment. Moreover, participants with missing data were omitted from the analysis, resulting in a data analysis bias according to the PROBAST tool[41].

Therefore, we argue, that the observed prognostic impact of dynamics in calprotectin concentrations needs to be validated in larger, multi-centre cohorts of ICU patients to fully understand the role of calprotectin in predicting patients' outcome.

In summary, serum calprotectin concentrations are significantly increased in critically ill patients with sepsis and associated with renal and respiratory dysfunction. High calprotectin concentrations at ICU admission predict long-term mortality risk. Strikingly, our study is the first to demonstrate a prognostic value of rising calprotectin concentrations during ICU treatment.

References

1. Striz I, Trebichavsky I. Calprotectin—a pleiotropic molecule in acute and chronic inflammation. Physiol. Res. 2004;53:245–253.
2. Fagerhol MK, Dale I, Andersson T. A radioimmunoassay for a granulocyte protein as a marker in studies on the turnover of such cells. Bull. Eur. De Physiopathol. Respir. 1980;16 Suppl:273–282.
3. Roseth AG, Aadland E, Jahnsen J, Raknerud N. Assessment of disease activity in ulcerative colitis by faecal calprotectin, a novel granulocyte marker protein. Digestion. 1997;58:176–180.
4. Sipponen T, Savilahti E, Kolho KL, Nuutinen H, Turunen U, Farkkila M. Crohn's disease activity assessed by fecal calprotectin and lactoferrin: Correlation with crohn's disease activity index and endoscopic findings. Inflamm. Bowel Dis. 2008;14:40–46.
5. Kang KY, Woo JW, Park SH. S100a8/a9 as a biomarker for synovial inflammation and joint damage in patients with rheumatoid arthritis. Korean J. Intern. Med. 2014;29:12–19.
6. Soyfoo MS, Roth J, Vogl T, Pochet R, Decaux G. Phagocyte-specific s100a8/a9 protein levels during disease exacerbations and infections in systemic lupus erythematosus. J. Rheumatol. 2009;36: 2190-2194.
7. De Jong HK, Achouiti A, Koh GC, Parry CM, Baker S, Faiz MA, van Dissel JT, Vollaard AM, van Leeuwen EM, Roelofs JJ, et al. Expression and function of s100a8/a9 (calprotectin) in human typhoid fever and the murine salmonella model. Plos Negl. Trop. Dis. 2015;9:e0003663.
8. Raju MS, Janavi V, Kamaraju RS, Sritharan V, Rajkumar K, Natarajan S, Kumar AD, Burgula S. Continuous evaluation of changes in the serum proteome from early to late stages of sepsis caused by klebsiella pneumoniae. Mol. Med. Rep. 2016;13:4835–4844.
9. Natesan M, Corea E, Krishnananthasivam S, Sathkumara HD, Dankmeyer JL, Dyas BK, Amemiya K, De Silva AD, Ulrich RG. Calprotectin as a biomarker for melioidosis disease progression and management. J. Clin. Microbiol. 2017;55:1205–1210.
10. Njunge, JM, Gwela A, Kibinge NK, Ngari M, Nyamako L, Nyatichi E, Thitiri J, Gonzales GB, Bandsma RHJ, Walson JL, et al. Biomarkers of post-discharge mortality among children with complicated severe acute malnutrition. Sci. Rep. 2019;9:5981.
11. Zhang LN, Wang XH, Wu L, Huang L, Zhao CG, Peng QY, Ai YH. Diagnostic and predictive levels of calcium-binding protein a8 and tumor necrosis factor receptor-associated factor 6 in sepsis-associated encephalopathy: A prospective observational study. Chin. Med. J. 2016;129:1674–1681.
12. Vogl T, Tenbrock K, Ludwig S, Leukert N, Ehrhardt C, van Zoelen MA, Nacken W, Foell D, van der Poll T, Sorg C, et al. Mrp8 and mrp14 are endogenous activators of toll-like receptor 4, promoting lethal, endotoxin-induced shock. Nat. Med. 2007;13:1042–1049.
13. Ehrchen JM, Sunderkotter C, Foell D, Vogl T, Roth J. The endogenous toll-like receptor 4 agonist s100a8/s100a9 (calprotectin) as innate amplifier of infection, autoimmunity, and cancer. J. Leukoc. Biol. 2009;86:557–566.
14. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, Bellomo R, Bernard GR, Chiche JD, Coopersmith CM, et al. The third international consensus definitions for sepsis and septic shock (sepsis-3). JAMA. 2016;315:801–810.
15. Liu VX, Lu Y, Carey KA, Gilbert ER, Afshar M, Akel M, Shah NS, Dolan J, Winslow C, Kipnis P, et al. Comparison of early warning scoring systems for hospitalized patients with and without infection at risk for in-hospital mortality and transfer to the intensive care unit. Jama Netw. Open. 2020;3:e205191.
16. Shahsavarinia K, Moharramzadeh P, Arvanagi RJ, Mahmoodpoor A. Qsofa score for prediction of sepsis outcome in emergency department. Pak. J. Med. Sci. 2020;36:668–672.
17. Larsson A, Tyden J, Johansson J, Lipcsey M, Bergquist M, Kultima K, Mandic-Havelka A. Calprotectin is superior to procalcitonin as a sepsis marker and predictor of 30-day mortality in intensive care patients. Scand. J. Clin. Lab. Investig. 2020;80:156–161.
18. Budczies J, Klauschen F, Sinn BV, Gyorffy B, Schmitt WD, Darb-Esfahani S, Denkert C. Cutoff finder: A comprehensive and straightforward web application enabling rapid biomarker cutoff optimization. PLoS One. 2012;7:e51862.
19. Decembrino L, De Amici M, Pozzi M, De Silvestri A, Stronati M. Serum calprotectin: A potential biomarker for neonatal sepsis. J. Immunol. Res. 2015;2015:147973.

20. Jonsson N, Nilsen T, Gille-Johnson P, Bell M, Martling CR, Larsson A, Martensson J. Calprotectin as an early biomarker of bacterial infections in critically ill patients: An exploratory cohort assessment. Crit Care Resusc. 2017;19:205–213.
21. Bartakova E, Stefan M, Stranikova A, Pospisilova L, Arientova S, Beran O, Blahutova M, Maca J, Holub M. Calprotectin and calgranulin c serum levels in bacterial sepsis. Diagn. Microbiol. Infect. Dis. 2019;93 219–226.
22. Huang L, Li J, Han Y, Zhao S, Zheng Y, Sui F, Xin X, Ma W, Jiang Y, Yao Y, et al. Serum calprotectin expression as a diagnostic marker for sepsis in postoperative intensive care unit patients. J. Interferon Cytokine Res. : Off. J. Int. Soc. Interferon Cytokine Res. 2016;36:607–616.
23. Pedersen L, Nybo M, Poulsen MK, Henriksen JE, Dahl J, Rasmussen LM. Plasma calprotectin and its association with cardiovascular disease manifestations, obesity and the metabolic syndrome in type 2 diabetes mellitus patients. Bmc Cardiovasc. Disord. 2014;14:196.
24. Homann C, Garred P, Graudal N, Hasselqvist P, Christiansen M, Fagerhol MK, Thomsen AC. Plasma calprotectin: A new prognostic marker of survival in alcohol-induced cirrhosis. Hepatology 1995;21: 979–985.
25. Pouwels SD, Nawijn MC, Bathoorn E, Riezebos-Brilman A, van Oosterhout AJ, Kerstjens HA, Heijink IH. Increased serum levels of ll37, hmgb1 and s100a9 during exacerbation in copd patients. Eur Respir J. 2015;45:1482–1485.
26. Lorey MB, Rossi K, Eklund KK, Nyman TA, Matikainen S. Global characterization of protein secretion from human macrophages following non-canonical caspase-4/5 inflammasome activation. Mol. Cell. Proteom. 2017;16:S187-S199.
27. Lee CW, Kou HW, Chou HS, Chou HH, Huang SF, Chang CH, Wu CH, Yu MC, Tsai HI. A combination of sofa score and biomarkers gives a better prediction of septic aki and in-hospital mortality in critically ill surgical patients: A pilot study. World J. Emerg. Surg. 2018;13:41.
28. Mikacenic C, Moore R, Dmyterko V, West TE, Altemeier WA, Liles WC, Lood C. Neutrophil extracellular traps (nets) are increased in the alveolar spaces of patients with ventilator-associated pneumonia. Crit Care. 2018;22:358.
29. Kuipers MT, Vogl T, Aslami H, Jongsma G, van den Berg E, Vlaar AP, Roelofs JJ, Juffermans NP, Schultz MJ, van der Poll T, et al. High levels of s100a8/a9 proteins aggravate ventilator-induced lung injury via tlr4 signaling. PLoS One. 2013;8:e68694.
30. Shi H, Zuo Y, Yalavarthi S, Gockman K, Zuo M, Madison JA, Blair CN, Woodard W, Lezak SP, Lugogo NL, et al. Neutrophil calprotectin identifies severe pulmonary disease in covid-19. Medrxiv: Prepr. Serv. Health Sci. 2021;109:67-72
31. Gao S, Yang Y, Fu Y, Guo W, Liu G. Diagnostic and prognostic value of myeloid-related protein complex 8/14 for sepsis. Am. J. Emerg Med. 2015;33:1278–1282.
32. Pruenster M, Vogl T, Roth J, Sperandio M. S100a8/a9: From basic science to clinical application. Pharm. 2016;167:120–131.
33. Dreschers S, Ohl K, Lehrke M, Mollmann J, Denecke B, Costa I, Vogl T, Viemann D, Roth J, Orlikowsky T, et al. Impaired cellular energy metabolism in cord blood macrophages contributes to abortive response toward inflammatory threats. Nat. Commun. 2019;10:1685.
34. Coveney AP, Wang W, Kelly J, Liu JH, Blankson S, Wu QD, Redmond HP, Wang JH. Myeloid-related protein 8 induces self-tolerance and cross-tolerance to bacterial infection via tlr4- and tlr2-mediated signal pathways. Sci. Rep. 2015;5:13694.
35. Otsuka K, Terasaki F, Ikemoto M, Fujita S, Tsukada B, Katashima T, Kanzaki Y, Sohmiya K, Kono T, Toko H, et al. Suppression of inflammation in rat autoimmune myocarditis by s100a8/a9 through modulation of the proinflammatory cytokine network. Eur J. Heart Fail. 2009;11:229–237.
36. Lusitani D, Malawista SE, Montgomery RR. Calprotectin, an abundant cytosolic protein from human polymorphonuclear leukocytes, inhibits the growth of borrelia burgdorferi. Infect. Immun. 2003;71: 4711–4716.
37. Damo SM, Kehl-Fie TE, Sugitani N, Holt ME, Rathi S, Murphy WJ, Zhang Y, Betz C, Hench L, Fritz G, et al. Molecular basis for manganese sequestration by calprotectin and roles in the innate immune response to invading bacterial pathogens. Proc. Natl. Acad. Sci. United States Am. 2013;110:3841–3846.

38. Simard JC, Simon MM, Tessier PA, Girard D. Damage-associated molecular pattern s100a9 increases bactericidal activity of human neutrophils by enhancing phagocytosis. J. Immunol. 2011;186: 3622-3631.
39. Achouiti A, Vogl T, Urban CF, Rohm M, Hommes TJ, van Zoelen MA, Florquin S, Roth J, van 't Veer C, de Vos AF, et al. Myeloid-related protein-14 contributes to protective immunity in gram-negative pneumonia derived sepsis. Plos Pathog. 2012;8:e1002987.
40. Achouiti A, Vogl T, Van der Meer AJ, Stroo I, Florquin S, de Boer OJ, Roth J, Zeerleder S, van 't Veer C, de Vos AF, et al. Myeloid-related protein-14 deficiency promotes inflammation in staphylococcal pneumonia. Eur Respir J. 2015;46:464–473.
41. Wolff RF, Moons KGM, Riley RD, Whiting PF, Westwood M, Collins GS, Reitsma JB, Kleijnen J, Mallett S, Groupdagger P. Probast: A tool to assess the risk of bias and applicability of prediction model studies. Ann. Intern. Med. 2019;170:51–58.

Chapter 3

High Circulating Caspase-Cleaved Keratin 18 Fragments (M30) Indicate Short-Term Mortality in Critically Ill Patients

Alexander Koch
Eray Yagmur
Janine Linka
Fabienne Schumacher
Jan Bruensing
Lukas Buendgens
Ulf Herbers
Ger H. Koek
Ralf Weiskirchen
Christian Trautwein
Frank Tacke

Dis Markers. 2018:8583121

Abstract

Caspase-cleaved fragments of the intermediate filament protein keratin 18 (cytokeratin-18, CK18) can be detected in serum as M30 levels and may serve as a circulating biomarker indicating apoptosis of epithelial and parenchymal cells. In order to evaluate M30 as a biomarker in critical illness, we analysed circulating M30 levels in 243 critically ill patients (156 with sepsis, 87 without sepsis) at admission to the medical intensive care unit (ICU), in comparison to healthy controls (n=32).

M30 levels were significantly elevated in ICU patients compared with healthy controls. Circulating M30 was closely associated with disease severity, but did not differ between patients with sepsis and ICU patients without sepsis. M30 serum levels were correlated with biomarkers of inflammation, cell injury, renal failure and liver failure in critically ill patients.

Patients that died at the ICU showed increased M30 levels at admission, compared with surviving patients. A similar trend was observed for the overall survival. Regression analyses confirmed that M30 levels are associated with mortality, and patients with M30 levels above 250.8 U/L displayed an excessive short-term mortality. Thus, our data support the utility of circulating levels of the apoptosis-related keratin fragment M30 as a prognostic biomarker at ICU admission.

Background

Excessive systemic inflammation as a consequence of massive innate immune cell activation is a key characteristic in critically ill patients. This is triggered by infection-related molecules, termed pathogen-associated molecular patterns (PAMPs), and by the release of endogenous immunogenic signals, termed danger-associated molecular patterns (DAMPs)[1]. Activated innate immune cells release abundant inflammatory cytokines (e.g., tumor necrosis factor and interleukins) that initiate inflammatory and also cell death signaling cascades in immune cells and parenchymal cells[2]. While excessive systemic inflammation is well recognized as a major driver of multiple organ failure and mortality in critical illness[3], the resulting mode of cell death, such as necrosis, apoptosis, necroptosis, pyroptosis, or ferroptosis, is incompletely understood[4]. Nonetheless, targeting apoptotic pathways has been suggested as a potential novel therapeutic approach in sepsis therapy[5-7].

The systemic release of caspase-cleaved fragments of keratin 18 (K18), a major type I intermediate filament protein of the cytoskeleton, has been identified as a specific biomarker reflecting apoptotic cell death. K18 occurs in all single-layer epithelial cells such as the gut, urinary tract, or respiratory tract and in parenchymal cells like hepatocytes or cholangiocytes[8]. The caspase-cleaved K18 fragment exposes a circulating neoepitope that is recognized by the specific monoclonal antibody M30, which has been widely described as a valid biomarker in the context of acute and chronic liver diseases[8,9].

Few data exist on M30 levels in critical illness. Four independent observational studies in patients with sepsis, two of them conducted in small patient cohorts, reported consistently elevated M30 levels compared with controls as well as an association with adverse clinical outcome[10-13]. However, it is currently less well defined if the clinical utility and prognostic relevance of M30 as a biomarker can be extrapolated to heterogeneous cohorts of critically ill patients, in which not only infectious threats (PAMPs) but also sterile inflammation (DAMPs) trigger cell death mechanisms[1].

We therefore studied circulating M30 in a large cohort of 243 critically ill patients at a medical intensive care unit (ICU), in comparison to healthy controls, and assessed its potential as a diagnostic and prognostic biomarker in medical ICU patients.

Methods

Study design and patient characteristics

In our single- center prospective observational trial, we serially gathered data and blood samples of consecutive patients upon admission to the medical ICU in the University Hospital Aachen, Germany. We excluded patients who had an elective procedure or were admitted for postinterventional observational stays[14].

The study protocol was approved by the local ethics committee and undertaken in accordance with the ethical standards laid down in the Declaration of Helsinki (ethics committee of the University Hospital Aachen, RWTH Aachen University, Aachen, Germany, reference number EK 150/06).

We secured written informed consent from the patient, the spouse, or the legal guardian according to the German civil code BGB §1896. The patients were categorized as sepsis and nonsepsis according to the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3)[15] and were treated following the current guidelines for treatment of sepsis (Surviving Sepsis Campaign)[16]. As a control cohort, we included healthy blood donors with normal blood count, normal values of liver enzymes, and a negative serology for viral hepatitis and HIV.

To assess the patients' outcome, we defined the ICU mortality as well as overall mortality, which was based on contacting the patients, relatives, and/or their general practitioners in approximately 6-month intervals after discharge from hospital for two years[17]. Short-term mortality was defined as mortality within 30 days after admission to the ICU, independent from whether the patients died at the ICU, in the hospital, or outside the hospital.

M30 measurements

We obtained blood samples immediately at the time of admission to avoid influence of any therapeutic procedures. After centrifugation, serum was stored at -80°C. M30 was analyzed with a commercial enzyme immunoassay (M30 Apoptosense® ELISA, cat. no. 10011, TECOmedical Group, Sissach, Germany).

Statistical analysis

Because most samples were not normally distributed, the Mann–Whitney U test was applied to test for statistically significant differences between two groups. Correlations were assessed by Spearman's rank correlation method. All values, including outside

values as well as far-out values, were included. p values less than 0.05 were considered as statistically significant.

The Cox regression model was employed to evaluate the prognostic value of M30 on the outcome. Furthermore, the survival was assessed using Kaplan-Meier analysis with a M30 cut-off level that had been calculated via the Youden Index[18]. All analyses were conducted using IBM SPSS Statistics (SPSS; Chicago, Illinois).

Results

M30 levels are significantly elevated in ICU patients compared with healthy controls, but are not related to sepsis

Circulating M30 levels were significantly increased in critically ill patients (n=243, median 178.3 U/L, range 16.7-1001 U/L, Table 3.1) compared to the healthy control group (n=32, median 107.1 U/L, range 48.3-217.1 U/L, p<0.001, Figure 3.1A). A total of n=156 patients fulfilled the sepsis criteria, mostly with a pulmonary focus (n=84).

Non-sepsis patients (n=87) were admitted due to cardio-pulmonary disorders (n=29), decompensated liver cirrhosis (n=13), acute pancreatitis (n=11) and other conditions (Table 3.2). M30 levels did not differ between patients with sepsis (median 161.8 U/L, range 21.6-1000 U/L) and critical illness without sepsis (median 193.6 U/L, range 16.7-1001 U/L, Table 3.1, Figure 3.1B).

Table 3.1 Patient characteristics and **M30** serum measurements at ICU admission.

Parameter	All patients	Non-sepsis	Sepsis
Number	243	87	156
Sex (male/female)	154 / 89	55 / 32	99 / 57
Age median (range) [years]	64 (18-90)	61 (18-85)	65 (21-90)
APACHE-II score median (range)	18 (2-43)	17 (2-34)	19 (3-43)
ICU days median (range)	7 (1-70)	5 (1-44)	10 (1-70)
Death during ICU n(%)	64 (26%)	15 (17%)	49 (31%)
Death during follow-up (total) n(%)	115 (47%)	29 (33%)	86 (55%)
Mechanical ventilation n(%)	168 (69%)	55 (63%)	113 (72%)
pre-existing diabetes n(%)	73 (30%)	30 (35%)	43 (28%)
pre-existing cirrhosis n(%)	25 (10%)	16 (18%)	9 (6%)
BMI median (range) [m^2/kg]	25.9 (15.3-86.5)	25.4 (15.9-53.3)	26.0 (15.3-86.5)
WBC median (range) [$x10^3/\mu l$]	12.8 (0-208)	11.9 (2.5-27.7)	13.1 (0-208)
CRP median (range) [mg/dl]	93 (0-230)	18 (5-230)	153.3 (0-230)
Procalcitonin median (range) [µg/l]	1.2 (0-207.5)	0.35 (0.03-100)	3.4 (0-207.5)
Creatinine median (range) [mg/dL]	1.3 (0-21.6)	1.0 (0.2-15)	1.6 (0-21.6)
INR median range)	1.17 (0-133)	1.15 (0.9-6.73)	1.18 (0-133)
M30 median (range) [U/L]	178.3 (16.7-1001)	161.8 (21.6-1000)	193.6 (16.7-1001)

For quantitative variables, median and range (in parenthesis) are given. Abbreviations: APACHE: Acute Physiology And Chronic Health Evaluation; BMI: body mass index; CRP: C-reactive protein; ICU: intensive care unit; INR: international normalized ratio; WBC: white blood cell.

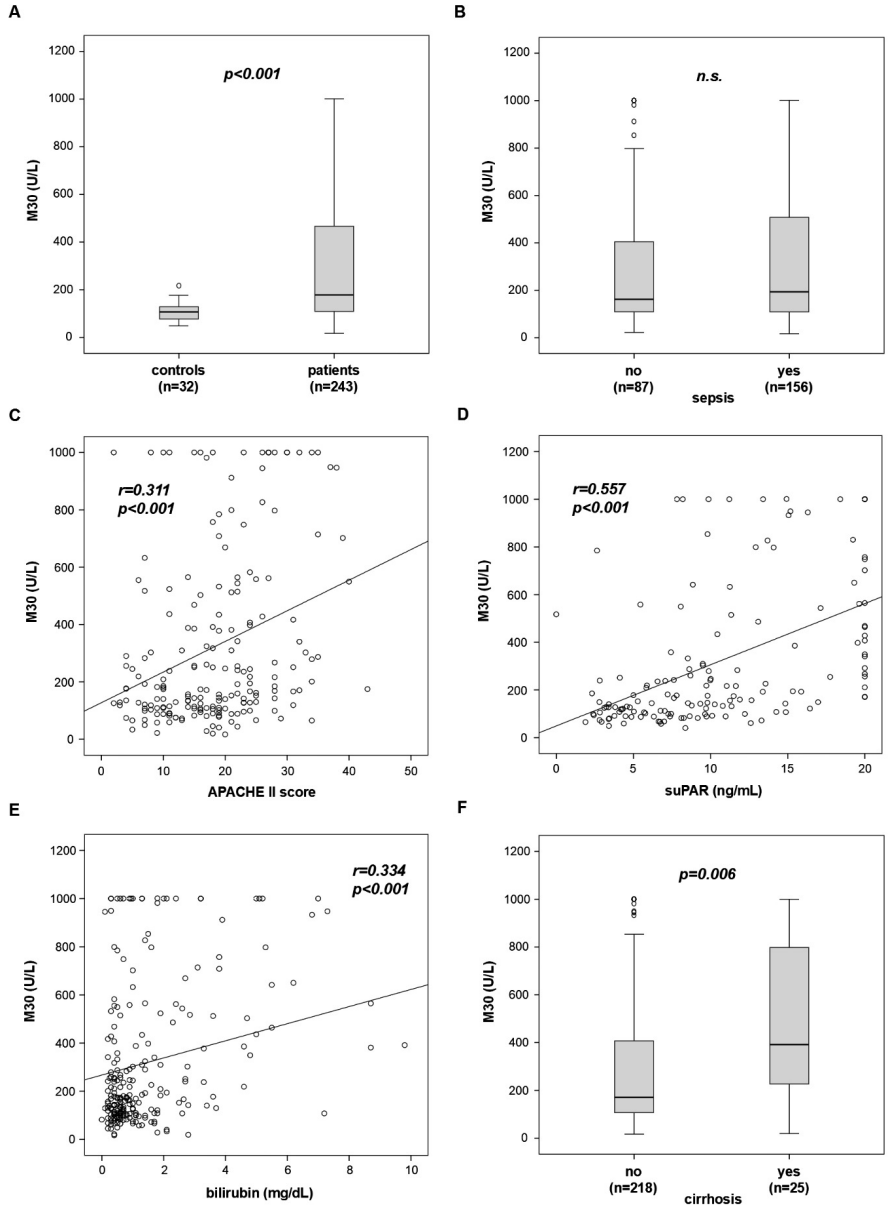


Figure 3.1 Serum M30 levels in critically ill patients. (**A**) Serum levels of M30, at the time of admission to the ICU, were significantly higher in critically ill patients than in healthy controls (p<0.001; U-test). (**B**) M30 levels did not differ between ICU patients with or without sepsis. (**C-D**) M30 levels correlated with disease severity, as assessed by the Apache-II score (**C**) or serum concentrations of soluble urokinase plasminogen activator receptor (suPAR, D). **(E-F**) M30 levels in critically ill patients correlated with serum bilirubin (E) and were particularly elevated in ICU patients with liver cirrhosis (**F**).

Table 3.2 Disease etiology of the study population leading to ICU admission.

	Sepsis	**Non-sepsis**
	156	**87**
Etiology of sepsis critical illness		
Site of infection n (%)		
Pulmonary	84 (54%)	
Abdominal	27 (17%)	
Urogenital	10 (6%)	
Other	35 (23%)	
Etiology of non-sepsis critical illness n (%)		
Cardio-pulmonary disorder		29 (33%)
Exacerbated chronic obstructive pulmonary disease		3 (3.5%)
Acute pancreatitis		11 (13%)
Acute liver failure		2 (2%)
Decompensated liver cirrhosis		13 (15%)
Severe gastrointestinal hemorrhage		7 (8%)
Neurological diseases		4 (4.5%)
Intoxication		3 (3.5%)
Ketoacidosis / diabetic coma		5 (6%)
Vasculitis		3 (3.5%)
Non-sepsis other		7 (8%)

M30 serum concentrations are associated with disease severity

To identify critical factors that impact M30 levels we conducted correlation analyses with scoring systems for disease severity as well as with clinically established laboratory parameters representing disease severity (Table 3.3).

We observed significant correlations between circulating M30 levels at admission and clinical scores for disease severity such as Acute Physiology And Chronic Health II [APACHE II] ($r=0.311$, $p<0.001$, Figure 3.1C), Sequential Organ Failure Assessment [SOFA] or Simplified Acute Physiology Score 2 [SAPS2] (Table 3.3). A positive association was also observed between M30 levels and soluble urokinase plasminogen activator receptor (suPAR, Figure 3.1D), which had been identified as a marker of systemic inflammation and poor prognosis in critically ill patients[19].

M30 levels are correlated with biomarkers of liver failure, renal failure, inflammation and cell injury in critically ill patients

Furthermore, we found strong correlations between M30 levels and biomarkers that reflect hepatic and renal dysfunction (Table 3.3). More precisely, circulating M30 levels correlated with markers indicating the hepatic biosynthetic capacity (e.g., international normalized ratio [INR], antithrombin III, pseudocholinesterase), parenchymal damage (e.g., glutamate dehydrogenase [GLDH], aspartate and alanine transaminase) and parameters indicating cholestasis (e.g., gamma- glutamyltransferase, alkaline

phosphatase, bilirubin, Figure 3.1E) as well as markers tracing renal dysfunction (e.g., creatinine, cystatin C, glomerular filtration rate).

Table 3.3 Correlations of clinical scores and laboratory parameters with M30 serum concentrations at admission day (Spearman rank correlation test, only significant results are shown) *.

	ICU patients	
Parameters	**r**	**p**
Disease severity		
Apache II score	0.311	<0.001
SOFA score	0.390	<0.001
SAPS2 score	0.290	0.018
Inflammation		
Procalcitonin	0.362	<0.001
SuPAR	0.557	<0.001
Interleukin-6	0.163	0.023
TNF	0.441	0.002
Interleukin-10	0.369	<0.001
LDH	0.299	<0.001
Renal function		
Creatinine	0.235	<0.001
GFR (creatinine)	-0.224	0.003
Cystatin C	0.292	<0.001
GFR (cystatin C)	-0.285	<0.001
Urea	0.192	0.003
Uric acid	0.158	0.027
Liver function		
Protein	-0.269	<0.001
Albumin	-0.212	0.015
Pseudocholinesterase	-0.275	<0.001
Bilirubin	0.334	<0.001
Bilirubin (conjugated)	0.515	<0.001
Gamma GT	0.337	<0.001
Alkaline phosphatase	0.300	<0.001
AST	0.427	<0.001
ALT	0.358	<0.001
GLDH	0.466	<0.001
INR	0.402	<0.001
Prothrombin time	-0.391	<0.001
aPTT	0.389	<0.001
D-dimers	0.498	<0.001
Antithrombin III	-0.420	<0.001
Platelets	-0.185	0.004

*Non-significant correlations were noted for M30 levels with blood count, sodium, potassium, magnesium, amylase, lipase, creatine kinase, C-reactive protein, thyroid stimulating hormone, vitamin D, parameters of mechanical ventilation, central venous pressure

In addition, circulating M30 levels showed to correlate with parameters of systemic inflammation (e.g., tumor necrosis factor, interleukins 6 and 10, procalcitonin) and to the general cell injury marker lactate dehydrogenase (LDH, r=0.299, p<0.001).

Patients with underlying liver cirrhosis have significantly elevated M30 levels

M30 was indicated to be increased in patients with hepatic dysfunction[11,20]. In accordance, patients admitted to the ICU with liver cirrhosis (n=25, median 391.4 U/L, range 19.4-1000) had significantly increased M30 levels compared to critically ill patients without cirrhosis (n=218, median 171 U/L, range 16.7-1001, p=0.006, Figure 3.1F).

Although M30 levels had been specifically linked to non-alcoholic steatohepatitis[9], M30 serum concentrations in our study were not associated with obesity (M30 in patients with BMI >30kg/m² median 155.7 U/L vs. BMI <30 kg/m² median 177.8 U/L, not significant).

High M30 serum concentrations are associated with excessive short-term mortality

We found increased M30 levels at admission in those patients, who died at the ICU (n=64, median 324.9 U/L, range 16.7-1000 U/L), compared with surviving patients (n=179, median 166.5 U/L, range 21.6-1001 U/L, p<0.001; Figure 3.2A). Using Cox regression analysis, high M30 levels significantly predicted ICU mortality (p=0.005). Kaplan-Meyer curves were generated by applying the optimal cut-off value (M30 of 250.8 U/L) for the best ratio of sensitivity and specificity for mortality using the Youden-index, displaying the prognostic value of high M30 levels for short-term mortality (Figure 3.2B).

We detected a trend, but no significant difference in M30 levels regarding the overall mortality (non-survivors n=115, median 251 U/L, range 16.7-1000 U/L vs. survivors n=115, median 166.5 U/L, range 21.6-1001 U/L, p=0.059; Figure 3.2C). However, Cox regression analysis remained significant for predicting overall survival as well (p=0.004), and Kaplan-Meier curves displayed a separation between patients with high vs. low M30 levels in their overall survival (Figure 3.2D).

The validity and performance of M30 as a biomarker to predict ICU or overall survival in critically ill patients are summarized in Table 3.4.

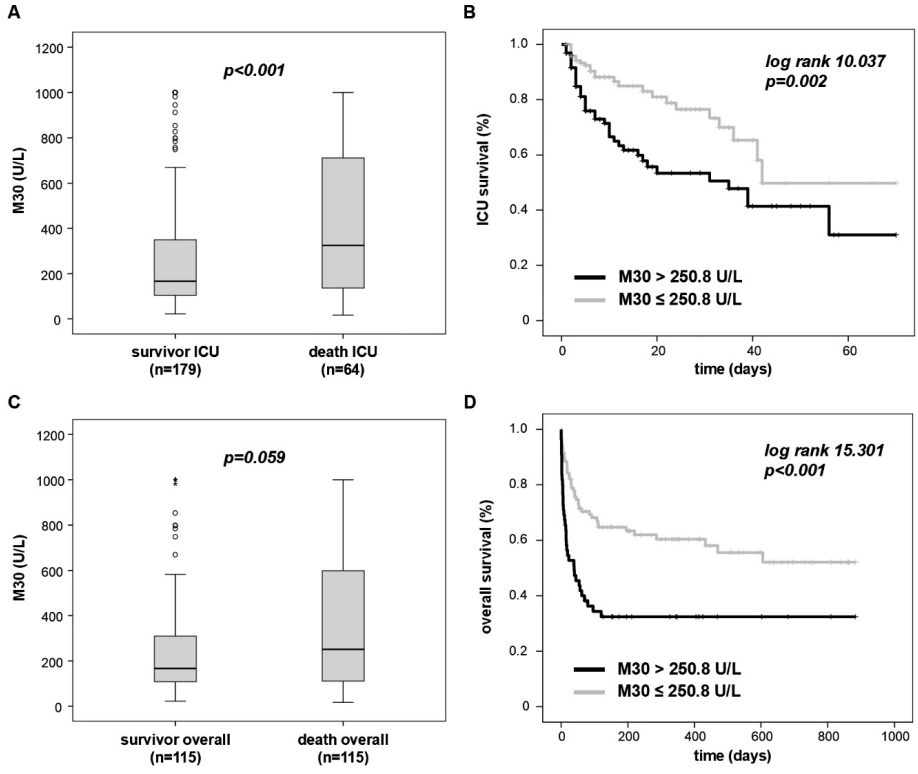


Figure 3.2 **Prediction of mortality by M30 serum levels.** (**A**) Patients that died during the course of ICU treatment had significantly higher serum M30 levels on ICU admission than survivors (p<0.001). (**B**) On Kaplan-Meier survival curve analysis, ICU patients with M30 levels above 250.8 U/L had increased ICU mortality. (**C**) Patients that died during the total observation period displayed a trend towards higher serum M30 levels at admission to the ICU than survivors (p=0.059). (**D**) On Kaplan-Meier survival curve analysis, ICU patients with M30 levels above 250.8 U/L had increased overall mortality, which was apparent especially during the first 30 days after admission.

Table 3.4 Serum **M30** performance as a biomarker to predict ICU or overall mortality.

	ICU mortality	**Overall mortality**
M30 [U/L] optimal cut-off	250.8	250.8
Sensitivity	0.61	0.54
Specificity	0.68	0.70
Positive predictive value	0.41	0.63
Negative predictive value	0.83	0.59
Youden-index	0.29	0.21
LHR+	1.92	1.82
LHR-	0.57	0.65
Diagnostic odds ratio	3.34	2.79

Abbreviation: LHR, likelihood ratio.

As visible from the Kaplan Meier curve analyses, the majority of deaths that separated patients with high from low M30 levels occurred within the first 30 days. In fact, patients that died within the first 30 days had significantly higher M30 levels (n=74, median 294.6 U/L, range 16.7-1000 U/L) than patients that survived (n=169, median 166.5 U/L, range 21.6-1001 U/L, p=0.001). This difference remained significant at later time-points (e.g., 60 days, 90 days, 180 days, 360 days mortality), but was mainly driven by the difference within the first 30 days (detailed data not shown). In addition, this difference regarding M30 levels and 30 days mortality was significant also in the subgroup of sepsis patients (p=0.007), while the smaller subgroup of non-sepsis patients showed a clear trend towards higher M30 levels in patients that died within 30 days after ICU admission (p=0.066).

Discussion

During the apoptotic mode of cell death, caspases, intracellular proteases that cleave aspartate residues, become activated either via the intrinsic (mitochondrial release of cytochrome C) or the extrinsic pathway (inflammatory cytokines and death receptors). The caspases 3, 7, and 9 mediate the early cleavage of the intermediate filament protein cytokeratin 18 in position 396DALD-S, which results in a neoepitope formation that can be detected in serum by the M30 antibody[8].

Our study revealed significantly elevated M30 serum levels in a heterogeneous cohort of critically ill medical patients in close association with disease severity, indicating that apoptosis is a common feature in critical illness and impacts prognosis. As expected, M30 levels correlated with biomarkers reflecting systemic inflammation, supporting that excessive innate immune cell activation is a main driver of programmed cell death. Our study clearly demonstrates that the serum apoptosis marker M30 is independent from the concomitant presence of sepsis. This contrasts previous reports from ICU patients, which have uniformly linked elevated M30 to the clinical course of sepsis[10-13]. Independent from an infectious or noninfectious origin of critical illness, M30 levels correlated with markers of organ dysfunction and disease severity in our study. While these data suggest that hepatocyte apoptosis contributes to circulating M30 in critical illness[2], additional cellular sources might include other epithelial cells, the kidney[21,22], or the gastrointestinal epithelium[23].

As demonstrated in mouse models[24], a substantial quantity of apoptosis in critical illness proceeds in the gut[25], supporting a role of the gut in critical illness by translocation of bacteria through a disrupted intestinal epithelial barrier, alteration of intes- tinal immune tissue, and changes in intestinal microflora[23,26].

Altogether, our data emphasize that not only hypoxia- or hypoperfusion-triggered necroses determine the prognosis in critically ill patients, but also apoptotic pathways that are being boosted by systemic inflammation.

Notably, M30 levels are already quite widely used as a biomarker in chronic liver disorders, especially in nonalcoholic fatty liver disease[8]. In our study, we observed the highest M30 levels in patients with hepatic dysfunction and/or liver cirrhosis. This is well in line with previous studies reporting massively elevated M30 levels in patients with acute liver failure[27], acute-on-chronic liver failure[20], and decompensated liver cirrhosis[9]. These associations emphasize the crucial role of the hepatic function for the prognosis in critical illness, as the assessment of liver failure is already included in several prognostic ICU scores such as the SOFA score[2].

In our study, circulating M30 was an early predictor of adverse outcome upon admission of medical patients to the ICU. M30 levels are correlated to disease severity, organ failure and short-term mortality at the ICU, independent of the presence of sepsis. Our findings indicate a broad clinical relevance of apoptosis in critically ill patients and give impulses for further research. With its strong prognostic value already at ICU admission, M30 is likely to improve risk assessment, if included in novel multi-marker-panels or clinical scoring systems.

Furthermore, the association between high circulating M30 and increased mortality at the ICU in our study implies that a transient inhibition of apoptotic pathways could potentially reduce the excessive short-term mortality in these patients.

While no such clinical trials, stratified by M30 levels, are available in critically ill patients, experimental evidence from animal models suggests that prevention of apoptosis might improve survival in endotoxemia and sepsis[7]. A selective caspase 3 as well as a pan-caspase inhibitor reduced mortality in a mouse model of polymicrobial sepsis, but this effect has been primarily linked to apoptosis of lymphocytes[28].

Similarly, hydrodynamic injection of small interfering RNA (siRNA) against either the Fas death receptor or caspase-8, which effectively targets hepatocytes, reduced mortality in septic mice[29]. On the other hand, apoptosis is a physiological process essential for tissue regeneration as well as a crucial regulator preventing persistent (over-) activation of immune cells, making it challenging to therapeutically interfere with this complex and incompletely understood network[5,30].

Conclusions

Our study demonstrated that circulating levels of the apoptosis-related keratin fragment M30 are significantly elevated in critically ill patients as compared with healthy controls, independent of the presence of sepsis.

M30 levels are correlated with clinical scoring systems for disease severity as well as biomarkers indicating organ dysfunction and inflammation. The remarkably high levels in patients with cirrhosis and the association with liver function tests indicate that hepatocyte apoptosis might contribute substantially to high circulating M30 in critically ill patients. M30 levels above 250.8 U/L at admission to the ICU indicate an unfavourable short-term prognosis.

Further research may explore how this biomarker could be implemented in a multi-marker risk assessment panel at the ICU or help in guiding interventional strategies targeting apoptotic pathways in critical illness.

References

1. Ma KC, Schenck EJ, Pabon MA, Choi AMK. The Role of Danger Signals in the Pathogenesis and Perpetuation of Critical Illness. Am J Respir Critical Care Med 2018;197:300-309.
2. Strnad P, Tacke F, Koch A, Trautwein C. Liver - guardian, modifier and target of sepsis. Nat Rev Gastroenterol Hepatol. 2017;14(1):55-66.
3. Chousterman BG, Swirski FK, Weber GF. Cytokine storm and sepsis disease pathogenesis. Semin Immunopathol. 2017;39:517-528.
4. Aziz M, Jacob A, Wang P. Revisiting caspases in sepsis. Cell Death Dis. 2014;5:e1526.
5. Wesche-Soldato DE, Swan RZ, Chung CS, Ayala A. The apoptotic pathway as a therapeutic target in sepsis. Curr Drug Targets. 2007;8(4):493-500.
6. Huttunen R, Aittoniemi J. New concepts in the pathogenesis, diagnosis and treatment of bacteremia and sepsis. J Infect. 2011;63(6):407-419.
7. Harjai M, Bogra J, Kohli M, Pant AB. Is suppression of apoptosis a new therapeutic target in sepsis? Anaesth Intensive Care. 2013;41(2): 175-183.
8. Ku NO, Strnad P, Bantel H, Omary MB. Keratins: Biomarkers and modulators of apoptotic and necrotic cell death in the liver. Hepatology. 2016;64(3): 966-976.
9. Mazzolini G, Sowa JP, Canbay A. Cell death mechanisms in human chronic liver diseases: a far cry from clinical applicability. Clin Sci. 2016;130(23): 2121-2138.
10. Roth GA, Krenn C, Brunner M, Moser B, Ploder M, Spittler A, et al. Elevated serum levels of epithelial cell apoptosis-specific cytokeratin 18 neoepitope m30 in critically ill patients. Shock. 2004;22(3): 218-220.
11. Hofer S, Brenner T, Bopp C, Steppan J, Lichtenstern C, Weitz J, et al. Cell death serum biomarkers are early predictors for survival in severe septic patients with hepatic dysfunction. Crit Care. 2009;13(3): R93.
12. Lorente L, Martin MM, Gonzalez-Rivero AF, Ferreres J, Sole-Violan J, Labarta L, et al. Serum levels of caspase-cleaved cytokeratin-18 and mortality are associated in severe septic patients: pilot study. PloS One. 2014;9(10): e109618.
13. Moore DJ, Greystoke A, Butt F, Wurthner J, Growcott J, Hughes A, et al. A pilot study assessing the prognostic value of CK18 and nDNA biomarkers in severe sepsis patients. Clin Drug Invest. 2012;32(3): 179-187.
14. Koch A, Weiskirchen R, Kunze J, Duckers H, Bruensing J, Buendgens L, et al. Elevated asymmetric dimethylarginine levels predict short- and long-term mortality risk in critically ill patients. J Crit Care. 2013;28(6): 947-953.
15. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA. 2016;315(8): 801-810.
16. Rhodes A, Evans LE, Alhazzani W, Levy MM, Antonelli M, Ferrer R, et al. Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock: 2016. Intensive Care Med. 2017;43(3): 304-377.
17. Koch A, Voigt S, Kruschinski C, Sanson E, Duckers H, Horn A, et al. Circulating soluble urokinase plasminogen activator receptor is stably elevated during the first week of treatment in the intensive care unit and predicts mortality in critically ill patients. Crit Care. 2011;15(1): R63.
18. Buendgens L, Yagmur E, Bruensing J, Herbers U, Baeck C, Trautwein C, et al. C-terminal proendothelin-1 (CT-proET-1) is associated with organ failure and predicts mortality in critically ill patients. J Intensive Care. 2017;5: 25.
19. Backes Y, van der Sluijs KF, Mackie DP, Tacke F, Koch A, Tenhunen JJ, et al. Usefulness of suPAR as a biological marker in patients with systemic inflammation or infection: a systematic review. Intensive Care Med. 2012;38(9): 1418-1428.
20. Adebayo D, Morabito V, Andreola F, Pieri G, Luong TV, Dhillon A, et al. Mechanism of cell death in acute-on-chronic liver failure: a clinico-pathologic-biomarker study. Liver Int. 2015;35(12): 2564-2574.
21. Chu PG, Weiss LM. Keratin expression in human tissues and neoplasms. Histopathology. 2002;40(5): 403-439.
22. Roth GA, Lebherz-Eichinger D, Ankersmit HJ, Hacker S, Hetz H, Vukovich T, et al. Increased total cytokeratin-18 serum and urine levels in chronic kidney disease. Clin Chim Acta 2011;412: 713-717.

23. Hotchkiss RS, Schmieg RE, Jr., Swanson PE, Freeman BD, Tinsley KW, Cobb JP, et al. Rapid onset of intestinal epithelial and lymphocyte apoptotic cell death in patients with trauma and shock. Crit Care Med. 2000;28(9): 3207-3217.
24. Hiramatsu M, Hotchkiss RS, Karl IE, Buchman TG. Cecal ligation and puncture (CLP) induces apoptosis in thymus, spleen, lung, and gut by an endotoxin and TNF-independent pathway. Shock. 1997;7(4): 247-253.
25. Clark JA, Coopersmith CM. Intestinal crosstalk: a new paradigm for understanding the gut as the "motor" of critical illness. Shock. 2007;28(4): 384-393.
26. Haak BW, Wiersinga WJ. The role of the gut microbiota in sepsis. Lancet Gastroenterol Hepatol. 2017;2(2): 135-143.
27. Bechmann LP, Jochum C, Kocabayoglu P, Sowa JP, Kassalik M, Gieseler RK, et al. Cytokeratin 18-based modification of the MELD score improves prediction of spontaneous survival after acute liver injury. J Hepatol. 2010;53(4): 639-647.
28. Hotchkiss RS, Chang KC, Swanson PE, Tinsley KW, Hui JJ, Klender P, et al. Caspase inhibitors improve survival in sepsis: a critical role of the lymphocyte. Nat. Immunol. 2000;1(6): 496-501.
29. Wesche-Soldato DE, Chung CS, Lomas-Neira J, Doughty LA, Gregory SH, Ayala A. In vivo delivery of caspase-8 or Fas siRNA improves the survival of septic mice. Blood. 2005;106(7): 2295-2301.
30. Hotchkiss RS, Nicholson DW. Apoptosis and caspases regulate death and inflammation in sepsis. Nat Rev Immunol. 2006;6(11): 813-822.

Chapter 4

High-mobility Group Box 1 (HMGB1) as a Biomarker in Critically Ill Patients

Eray Yagmur
Lukas Buendgens
Ulf Herbers
Anne Beeretz
Ralf Weiskirchen
Ger H. Koek
Christian Trautwein
Frank Tacke
Alexander Koch

J Clin Lab Anal. 2018:32(8):e22584

Abstract

Background
Extracellular release of high-mobility group box 1 (HMGB1) acts as a danger-associated molecular pattern (DAMP), thereby "alarming" the immune system and promoting systemic inflammation. We investigated plasma HMGB1 concentrations as a potential diagnostic and prognostic biomarker in critical illness.

Methods
Our study included 218 critically ill patients (145 with sepsis, 73 without sepsis), of whom blood samples were obtained at the time-point of admission to the medical intensive care unit (ICU).

Results
HMGB1 levels were significantly elevated in critically ill patients (n=218) compared with healthy controls (n=66). Elevated HMGB1 plasma levels were independent from the presence of sepsis. Moreover, HMGB1 was not associated with disease severity, organ failure or mortality in the ICU. We observed a trend towards lower HMGB1 levels in ICU patients with pre-existing obesity, type 2 diabetes and end-stage renal disease patients on chronic hemodialysis.

Conclusion
In conclusion, our study did not reveal significant associations between HMGB1 levels at ICU admission and clinical outcomes in critically ill patients. Due to the pathogenic role of HMGB1 in the late phases of experimental sepsis, future studies might assess the potential value of HMGB1 by measuring its plasma concentrations at later time points during the course of critical illness.

Introduction

High-mobility group box 1 (HMGB1) protein (OMIM: 163905) was initially identified as a nuclear protein, but it gained tremendous attention as an extracellular molecule acting as a danger-associated molecular pattern (DAMP).

As an intracellular protein, HMGB1 is involved in DNA replication, transcription and repair mechanisms[1-3]. As an extracellular mediator, it plays a key role in activating cascades of inflammatory processes and diverse additional extracellular biological activities[4,5]. Notably, in critical illness, it is therefore assumed that HMGB1 mediates the cellular stress response associated with organ failure, systemic inflammation and infection[6-8]. Thus, HMGB1 is widely considered as the prototypic DAMP or alarmin[9,10].

HMGB1 itself has cytokine, chemokine and growth factor activity, regulating the inflammatory and immunological response as a ligand for toll-like receptor (TLR) 2/4, the chemokine CXCL12 and for receptor for advanced glycation end-products (RAGE)[11,12]. Tissue macrophages are the main cellular target of HMGB1 and other alarmins[13]. HMGB1 is released in response to pathogenic infection and actively secreted for chemotactic and cytokine-like function, but also supports tissue repair mechanisms, including angiogenesis, fibrosis and tissue regeneration (1,5). In experimental models of sepsis, HMGB1 has been described as a late mediator of sepsis, and its blockade in murine sepsis models improved mortality[14,15]. All these characteristics fuelled the hypothesis that circulating HMGB1 could be a valuable diagnostic tool in critically ill patients in terms of systemic inflammation, severity of disease and mortality[6,16,17].

Although prior studies have unanimously reported elevated HMGB1 levels in the setting of critical illness, the associations between HMGB1 and clinical outcome are controversial[18-20]. In addition, it remained unclear, whether the presence of critical illness or the presences of severe infection or sepsis are the main determinants of increased HMGB1 plasma levels[19].

This prompted us to revisit the role of HMGB1 in a large, well characterized cohort of critically ill patients with and without sepsis at the medical intensive care unit (ICU).

Methods

Study design and patient characteristics

Critically ill patients were included at admission to the medical ICU at the RWTH University Hospital Aachen, Germany. Patients, who were admitted for post-interventional observational stay or underwent an elective procedure, were excluded[21]. The local ethics committee approved our study in accordance to the ethical standards laid down in the Declaration of Helsinki (reference number EK 150/06).

The patients were categorized as sepsis and non-sepsis according to the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3)[22], and were treated following the current guidelines for treatment of sepsis (Surviving Sepsis Campaign)[23]. As a healthy control group, we analysed blood donors with normal blood counts, normal values of liver enzymes and a negative serology for viral hepatitis and HIV[24].

In order to determine long-term outcome, we contacted the patients, their relatives and/or the general practitioner in approximately 6-months intervals after discharge from the hospital for two years[24].

Measurements of HMGB1 plasma levels

Blood samples were collected at the time of admission (before specific therapeutic measures), centrifuged, and plasma was stored at -80°C. Plasma HMGB1 concentrations were determined using a quantitative sandwich enzyme immunoassay (ELISA), according to manufacturer`s instructions (HMGB1 ELISA, #ST51011, IBL International, Hamburg, Germany).

Statistical analysis

Due to the skewed distribution of the parameters, data are given as median and range, and graphically displayed by box-and-whiskers plots. The degree of association between two variables was assessed by the Spearman rank correlation test. Comparisons of parameters between two different groups were conducted with the Mann-Whitney U-test.

All values, including outside values as well as far out values, were included. The prognostic value of HMGB1 was explored using three groups, consisting of the patients with HMGB1 from the lowest quartile, from the middle 50% and from the highest quartile, by Kaplan Meier curves for ICU as well as for overall survival[24]. P-values less

than 0.05 were considered as statistically significant. All analyses were performed with IBM SPSS Statistics (SPSS; Chicago, Illinois).

Results

HMGB1 plasma levels are significantly elevated in critically ill patients as compared with healthy controls

HMGB1 plasma levels were significantly elevated in a large cohort of 218 critically ill medical patients (median 10.37 ng/ml, range 0.25-80 ng/ml; Table 4.1) at admission to the ICU, as compared with 66 healthy controls (median 2.67 ng/ml, range 0.25-10.47 ng/ml, p<0.001; Figure 4.1A).

Among the critically ill patients, there was no clear association between HMGB1 plasma concentrations and different disease aetiologies leading to ICU admission (data not shown).

Table 4.1 Baseline patient characteristics and **HMGB1** plasma measurements.

Parameter	All Patients	Non-Sepsis	Sepsis	*p-value**
Number	218	73	145	
Sex (male/female)	133 / 85	48 / 25	85 / 60	*0.378*
Age median (range) [years]	64 (18-90)	61 (18-85)	65 (20-90)	*0.275*
APACHE-II score median (range)	18 (2-43)	13.5 (2-33)	19 (4-43)	*<0.001*
ICU days median (range)	7 (1-137)	6 (1-45)	9 (1-137)	*0.004*
Death during ICU n(%)	49 (22.5%)	9 (12.3%)	40 (27.6%)	*0.010*
Death during follow-up (total) n(%)	89 (40.8%)	22 (30.1%)	67 (46.2%)	*0.028*
Mechanical ventilation n(%)	145 (66.5%)	46 (63%)	99 (68.3%)	*0.451*
Ventilation time median (range) [h]	86 (0-3628)	31 (0-3628)	117 (0-2966)	*0.027*
Pre-existing diabetes n(%)	63 (28.9%)	22 (30.1%)	41 (28.3%)	*0.874*
BMI median (range) [m^2/kg]	25.8 (14-86)	25.7 (15.9-40.5)	28.9 (14-86.5)	*0.539*
WBC median (range) [x10^3/µl]	13.1 (0.1-208)	12.5 (1.8-29.6)	14.1 (0.1-208)	*0.017*
CRP median (range) [mg/dl]	100.5 (5-230)	17 (5-230)	164 (5-230)	*<0.001*
Procalcitonin median (range) [µg/l]	0.7 (0.03-207.5)	0.2 (0.03-100)	2.2 (0.1-207.5)	*<0.001*
Creatinine median (range) [mg/dl]	1.3 (0.1-15)	1.0 (0.2-15)	1.5 (0.1-10.7)	*0.013*
GFR Cystatin C median (range) [ml/min]	34.5 (3-379)	59 (5-379)	22 (3-218)	*0.001*
AST median (range) [U/l]	42 (7-20332)	47 (11-20332)	41 (7-7832)	*0.165*
ALT median (range) [U/l]	30 (3-7867)	37 (7-7867)	25 (3-5890)	*0.064*
Bilirubin median (range) [mg/dl]	0.7 (0.1-20.8)	0.7 (0.1-20.8)	0.7 (0.1-18.9)	*0.952*
INR median (range)	1.16 (0.92-13)	1.17 (0.95-6.73)	1.16 (0.92-13)	*0.864*
HMGB1 day 1 median (range) [ng/ml]	10.37 (0.20-80)	11.21 (0.20-76.04)	10.23 (0.20-80)	*0.336*

For quantitative variables, median and range (in parenthesis) are given. *p-values for the comparison of sepsis and non-sepsis patients are given (U-test for quantitative variables or chi-square test for qualitative parameters). Abbreviations: ALT, alanine aminotransferase; APACHE: Acute Physiology And Chronic Health Evaluation; AST, aspartate aminotransferase; BMI: body mass index; CRP: C-reactive protein; ICU: intensive care unit; INR: international normalized ration; WBC: white blood cell.

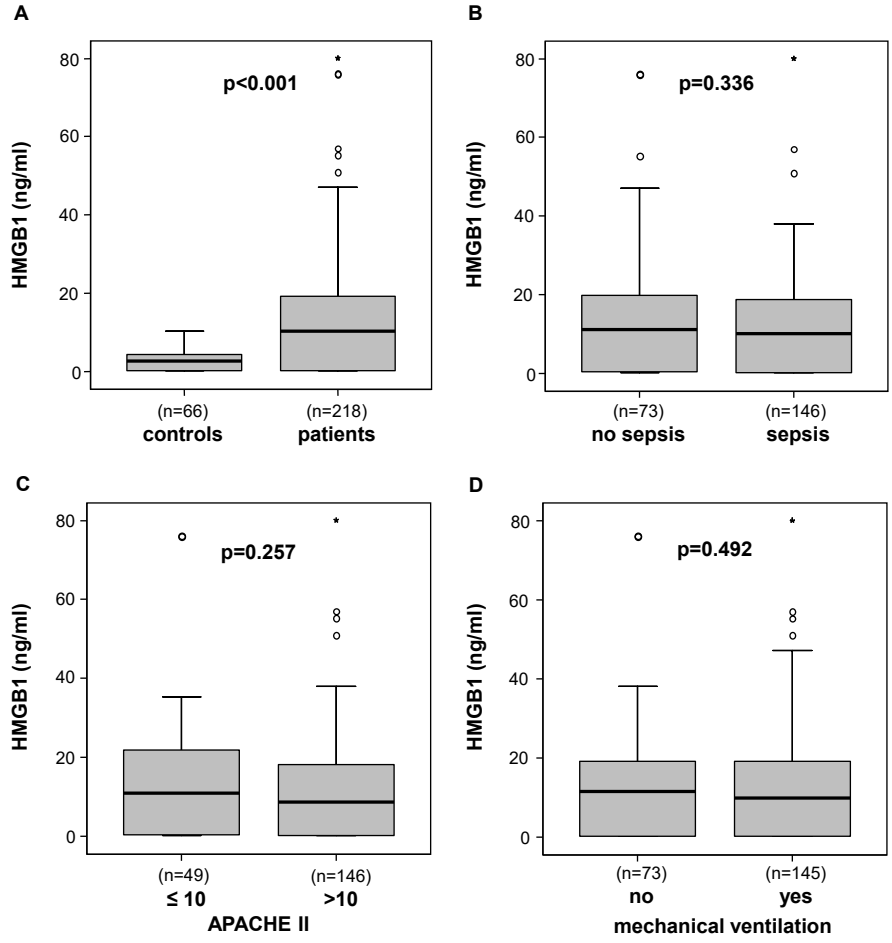


Figure 4.1 HMGB1 levels in critically ill patients. (**A**) HMGB1 plasma concentrations are significantly elevated in critically ill patients (n=218) compared with healthy controls (n=66). (**B**) HMGB1 levels do not differ between ICU patients with (n=146) or without sepsis (n=73). (**C**) High disease severity, as defined by an APACHE-II score above 10 (n=146), is not associated with elevated plasma HMGB1. (**D**) The need of mechanical ventilation (n=145) was not associated with HMGB1 levels at ICU admission. P-values (U-test) are given in the figure.

Elevated HMGB1 plasma levels in critically ill patients are independent of the presence of sepsis

Elevated HMGB1 have been previously reported in patients with bacteraemia, sepsis and septic shock[18-20]. Within the cohort of ICU patients, HMGB1 levels did not differ between patients with sepsis (n=146, median HMGB1 10.23 ng/ml, range 0.2-80 ng/ml)

and patients without sepsis (n=73, median 11.21 ng/ml, range 0.2-76.04 ng/ml; Figure 4.1B).

Typical sites of infection in sepsis were pneumonia, abdominal and urogenital tract, while non-sepsis causes of critical illness included, among others, cardiopulmonary diseases, acute pancreatitis and decompensated liver cirrhosis (detailed data not shown).

HMGB1 plasma levels are not associated with disease severity or mechanical ventilation

Circulating HMGB1 has been previously suggested as a biomarker for disease severity in various clinical settings[25]. Plasma HMGB1 concentrations were not associated with disease severity, determined by the Acute Physiology And Chronic Health Evaluation-II (APACHE-II) score. Patients with a high APACHE-II score (above 10) did not have higher HMGB1 levels than patients with an APACHE-II score below or equal to 10 (Figure 4.1C).

In addition, there was also no significant difference in HMGB1 plasma levels between ventilated or non-ventilated critically ill patients (median 9.9 ng/ml vs. median 11.5 ng/ml no ventilation, p=0.492) (Figure 4.1D).

Association of HMGB1 plasma levels in critically ill patients with metabolic and renal comorbidities

HMGB1 has been associated with metabolic disorders[26,27]. We therefore assessed whether metabolic comorbidities, including pre-existing obesity or diabetes impacted HMGB1 levels at ICU admission.

Interestingly, patients with pre-existing obesity, defined as a body mass index above 30 kg/m², showed a trend towards lower HMGB1 levels at ICU admission (median 5.93 ng/ml vs. median 11.6 ng/ml in non-obese patients, p=0.052) (Figure 4.2A). Patients with pre-existing type 2 diabetes have slightly lower HMGB1 levels (median 8.29 ng/ml in diabetics vs. median 11.1 ng/ml in non-diabetics, not significant; Figure 4.2B). This finding corresponds to the inverse correlation between HMGB1 and blood glucose at ICU admission (Table 4.2).

We next investigated the potential association between HMGB1 and renal diseases, based on its involvement in chronic renal disorders[28]. A small subgroup of our cohort consisted of end-stage renal disease patients requiring chronic hemodialysis (n=9). These patients showed a clear trend towards lower HMGB1 levels at ICU admission (median 3.4 ng/ml in patients with vs. median 9.9 ng/ml in patients without chronic renal replacement therapy, p=0.259) (Figure 4.2C).

However, in the whole cohort of critically ill patients, we did not observe a correlation between HMGB1 and classical markers of renal failure, such as creatinine or cystatin C. HMGB1, however, correlated inversely with urea, likely reflecting renal function, metabolism and nutritional status (**Table 4.2**).

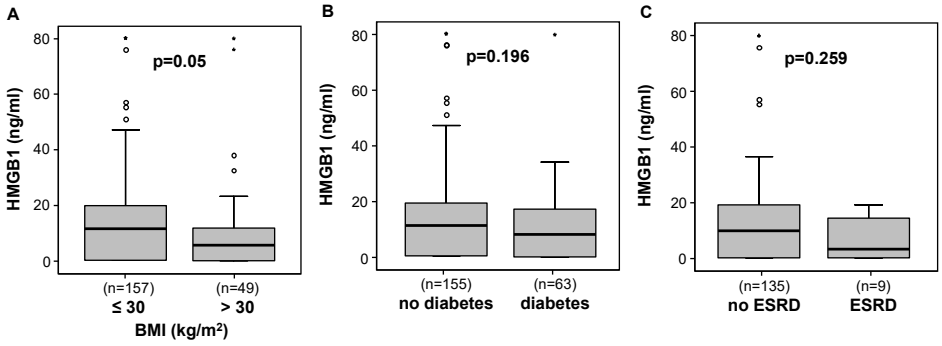


Figure 4.2 Impact of metabolic comorbidities on HMGB1 levels. HMGB1 plasma concentrations did not differ between ICU patients with or without obesity, as defined by a body-mass index (BMI) above 30 kg/m^2 (n=49, **A**), pre-existing type 2 diabetes (n=63, **B**) or pre-existing end-stage renal disease (n=9, ESRD, **C**).

Table 4.2 Correlations with HMGB1 plasma concentrations at ICU admission day (Spearman rank correlation test, only significant results are shown).

	ICU patients	
Parameters	**r**	***p***
Age	-0.238	*<0.001*
BMI	-0.161	*0.021*
Lactate	0.144	*0.035*
Urea	-0.173	*0.044*
Bilirubin	0.176	*0.010*
Bilirubin conjugated	0.213	*0.017*
Glucose	-0.167	*0.014*
HDL cholesterol	-0.324	*0.008*
Haemoglobin	0.164	*0.015*
Haematocrit	0.159	*0.018*

Abbreviations: BMI: body mass index; HDL cholesterol; high density lipoprotein cholesterol.

High HMGB1 plasma concentrations at ICU admission are not associated with adverse prognosis

In critically patients, who subsequently died during the ICU treatment (n=49), we did not find significantly altered HMGB1 levels at admission to the ICU, suggesting that HMGB1 is not a prognostic biomarker in critical diseases[16].

Nevertheless, we observed a trend towards increased HMGB1 levels in the deceased patients compared to the surviving patients (median 12.89 ng/ml vs. median 9.65 ng/ml in ICU survivors) (Figure 4.3A), while there was no significant difference for the overall mortality either (Figure 4.3B).

By Kaplan-Meier curve analysis, patients with HMGB1 levels of the highest quartile (>19.2 ng/ml) showed a tendency towards improved ICU (Figure 4.3C) or overall survival (Figure 4.3D), but did not reach statistical significance.

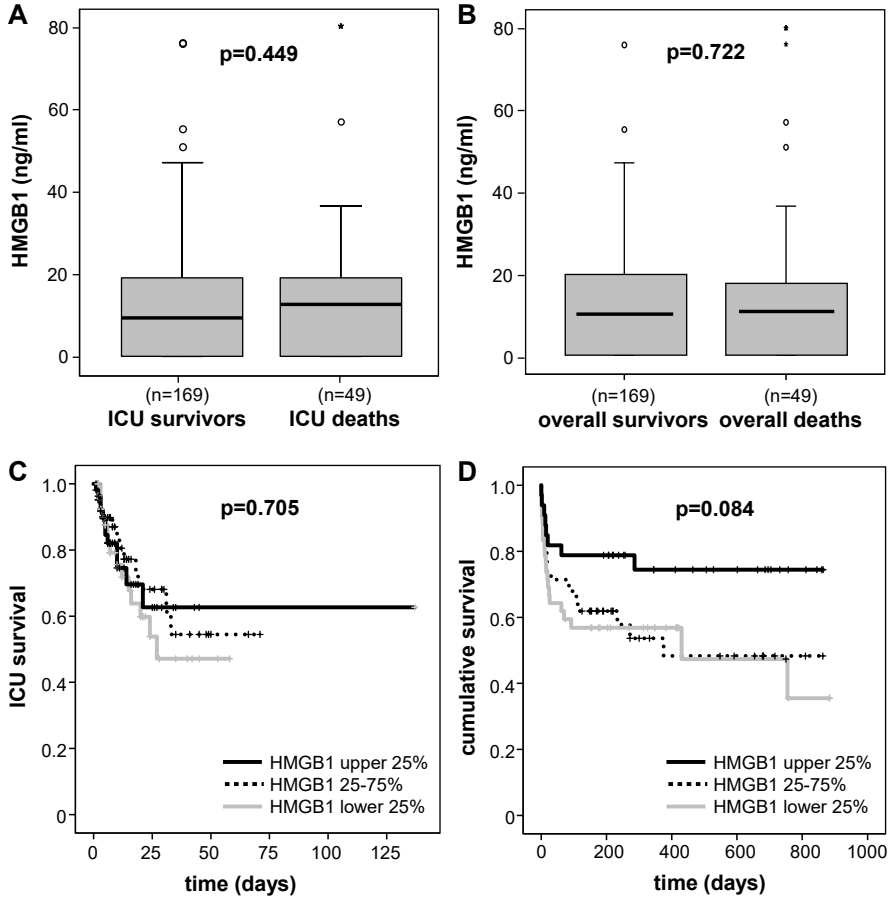


Figure 4.3 HMGB1 is not a prognostic biomarker for mortality in critically ill patients. (**A-B**) Patients that died during the course of ICU treatment (n=49, **A**) and/or during follow-up (n=89, **B**) are characterized by a tendency towards higher plasma HMGB1 concentrations already at ICU admission. (**C-D**) Kaplan-Meier survival curves of ICU patients are displayed, showing that patients with HMGB1 levels of upper quartile (on admission >19.2 ng/mL; black) had a tendency towards higher ICU (**C**) as well as overall survival (**D**) as compared to patients with HMGB1 serum concentrations of lower quartile (on admission <0.2 ng/ml; grey) or middle 50% (dotted line). P-values are given in the figure.

Discussion

Hypoxic, stressed, injured or dying cells release DAMPs, or alarmins, to activate the immune system, promote inflammation, but also initiate tissue repair[29]. These processes are presumed to be hyper-activated in critical illness, where cell death, systemic inflammation, tissue hypoperfusion and infection determine the clinical course in the ICU[30].

HMGB1 is a blueprint of DAMPs, because it can be actively released by innate immune cells in response to exogenous bacterial products or endogenous inflammatory stimuli and can be passively released from damaged parenchymal cells[5,12,31]. Animal models supported the hypothesis that circulating HMGB1 is involved in the pathogenesis of sepsis[7].

However, unlike classical inflammatory cytokines (e.g., TNF), an increase in HMGB1 is not observed within the first hours after sepsis induction, but was a characteristic of the late phase of sepsis associated with lethality[14,15,32-34]. In turn, neutralization of HMGB1 during the late phase of sepsis using specific antibodies prevented rodents from sepsis-related death[33,35]. Based on its pathogenic role, circulating HMGB1 has been suggested as a biomarker for the assessment of sepsis, disease severity and mortality[6,16,17].

However, our study with a prospectively enrolled, large and heterogeneous cohort of critically ill medical patients clearly demonstrated that HMGB1 has limited value as a biomarker in the ICU setting. Although we did confirm elevated HMGB1 plasma levels in critically ill patients as compared to healthy controls, in line with previous reports[18-20], HMGB1 (sampled at ICU admission) was not an indicator of disease severity, organ failure or mortality.

Even more surprising, patients with sepsis did not display different HMGB1 concentrations as compared to ICU patients without sepsis. Earlier studies from Denmark that included 194 and 185 patients had found higher levels of HMGB1 in infectious versus non-infectious diseases[18,36].

On the contrary, HMGB1 was not able to discriminate sepsis from non-infectious disease in the setting of an Emergency Department, as identified in a cohort of 631 patients with heterogeneous diseases[37]. Similar controversies have been reported regarding the potential association of HMGB1 to disease severity and mortality. HMGB1 was reported to be elevated in patients with severe sepsis and septic shock compared to patients with sepsis[19,36], but did not differ between survivors or non-survivors in an observational study that included 247 patients from 24 ICUs in Finland[19].

Interestingly, while HMGB1 levels at ICU admission did not predict mortality in a French study on 42 critically ill patients with septic shock, HMGB1 levels at day 3 were able to discriminate between survivors and non-survivors in a medical ICU setting[20]. In a study from the Karolinska institute that analyzed 64 patients (including 33 with septic shock), HMGB1 remained high in patients for 1 week after ICU admission[16], supporting that HMGB1 is a downstream and late mediator of inflammation. Thus, the lack of clinical utility as a biomarker in the early phase of critical disease at ICU admission, as demonstrated by our study, does not preclude its potential value in later phases of the clinical course. Future studies should particularly focus on longitudinal measurements of HMGB1 in the ICU.

In addition, circulating HMGB1 in patient's plasma might not necessarily reflect the full biological activity during critical illness. On the one hand, local concentrations in ischemic or injured tissue might be higher - and immediately bind to TLR2, TLR4 or RAGE on local macrophages[25]. On the other hand, a 30-kDa low molecular weight HMGB1 variant was detected in sepsis patients[5,14], suggesting that HMGB1 may form large complexes with other serum components that might not be detected by conventional ELISA systems.

These unidentified HMGB1-binding molecules or chemical modifications may affect the biological activities or immunodetection of HMGB1. For instance, a recent study indicated that reactive oxygen species (ROS) may oxidise HMGB1 and consequently abolish HMGB1-mediated immunostimulatory activities[38,39].

Moreover, exogenous factors might influence HMGB1 concentrations in the circulation. For instance, metformin or statins have been associated with decreased plasma HMGB1[40].

Nonetheless, it is important to note the limitations of our study. We conducted a monocentric, observational study with relatively broad inclusion criteria. While this reflects a "real-world situation" of critically ill patients at a medical ICU, it introduces a substantial heterogeneity regarding patient characteristics and therefore resulting in relatively small subgroups, which may be underpowered for further subgroup analyses.

Due to the prospective inclusion study design with an uncertain further course in the ICU, a substantial fraction of the patients had a rather low APACHE-II score, which may have reduced the discriminant role of HMGB1 in our cohort.

Conclusion

In conclusion, we herein demonstrated significantly elevated HMGB1 plasma concentrations in critically ill patients, corroborating the extracellular properties of HMGB1 as an alarmin signal.

However, our study did not reveal a significant association between HMGB1 levels at ICU admission and clinical outcomes in critically ill patients. This overt discrepancy of HMGB1's important pathogenic role in experimental sepsis and its poor performance as a clinical biomarker for prediction of outcome in critical illness might be related to its function during late stages of critical disease, which cannot be accurately captured at admission to the ICU.

Prospective studies should investigate the practical clinical value of longitudinal HMGB1 plasma concentrations during the course of critical illness.

References

1. Ding J, Cui X, Liu Q. Emerging role of HMGB1 in lung diseases: friend or foe. J Cell Mol Med. 2017;21: 1046-1057.
2. Lu B, Wang H, Andersson U, Tracey KJ. Regulation of HMGB1 release by inflammasomes. Protein Cell. 2013;4;163-167.
3. Ferrari S, Finelli P, Rocchi M, Bianchi ME. The active gene that encodes human high mobility group 1 protein (HMG1) contains introns and maps to chromosome 13. Genomics. 1996;35:367-371.
4. Kang R, Chen R, Zhang Q, et al. HMGB1 in health and disease. Mol Aspects Med. 2014;40:1-116.
5. Wang H, Zhu S, Zhou R, Li W, Sama AE. Therapeutic potential of HMGB1-targeting agents in sepsis. Expert Rev Mol Med. 2008;10: e32
6. Gentile LF, Moldawer LL. HMGB1 as a therapeutic target for sepsis: it`s all in the timing! Expert Opin Ther Targets. 2014;18:243-245.
7. Wang H, Ward MF, Sama AE. Targeting HMGB1 in the treatment of sepsis. Expert Opin Ther Targets. 2014;18:257-268.
8. Huang W, Tang Y, Li L. HMGB1, a potent proinflammatory cytokine in sepsis. Cytokine. 2010;51:119-126.
9. Keyel PA. How is inflammation initiated? Individual influences of IL-1, IL-18 and HMGB1. Cytokine. 2014;69:136-145.
10. Lu B, Wang C, Wang M, et al. Molecular mechanism and therapeutic modulation of high mobility group box 1 release and action: an updated review. Expert Rev Clin Immunol. 2014;10:713-727.
11. Chiba S, Baghdadi M, Akiba H, et al. Tumor-infiltrating DCs suppress nucleic acid-mediated innate immune responses through interactions between the receptor TIM-3 and the alarmin HMGB1. Nat Immunol. 2012;13:832-842.
12. Chen GY, Tang J, Zheng P, Liu Y. CD24 and Siglec-10 selectively repress tissue damage-induced immune responses. Science. 2009;323:1722-1725.
13. Krenkel O, Tacke F. Liver macrophages in tissue homeostasis and disease. Nat Rev Immunol. 2017; 17(5):306-321.
14. Wang H, Bloom O, Zhang M, et al. HMG-1 as a late mediator of endotoxin lethality in mice. Science. 1999;285:248-251.
15. Abraham E, Arcaroli J, Carmody A, Wang H, Tracey KJ. HMG-1 as a mediator of acute lung inflammation. J Immunol. 2000;165:2950-2954.
16. Sundèn-Cullberg J, Norrby-Teglund A, Rouhiainen A, et al. Persistent elevation of high mobility group box-1 protein (HMGB1) in patients with severe sepsis and septic shock. Crit Care Med. 2005;33:564-573.
17. Angus DC, Yang L, Kong L, et al. Circulating high-mobility group box 1 (HMGB1) concentrations are elevated in both uncomplicated pneumonia and pneumonia with severe sepsis. Crit Care Med. 2007;35:1061-1067.
18. Gaïni S, Pedersen SS, Koldkjaer OG, Pedersen C, Møller HJ. High mobility group box-1 protein in patients with suspected community-acquired infections and sepsis: a prospective study. Crit Care. 2007;11:R32.
19. Karlsson S, Pettilä V, Tenhunen J, Laru-Sompa R, Hynninen M, Ruokonen E. HMGB1 as a predictor of organ dysfunction and outcome in patients with severe sepsis. Intensive Care Med. 2008;34:1046-1053.
20. Gibot S, Massin F, Cravoisy A, et al. High-mobility group box 1 protein plasma concentrations during septic shock. Intensive Care Med. 2007;33:1347-1353.
21. Koch A, Weiskirchen R, Kunze J, et al. Elevated asymmetric dimethylarginine levels predict short- and long-term mortality risk in critically ill patients. J Crit Care. 2013;28:947-953.
22. Buendgens L, Yagmur E, Bruensing J, et al. Growth Differentiation Factor-15 Is a Predictor of Mortality in Critically Ill Patients with Sepsis. Dis Markers. 2017;5271203.
23. Rhodes A, Evans LE, Alhazzani W, et al. Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock: 2016. Intensive Care Med. 2017;43:304-377.
24. Koch A, Voigt S, Kruschinski C, et al. Circulating soluble urokinase plasminogen activator receptor is stably elevated during the first week of treatment in the intensive care unit and predicts mortality in critically ill patients. Crit Care. 2011;15:R63.
25. Venereau E, De Leo F, Mezzapelle R, Careccia G, Musco G, Bianchi ME. HMGB1 as biomarker and drug target. Pharmacol Res. 2016;111:534-544.

26. Wang H, Zhu S, Ward MF, Gong J, Sama AE. Hyperglycemia aggravates endotoxin-induced high mobility group box 1 protein release: yet another reason not to be too sweet. Crit Care Med. 2008;36: 2475-2476.
27. Hagiwara S, Iwasaka H, Hasegawa A, Koga H, Noguchi T. Effects of hyperglycemia and insulin therapy on high mobility group box 1 in endotoxin-induced acute lung injury in a rat model. Crit Care Med. 2008;36:2407-2413.
28. Zhou TB. Role of high mobility group box 1 and its signaling pathways in renal diseases. J Recept Signal Transduct Res. 2014;34:348-350.
29. Timmermanns K, Kox M, Scheffer GJ, Pickkers P. Danger in the intensive care unit: damps in critically ill patients. Shock. 2016;45:108-116.
30. Hotchkiss RS, Moldawer LL, Opal SM. Sepsis and septic shock. Nat Rev Dis Primers. 2016;2:16045. doi: 10.1038/nrdp.2016.45.
31. Andersson U, Tracey KJ. HMGB1 is a therapeutic target for sterile inflammation and infection. Annu Rev Immunol. 2011;29:139-162.
32. Tracey KJ, Fong Y, Hesse DG, et al. Anti-cachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteraemia. Nature. 1987;330:662-664.
33. Yang H, Ochani M, Li J, et al. Reversing established sepsis with antagonists of endogenous high-mobility group box 1. Proc Natl Acad Sci U S A. 2004;101:296-301.
34. Hotchkiss RS, Karl IE. The pathophysiology and treatment of sepsis. N Engl J Med. 2003;348:138-150.
35. Qin S, Wang H, Yuan R, et al. Role of HMGB1 in apoptosis-mediated sepsis lethality. J Exp Med. 2006;203:1637-1642.
36. Gaïni S, Koldkjaer OG, Møller HJ, Pedersen C, Pedersen SS. A comparison of high-mobility group box-1 protein, lipopolysaccharide-binding protein and procalcitonin in severe community-acquired infections and bacteremia: a prospective study. Crit Care. 2007;11:R76.
37. Gámez-Díaz L, Enriquez LE, Matute JD, et al. Diagnostic accuracy of HMGB-1, sTREM-1, and CD64 as markers of sepsis in patients recently admitted to the emergency department. Acad Emerg Med. 2011;18:807-815.
38. Tang D, Kang R, Zeh HJ 3rd, Lotze MT. High-mobility group box 1, oxidative stress, and disease. Antioxid Redox Signal. 2011;14:1315-1335.
39. Li G, Tang D, Lotze MT. Mènage à Trois in stress: DAMPs, redox and autophagy. Semin Cancer Biol. 2013;23:380-390.
40. de Souza AW, van der Geest KS, Brouwer E, Pinheiro FA, Oliveira AC, Sato EI, Andrade LE, Bijl M, Westra J, Kallenberg CG. High mobility group box 1 levels in large vessel vasculitis are not associated with disease activity but are influenced by age and statins. Arthritis Res Ther. 2015;17:158.

Part II

Role of Specific Adipokines in Critical Ill Patients

Chapter 5

Visfatin serum levels predict mortality in critically ill patients

Alexander Koch
Ralf Weiskirchen
Alexander Krusch
Jan Bruensing
Lukas Buendgens
Ulf Herbers
Eray Yagmur
Ger H. Koek
Christian Trautwein
Frank Tacke

Dis Markers. 2018:7315356

Abstract

The adipokine visfatin, also termed pre-B-cell colony enhancing factor (PBEF), is mainly derived from adipose tissue, but has been implicated in the regulation of innate immune responses.

We hypothesized that visfatin could be a potential circulating biomarker in critical illness and sepsis. We therefore measured serum levels of visfatin in a cohort of 229 critically ill medical patients upon admission to the intensive care unit (ICU). In comparison to 53 healthy controls, visfatin levels were significantly elevated in medical ICU patients, especially in patients with sepsis. Visfatin serum concentrations were strongly associated with disease severity and organ failure, but did not differ between patients with or without obesity or type 2 diabetes.

Visfatin levels correlated with biomarkers of renal failure, liver dysfunction and other adipokines (e.g., resistin, leptin, adiponectin) in critically ill patients. High visfatin levels at ICU admission indicated an increased mortality, both at the ICU and during long-term follow-up of approximately two years.

Our data therefore demonstrate that circulating visfatin is a valuable biomarker for risk and prognosis assessment in critically ill patients. Furthermore, visfatin seems to be involved in the pathogenesis of excessive systemic inflammation, supporting further research on visfatin as a therapeutic target.

Introduction

Besides their important roles in metabolism, adipocytokines or adipokines, i.e. hormones released from adipose tissue, are increasingly recognized as important regulators of immunity[1]. It has been suggested that adipokines contribute to the excessive systemic inflammatory reaction commonly observed in critical illness. We and others have previously shown that serum levels of the adipokines resistin and adiponectin are significantly elevated in critically ill patients and are associated with their mortality[2-6].

Relatively few data exist on visfatin in the setting of critical illness. The adipokine visfatin was initially identified in lymphocytes and is therefore also called pre-B-cell colony enhancing factor (PBEF)[7]. Leukocytes have been identified as a major source of circulating visfatin[8]. Moreover, visfatin is also involved in activation and attraction of inflammatory cells.

Experimental data obtained from human cells and mouse models revealed that visfatin is a chemoattractant for neutrophils[9], promotes neutrophil survival[10] and induces the dose-dependent release of cytokines in monocytes[11]. Interesting findings obtained from smaller trials demonstrated elevated visfatin serum levels in patients with respiratory diseases[12-14] and neonatal sepsis[15] as well as in patients with severe trauma or with critical neurological diseases[2].

Based on these findings, we analysed circulating visfatin levels in a large cohort of 229 prospectively enrolled critically ill patients at our medicial intensive care unit (ICU) in order to define the potential pathogenic role of visfatin in critical illness and its utility as a clinical biomarker in the ICU setting.

Materials and methods

Study design and patient characteristics

Critically ill patients were included at admission to the medical ICU at the University Hospital Aachen, Germany. Patients, who were admitted for post-interventional observational stay or underwent an elective procedure, were excluded[16].

The local ethics committee approved our study in accordance to the ethical standards laid down in the Declaration of Helsinki (reference number EK 150/06). The patients were categorized as sepsis and non-sepsis according to the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3)[17], and were treated

following the current guidelines for treatment of sepsis (Surviving Sepsis Campaign)[18]. As a healthy control group, we analyzed blood donors (36 male, 17 female, median age 37 years, range 25-67 years, BMI median 25 kg/m², range 19-34 kg/m²) with normal blood counts, normal values of liver enzymes and a negative serology for viral hepatitis and HIV[19].

In order to determine long-term outcome, we contacted the patients, their relatives and/or the general practitioner in approximately 6-months intervals after discharge from hospital for two years[19].

Measurements of visfatin and adipokines

Blood samples were collected at the time of admission (before specific therapeutic measures had been started at the ICU), centrifuged, and serum was stored at -80°C. Visfatin was analyzed with a commercial ELISA kit (USCN Life Science, #E90638Hu, BIOZOL Diagnostica, Eching, Germany).

Measurements of the other adipocytokines and related proteins resistin, adiponectin, leptin and leptin receptor were included as previously reported[3,4,20].

Statistical analysis

Due to the high range of visfatin values, especially comparing healthy controls and critically ill patients, all visfatin serum concentrations are presented as logarithmic values. The Mann-Whitney U-test was used to test differences between two groups, correlations were tested according to the Spearman's rank correlation method.

All values, including outside values as well as far out values, were included. P-values less than 0.05 were considered as statistically significant.

The prognostic value of visfatin on the outcome was evaluated by Cox regression models. Survival curves were generated by Kaplan-Meier analyses with a visfatin cut-off level calculated via the Youden-Index[21]. All analyses were performed with IBM SPSS Statistics (SPSS; Chicago, Illinois).

Results

Visfatin serum levels are significantly elevated in critically ill patients as compared with healthy controls

Visfatin serum levels were measured in a prospectively recruited cohort of 229 critically ill medical patients. Visfatin serum concentrations were approximately one log-fold higher in critically ill patients (median visfatin log 2.61 ng/ml, range 0.78-4.25, Table 5.1) compared to healthy controls (n=53, median visfatin log 1.66 ng/ml, range 0.30-3.21, p<0.001, Figure 5.1A).

Visfatin levels did not correlate with the age, neither in patients (r=0.24, p=0.723) nor in healthy controls (r=0.101, p=0.474). Of the 229 ICU patients, 142 were admitted due to sepsis, while 87 patients had a critical illness due to other origin such as cardio-pulmonary, gastrointestinal or hepatic disorders (Table 5.2). Patients with sepsis had further elevated visfatin levels compared to non-sepsis ICU patients (visfatin log 2.70 ng/ml vs. 2.51 ng/ml, p=0.04, Figure 5.1B). Within the sepsis patients, the site of infection (e.g., pneumonia, bloodstream, abdominal, urogenital, others) did not affect visfatin concentrations.

Table 5.1 Baseline patient characteristics and **visfatin** serum measurements.

Parameter	All Patients	Non-Sepsis	Sepsis
Number	229	87	142
Sex (male/female)	133 / 96	51 / 36	82 / 60
Age median (range) [years]	63 (18-90)	61 (18-85)	64 (20-90)
APACHE-II score median (range)	16 (2-43)	14.5 (2-33)	18 (3-43)
ICU days median (range)	7 (1-137)	5 (1-45)	9.5 (1-137)
Death during ICU n(%)	60 (26%)	15 (17%)	45 (32%)
Death during follow-up (total) n(%)	107 (47%)	31 (36%)	76 (54%)
Mechanical ventilation n(%)	157 (69%)	53 (61%)	104 (73%)
pre-existing diabetes n(%)	73 (32%)	27 (31%)	46 (32%)
pre-existing cirrhosis n(%)	23 (10%)	16 (18%)	7 (5%)
BMI median (range) [m^2/kg]	25.9 (15.9-86.5)	25.5 (15.9-53.9)	26.0 (17.1-86.5)
WBC median (range) [x10^3/µl]	12.8 (0-149)	12.0 (1.8-29.6)	14.0 (0-149)
CRP median (range) [mg/dl]	92 (5-230)	17 (5-230)	153 (5-230)
Procalcitonin median (range) [µg/l]	0.7 (0.03-207.5)	0.2 (0.03-100)	2.3 (0.10-207.5)
Creatinine median (range) [mg/dL]	1.35 (0.1-21.6)	1.0 (0.2-15.0)	1.7 (0.1-21.6)
INR median (range)	1.18 (0.9-13)	1.17 (0.9-6.7)	1.18 (0.9-13)
Log visfatin median (range) [ng/mL]	2.61 (0.78-4.25)	2.51 (0.78-3.89)	2.70 (1.08-4.25)

For quantitative variables, median and range (in parenthesis) are given. Abbreviations: APACHE: Acute Physiology And Chronic Health Evaluation; BMI: body mass index; CRP: C-reactive protein; ICU: intensive care unit; INR: international normalized ratio; WBC: white blood cell.

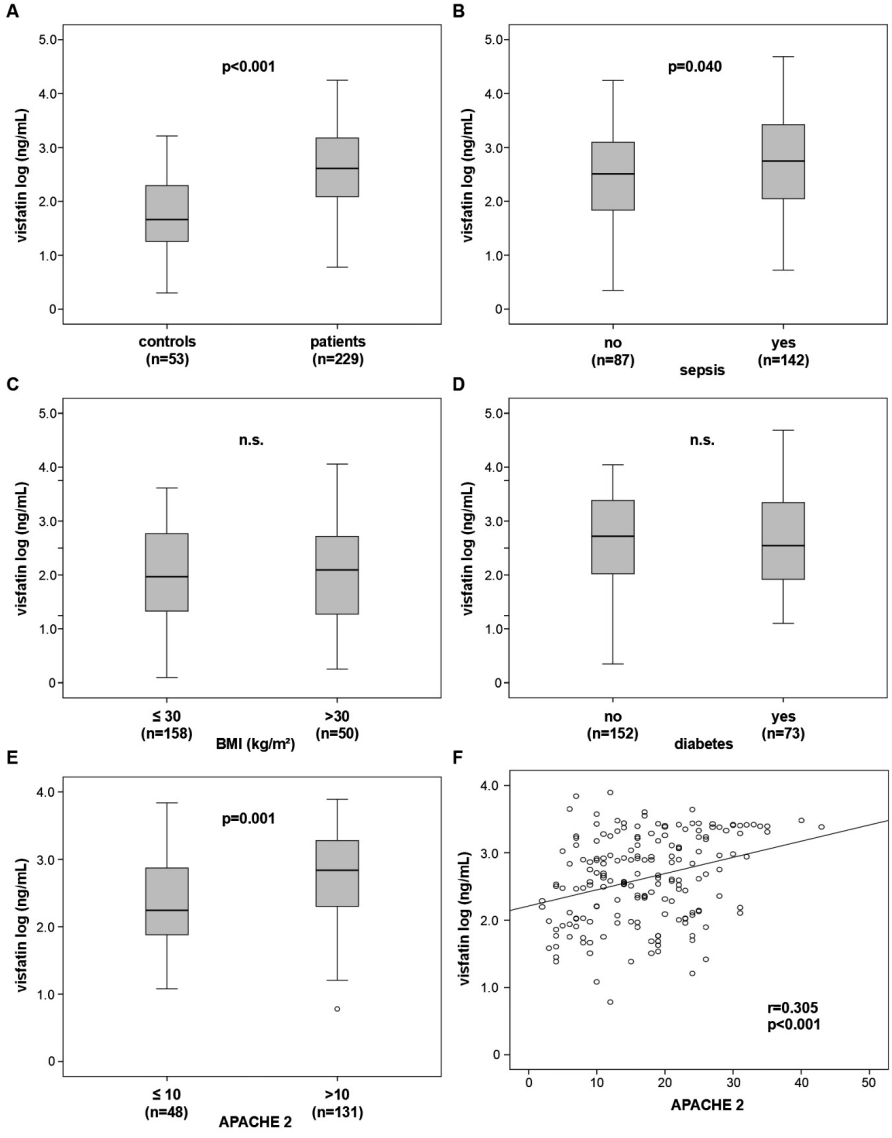


Figure 5.1 Visfatin levels in critically ill patients. (**A**) Visfatin serum concentrations (displayed as log visfatin) are significantly elevated in critically ill patients compared with controls. (**B-E**) Subgroup analyses of visfatin levels in critically ill patients, according to sepsis (**B**), obesity (**C**), defined by body mass index [BMI] above 30 kg/m², diabetes (**D**) or disease severity (APACHE 2 score above 10). (**F**) Visfatin levels correlate with APACHE II score in critically ill patients.

Table 5.2 Disease etiology of the study population leading to ICU admission.

	Sepsis	**Non-sepsis**
	142	**87**
Etiology of sepsis critical illness		
Site of infection n (%)		
Pulmonary	82 (58%)	
Abdominal	26 (18%)	
Urogenital	4 (3%)	
Other	30 (21%)	
Etiology of non-sepsis critical illness n (%)		
Cardio-pulmonary disorder		29 (33%)
Acute pancreatitis		11 (13%)
Acute liver failure		4 (5%)
Decompensated liver cirrhosis		15 (17%)
Severe gastrointestinal hemorrhage		6 (7%)
Non-sepsis other		22 (25%)

Diabetes or obesity did not impact visfatin levels at admission to the ICU

As high visfatin levels have been consistently associated with obesity, type 2 diabetes and the metabolic syndrome[7,22,23], we tested whether obesity or type 2 diabetes as a comorbidity at ICU admission impacted visfatin levels.

Unexpectedly, neither obesity as defined by a body-mass index (BMI) above 30 kg/m² (Figure 5.1C) nor pre-existing type 2 diabetes (Figure 5.1D) were associated with visfatin serum concentrations. Moreover, serum glucose at ICU admission or glycosylated haemoglobin A1 (HbA1c) did not correlate with visfatin levels in critically ill patients (data not shown).

In addition, n=23 patients admitted to the ICU had pre-existing liver cirrhosis. Their visfatin levels (median log visfatin 2.88, range 1.82-3.74) did not differ significantly from ICU patients without liver cirrhosis (median log visfatin 2.57, range 0.78-4.25, p=0.151).

Visfatin serum concentrations are strongly associated with disease severity

Based on our finding of high levels of visfatin in ICU patients, we next tested the potential association of visfatin with the severity of critical illness. In fact, patients with an Acute Physiology And Chronic Health II [APACHE II] score above 10 displayed significantly higher visfatin serum levels than patients with APACHE-II values below or equal to 10 (Figure 5.1E).

Moreover, visfatin levels directly correlated with APACHE-II scores (r=0.305, p<0.001, Figure 5.1F), Sequential Organ Failure Assessment [SOFA] or Simplified Acute Physiology Score 2 [SAPS2] scores (Table 5.3).

Table 5.3 Correlations with visfatin (log) serum concentrations at ICU admission (Spearman rank correlation test, only significant results are shown).

	ICU patients	
Parameters	**r**	**p**
Disease severity		
APACHE II score	0.305	<0.001
SOFA score	0.494	<0.001
SAPS2 score	0.406	<0.001
Inflammation		
C-reactive protein	0.256	<0.001
Procalcitonin	0.379	<0.001
suPAR	0.418	<0.001
White blood cell count	0.131	0.048
Interleukin-6	0.291	<0.001
TNF	0.331	0.003
Interleukin-10	0.423	<0.001
Renal function		
Creatinine	0.421	<0.001
GFR (creatinine)	-0.427	<0.001
Cystatin C	0.383	<0.001
GFR (cystatin C)	-0.372	<0.001
Urea	0.377	<0.001
Uric acid	0.231	<0.001
Liver function		
Protein	-0.352	<0.001
Albumin	-0.365	<0.001
Pseudocholinesterase	-0.316	<0.001
Bilirubin	0.167	0.012
Bilirubin (conjugated)	0.212	0.009
Alkaline phosphatase	0.218	0.001
AST	0.196	0.004
INR	0.315	<0.001
Prothrombin time	-0.336	<0.001
aPTT	0.283	<0.001
D-dimers	0.380	<0.001
Antithrombin III	-0.456	<0.001
Fibrinogen	-0.385	<0.001
Metabolism		
Leptin	-0.340	0.001
Leptin receptor	0.318	0.002
Adiponectin	0.235	0.02
Resistin	0.313	0.002

Abbreviations: APACHE: Acute Physiology And Chronic Health Evaluation; aPTT: activated prothrombin time; AST, aspartate aminotransferase; GFR: glomerular filtration rate; INR: international normalized ration; SAPS: Simplified Acute Physiology Score; SOFA: Sequential Organ Failure Assessment; suPAR: soluble urokinase plasminogen activator receptor; TNF: tumor necrosis factor

Visfatin levels are correlated with biomarkers of renal failure, liver failure and metabolic disturbances in critically ill patients

Due to the well-established role of circulating visfatin in systemic inflammation and cytokine release[24], we analysed correlations of visfatin in ICU patients with various biomarkers of inflammation, organ dysfunction and metabolism (Table 5.3).

Visfatin concentrations correlated closely with markers of inflammation including C-reactive protein, procalcitonin, interleukin-6 (IL-6) and other cytokines (Table 5.3), confirming observations obtained in neonatal sepsis[15]. Visfatin also correlated with soluble urokinase plasminogen activator receptor (suPAR, Figure 5.2A), a prognostic biomarker of inflammation in the ICU setting[25]. Circulating visfatin displayed a close association with renal dysfunction, as indicated by several markers including creatinine, cystatin C (Figure 5.2B) and their glomerular filtration rates (Table 5.3).

Similar results were noted for markers reflecting liver function like albumin (Figure 5.2C), bilirubin and coagulation factors (Table 5.3). Visfatin levels correlated with the other adipocytokines and related proteins assessed in our cohort, namely leptin, leptin receptor, adiponectin and resistin (Table 5.3).

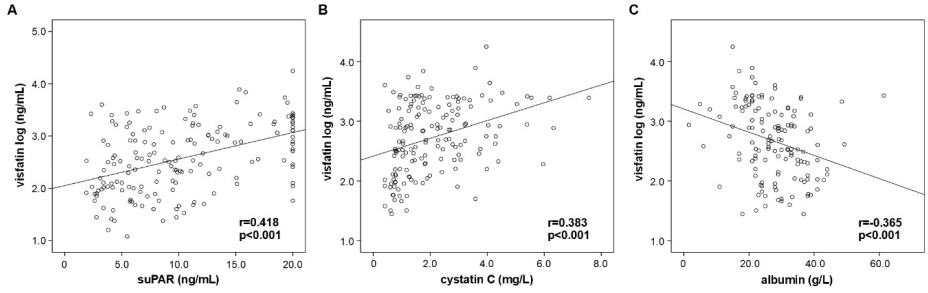


Figure 5.2 Visfatin levels correlate with inflammation and organ failure. (**A-C**) Correlation analyses revealed associations between serum visfatin and biomarkers of systemic inflammation (e.g., soluble urokinase plasminogen activator receptor [suPAR], **A**), renal failure (e.g., cystatin **C**, **B**) or hepatic dysfunction (e.g., albumin, **C**).

High visfatin serum concentrations at ICU admission are associated with adverse prognosis

In critically patients, who subsequently died during the ICU treatment (n=60), we found significantly elevated visfatin levels at admission to the ICU (Figure 5.3A), suggesting that visfatin might serve as a prognostic biomarker in critical diseases.

In fact, Cox regression analysis revealed that visfatin was robust predictor of ICU mortality ($p<0.001$). Kaplan-Meyer curves were calculated with a cut-off value of log

visfatin 2.89 ng/ml that showed the optimal ratio of sensitivity and specificity for mortality using the Youden-index. Here, visfatin levels clearly discriminated between survivors and non-survivors (Figure 5.3B).

Even patients that are successfully discharged from the ICU have a tremendous risk of mortality during the first years of follow-up[26]. We were able to assess long-term survival in 220 out of the 229 patients. Visfatin levels at ICU admission were significantly higher in patients that died during the follow-up period of approximately two years compared with survivors (Figure 5.3C).

Cox regression analysis confirmed the prognostic value of visfatin as a predictor of long-term mortality (p=0.001). Using the calculated optimal cut-off (log visfatin 3.01), patients with high visfatin demonstrated an unfavourable outcome, as depicted by Kaplan Meier survival curve analysis (Figure 5,3D).

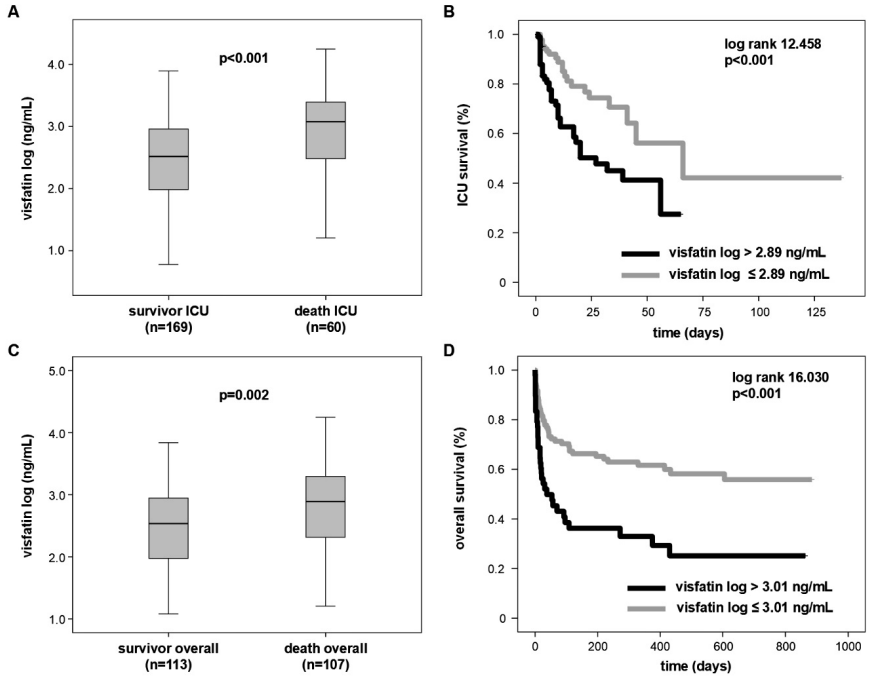


Figure 5.3 Visfatin is a biomarker for mortality in critically ill patients. (**A**) At the time of ICU admission, patients that died during the course of ICU treatment had significantly higher serum visfatin levels than survivors (p<0.001). (**B**) Patients with high or low visfatin levels displayed different ICU mortality by Kaplan-Meier survival curve analysis. (**C**) A similar observation was obtained when visfatin levels at ICU admission were compared between patients that died during the total observation period and survivors (p=0.002). (**D**) High visfatin levels at ICU admission predicted the overall mortality during long-term follow-up in critically ill patients (Kaplan-Meier survival curve analysis for the optimal visfatin cut-off is displayed).

The validity and performance of visfatin as a biomarker for the prediction of ICU or overall survival in critically ill patients are summarized in Table 5.4.

Notably, visfatin levels appeared more suited to predict outcome in comparison to other adipocytokines. By receiver operating characteristics (ROC) curve analyses, visfatin levels reached an area under the curve (AUC) to predict ICU mortality of 0.687, while resistin (0.562), adiponectin (0.623), leptin (0.404) and leptin receptor (0.580) demonstrated lower values.

For overall mortality, visfatin reached a higher AUC of 0.686 compared to resistin (0.563), adiponectin (0.638), leptin (0.407) and leptin receptor (0.609).

Table 5.4 Serum visfatin (log) performance as a biomarker to predict ICU or overall mortality.

	ICU mortality	Overall mortality
Visfatin (log) optimal cut-off	2.8882	3.0094
Sensitivity	0.63	0.45
Specificity	0.69	0.80
Positive predictive value	0.42	0.68
Negative predictive value	0.84	0.60
Youden-index	0.32	0.25
LHR+	2.02	2.20
LHR-	0.53	0.69
Diagnostic odds ratio	3.77	3.18

Abbreviation: LHR, likelihood ratio.

Discussion

The dysregulation of adipocytokines has been widely noted in critical illness and linked to systemic inflammation. Among interesting candidates of adipokines as biomarkers, leptin, adiponectin and resistin have been thoroughly investigated[1-4,20].

In this study, we focused on visfatin, an adipocytokine with several metabolic but also inflammation-orchestrating functions[24]. In a large cohort of prospectively enrolled critically ill medical patients, we demonstrate that visfatin serum levels are highly elevated compared to controls, associated with sepsis and disease severity, correlated to organ dysfunction and, most importantly, serve as a reliable predictor of mortality. Our findings are well in agreement with smaller trials reporting elevated visfatin and the association with poor outcome in patients with respiratory diseases[12-14] and neonatal sepsis[15]. Similar findings have also been reported from patients with severe trauma or with critical neurological diseases[2].

The close association between high visfatin levels and increased short- or long-term mortality in our study may be well explained by the strong correlations between visfatin and inflammatory mediators and cytokines, disease severity (e.g., clinical scores) and biomarkers reflecting organ failure. However, there is increasing evidence emerging that visfatin is directly involved in the pathogenesis of critical illness and systemic inflammation. Visfatin was found to be a chemoattractant for neutrophils[9] and has direct effects on neutrophil survival[10], which could jointly promote excessive release of cytokines[24], production of oxidative stress factors and subsequently result in tissue damage and organ failure[2].

In support of this hypothesis, the experimental inhibition of visfatin in mouse models of ventilator-associated lung injury reduced neutrophil infiltration, organ injury and mortality[9]. Moreover, distinct single-nucleotide polymorphisms (SNPs) in the visfatin gene have been identified in humans[27,28], of which the SNP-1543T was linked to a reduced risk of mortality, while the SNP-1001G was associated with a higher risk of mortality in patients with acute respiratory distress syndrome[29].

In our cohort, 24% of the critically ill medical patients were obese or morbidly obese, as defined by a BMI above 30 kg/m². This is in line with observations in the United States, where at least 25% of adult ICU patients are overweight, obese or morbidly obese[30,31].

Interestingly, we did not find dysregulated visfatin levels between ICU patients with or without obesity, supporting that circulating visfatin levels in critical illness are primarily attributable to the extent of inflammation and not adiposity itself. Nonetheless, visfatin levels were closely correlated with adiponectin, resistin and (inversely) leptin, indicating a concerted yet rectified activation of adipose tissue inflammation[1].

As outcome prediction is of major interest in the ICU setting, there is a high medical need to complement current prognostic models (e.g., APACHE-II, SAPS, SOFA) by additional biomarkers that could indicate the long-term prognosis beyond the acute critical illness[32].

Visfatin demonstrated in our study an exceptional value to predict the overall mortality during a two-years follow-up period. Thus, our data indicated that visfatin could be possibly used, either alone or in combination with other adipokines, for a more accurate prognostication in critical illness.

Conclusions

We demonstrate in our study comprising 229 critically ill medical patients that circulating levels of the adipokine visfatin were significantly elevated at admission to the ICU, as compared with healthy controls.

Visfatin serum concentrations were strongly associated with disease severity, organ failure and sepsis, but not with obesity or type 2 diabetes. High visfatin levels at ICU admission indicated an increased mortality, both at the ICU and during long-term follow-up.

Further research should aim at implementing visfatin as a prognostic biomarker in a comprehensive risk assessment algorithm at the ICU. Moreover, the close association between visfatin and prognosis as well as experimental data on visfatin neutralization in animal models support to explore visfatin as a therapeutic target in excessive systemic inflammation and sepsis.

References

1. Tilg H, Moschen AR. Adipocytokines: mediators linking adipose tissue, inflammation and immunity. Nat Rev Immunol. 2006;6(10):772-783.
2. Hajri T, Gharib M, Kaul S, Karpeh MS, Jr. Association between adipokines and critical illness outcomes. J Trauma Acute Care Surg. 2017;83(3):507-519.
3. Koch A, Gressner OA, Sanson E, Tacke F, Trautwein C. Serum resistin levels in critically ill patients are associated with inflammation, organ dysfunction and metabolism and may predict survival of non-septic patients. Crit Care. 2009;13(3):R95.
4. Koch A, Sanson E, Voigt S, Helm A, Trautwein C, Tacke F. Serum adiponectin upon admission to the intensive care unit may predict mortality in critically ill patients. J Crit Care. 2011;26(2):166-174.
5. Walkey AJ, Rice TW, Konter J, Ouchi N, Shibata R, Walsh K, et al. Plasma adiponectin and mortality in critically ill subjects with acute respiratory failure. Crit Care Med. 2010;38(12):2329-2334.
6. Sunden-Cullberg J, Nystrom T, Lee ML, Mullins GE, Tokics L, Andersson J, et al. Pronounced elevation of resistin correlates with severity of disease in severe sepsis and septic shock. Crit Care Med. 2007;35(6): 1536-1542.
7. Sonoli SS, Shivprasad S, Prasad CV, Patil AB, Desai PB, Somannavar MS. Visfatin--a review. Eur Rev Med Pharmacol Sci. 2011;15(1):9-14.
8. Friebe D, Neef M, Kratzsch J, Erbs S, Dittrich K, Garten A, et al. Leucocytes are a major source of circulating nicotinamide phosphoribosyltransferase (NAMPT)/pre-B cell colony (PBEF)/visfatin linking obesity and inflammation in humans. Diabetologia. 2011;54(5):1200-1211.
9. Hong SB, Huang Y, Moreno-Vinasco L, Sammani S, Moitra J, Barnard JW, et al. Essential role of pre-B-cell colony enhancing factor in ventilator-induced lung injury. Am J Respir Crit Care Med. 2008;178(6): 605-617.
10. Jia SH, Li Y, Parodo J, Kapus A, Fan L, Rotstein OD, et al. Pre-B cell colony-enhancing factor inhibits neutrophil apoptosis in experimental inflammation and clinical sepsis. J Clin Invest. 2004;113(9): 1318-1327.
11. Moschen AR, Kaser A, Enrich B, Mosheimer B, Theurl M, Niederegger H, et al. Visfatin, an adipocytokine with proinflammatory and immunomodulating properties. J Immunol. 2007;178(3):1748-1758.
12. Lee K, Huh JW, Lim CM, Koh Y, Hong SB. Clinical role of serum pre-B cell colony-enhancing factor in ventilated patients with sepsis and acute respiratory distress syndrome. Scand J Infect Dis. 2013;45(10): 760-765.
13. Lee KA, Gong MN. Pre-B-cell colony-enhancing factor and its clinical correlates with acute lung injury and sepsis. Chest 2011;140(2):382-390.
14. Juan X, Lu YM, Shi JD, Deng XQ, Long W. Visfatin levels in patients with severe pneumonia. World J Emerg Med. 2011;2(2):132-136.
15. Cekmez F, Canpolat FE, Cetinkaya M, Aydinoz S, Aydemir G, Karademir F, et al. Diagnostic value of resistin and visfatin, in comparison with C-reactive protein, procalcitonin and interleukin-6 in neonatal sepsis. Eur Cytokine Netw. 2011;22(2):113-117.
16. Koch A, Weiskirchen R, Kunze J, Duckers H, Bruensing J, Buendgens L, et al. Elevated asymmetric dimethylarginine levels predict short- and long-term mortality risk in critically ill patients. J Crit Care. 2013;28(6):947-953.
17. Buendgens L, Yagmur E, Bruensing J, Herbers U, Baeck C, Trautwein C, et al. Growth Differentiation Factor-15 Is a Predictor of Mortality in Critically Ill Patients with Sepsis. Dis Markers 2017;2017: 5271203.
18. Rhodes A, Evans LE, Alhazzani W, Levy MM, Antonelli M, Ferrer R, et al. Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock: 2016. Intensive Care Med. 2017; 43(3):304-377.
19. Koch A, Voigt S, Kruschinski C, Sanson E, Duckers H, Horn A, et al. Circulating soluble urokinase plasminogen activator receptor is stably elevated during the first week of treatment in the intensive care unit and predicts mortality in critically ill patients. Crit Care. 2011;15(1): R63.
20. Koch A, Weiskirchen R, Zimmermann HW, Sanson E, Trautwein C, Tacke F. Relevance of serum leptin and leptin-receptor concentrations in critically ill patients. Mediators Inflamm. 2010;2010:473540.

21. Buendgens L, Yagmur E, Bruensing J, Herbers U, Baeck C, Trautwein C, et al. C-terminal proendothelin-1 (CT-proET-1) is associated with organ failure and predicts mortality in critically ill patients. J Intensive Care. 2017;5:25.
22. Kaminska A, Kopczynska E, Bielinski M, Borkowska A, Junik R. Visfatin concentrations in obese patients in relation to the presence of newly diagnosed glucose metabolism disorders. Endokrynologia Polska 2015;66(2):108-113.
23. Stastny J, Bienertova-Vasku J, Vasku A. Visfatin and its role in obesity development. Diabetes Metab Syndr. 2012;6(2):120-124.
24. Luk T, Malam Z, Marshall JC. Pre-B cell colony-enhancing factor (PBEF)/visfatin: a novel mediator of innate immunity. J Leukoc Biol. 2008;83(4):804-816.
25. Backes Y, van der Sluijs KF, Mackie DP, Tacke F, Koch A, Tenhunen JJ, et al. Usefulness of suPAR as a biological marker in patients with systemic inflammation or infection: a systematic review. Intensive Care Med. 2012;38(9):1418-1428.
26. Wilson ME, Barwise A, Heise KJ, Loftsgard TO, Dziadzko M, Cheville A, et al. Long-Term Return to Functional Baseline After Mechanical Ventilation in the ICU. Crit Care Med. 2018;46(4):562-569.
27. Cardinal-Fernandez P, Ferruelo A, El-Assar M, Santiago C, Gomez-Gallego F, Martin-Pellicer A, et al. Genetic predisposition to acute kidney injury induced by severe sepsis. J Crit Care. 2013;28(4):365-370.
28. Ye SQ, Simon BA, Maloney JP, Zambelli-Weiner A, Gao L, Grant A, et al. Pre-B-cell colony-enhancing factor as a potential novel biomarker in acute lung injury. Am J Respir Crit Care Med. 2005;171(4): 361-370.
29. Bajwa EK, Yu CL, Gong MN, Thompson BT, Christiani DC. Pre-B-cell colony-enhancing factor gene polymorphisms and risk of acute respiratory distress syndrome. Crit Care Med. 2007;35(5):1290-1295.
30. Pepper DJ, Sun J, Welsh J, Cui X, Suffredini AF, Eichacker PQ. Increased body mass index and adjusted mortality in ICU patients with sepsis or septic shock: a systematic review and meta-analysis. Crit Care. 2016;20(1):181.
31. Gharib M, Kaul S, LoCurto J, Perez M, Hajri T. The obesity factor in critical illness: Between consensus and controversy. J Trauma Acute Care Surg. 2015;78(4): 866-873.
32. Vincent JL, Teixeira L. Sepsis biomarkers. Value and limitations. Am J Respir Crit Care Med. 2014; 190(10):1081-1082.

Chapter 6

Elevated CTRP1 Plasma Concentration Is Associated with Sepsis and Pre-Existing Type 2 Diabetes Mellitus in Critically Ill Patients

Eray Yagmur
David Buergerhausen
Ger H. Koek
Ralf Weiskirchen
Christian Trautwein
Frank Tacke
Alexander Koch

J Clin Med. 2019:8(5):661

Abstract

The adipokine family of C1q/TNF-like proteins (CTRP) plays a critical role in regulating systemic energy homeostasis and insulin sensitivity. It is involved in pathophysiological processes including inflammation and insulin-resistant obesity. Sepsis is associated with metabolic alterations and dysregulated adipokines, but the role of CTRP1 in critical illness and sepsis is unclear.

We investigated CTRP1 plasma concentrations in 145 septic and 73 non-septic critically ill patients at admission to the medical intensive care unit (ICU) in comparison to 66 healthy controls. We also assessed associations of CTRP1 with clinical characteristics, adipokine levels, metabolic and inflammatory parameters.

CTRP1 plasma concentration was significantly elevated in critically ill patients compared to healthy subjects. CTRP1 levels were significantly higher in ICU patients with sepsis. CTRP1 correlated strongly with markers of inflammatory response, renal function, liver damage and cholestasis.

Furthermore, CTRP1 levels were higher in ICU patients with type 2 diabetes mellitus, and correlated with HbA1c and body mass index. This study demonstrates significantly elevated levels of CTRP1 in critically ill patients, particularly with sepsis, and links circulating CTRP1 to inflammatory and metabolic disturbances.

Introduction

The highly conserved family of secreted C1q/TNF-related (glyco-)proteins (CTRP) is a paralogue of adiponectin with diverse functions in regulating metabolism and immunity[1–4]. Next to adiponectin, 15 additional CTRP family members have been identified to date[3]. The CTRP family is involved in a variety of clinical and pathophysiological processes including immune defense, inflammation, apoptosis, autoimmunity, cell differentiation, organogenesis and insulin-resistant obesity[5].

CTRP1 is a novel member of the CTRP family secreted by different tissues, mainly adipose tissue[2,6,7]. It is known that CTRP1 plays a critical role in regulating systemic energy homeostasis and insulin sensitivity[2,4,7–9]. For maintaining proper balance of energy substrate metabolism, CTRP1 depends on complex metabolic networks of secreted hormones (e.g., insulin, leptin, adiponectin)[4]. CTRP1 is reported to be involved in PI3K (phosphatidylinositol 3-kinase)/Akt (protein kinase B) signaling pathways to induce glucose transport by insulin[9,10]. Increase in Akt signaling affects glucose metabolism by increasing translocation of glucose transporters GLUT1 and GLUT4 to the plasma membrane and by activating glycolysis enzymes indirectly[11,12].

It is well known that infectious and inflammatory diseases such as sepsis and systemic inflammatory response syndrome are accompanied by metabolic alterations such as insulin resistance or dysregulated adipokines[13]. Conversely, metabolic diseases such as visceral obesity and type 2 diabetes are characterized by high levels of pro-inflammatory cytokines[3,14].

Regarding adipokines in critical illness and sepsis, these factors have gained great attention as potential biomarkers for disease severity and as potential targets for therapy. While several “inflammatory adipokines” are upregulated in critical illness, including visfatin or resistin[15,16], a surprisingly high number of circulating adipokines, such as leptin, omentin or adiponectin, do not differ between healthy controls and critically ill patients[13,17,18]. Nonetheless, even if not different from healthy controls, circulating adipokines like omentin or adiponectin have been associated with survival in critical illness[17,18].

Furthermore, CTRP1 is reported to be involved in blood pressure regulation and cardiovascular function[9,10]. In addition to effects in glucose metabolism, the CTRP1-dependent Akt signaling activates the Ras homolog gene family (Rho)/Rho kinase (ROCK) signaling pathways resulting in aldosterone secretion and vasoconstriction[9]. Moreover, the stimulation of human vascular smooth muscle cells by CTRP1 results in upregulated expression of pro-inflammatory cytokines such as interleukin 6 (IL-6), monocyte chemoattractant protein 1 (MCP1) and intracellular adhesion molecule 1

(ICAM1)[19]. These genes were proposed as potential key modulators regarding the function of CTRP1 in inflammatory diseases[19].

Critically ill patients are closely associated with the entire spectrum of metabolic-related disorders, including diabetes and inflammation such as sepsis and severe inflammatory response[6]. In sepsis, the role of CTRP1, with regard to its critical role in regulating systemic energy homeostasis and insulin sensitivity, is currently not fully understood.

Moreover, the potential value of circulating CTRP1 in critically ill patients is unknown. We therefore assessed plasma CTRP1 concentration in a large cohort of critically ill medical patients admitted to the intensive care unit (ICU) in order to investigate the diagnostic and clinical relevance of circulating CTRP1.

Experimental section

Critically ill patients were included at admission to the medical ICU at the RWTH University Hospital in Aachen, Germany. The current cohort of patients was collected from an ongoing, prospective observational trial in our ICU at the RWTH University Hospital, in which patients were included consecutively.

For the current analysis, we randomly enrolled n=218 patients that had been treated between 2006 and 2011 from the existing biobank. Patients who were admitted for post-interventional observational stay or underwent an elective procedure were excluded, according to an established protocol[20].

The patients were categorized as sepsis and non-sepsis according to the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3)[21], and were treated following the current guidelines for treatment of sepsis (Surviving Sepsis Campaign)[22]. As a healthy control group, we analysed n = 66 blood donors (43 male, 23 female, median age 29.5 years, range 18-67 years, BMI median 25.4 kg/m², range 17.9-37 kg/m²) with normal blood counts, normal values of liver enzymes and a negative serology for viral hepatitis and HIV[23].

The local ethics committee approved the study in accordance to the ethical standards laid down in the Declaration of Helsinki (reference number EK 150/06). All included participants provided written informed consent.

Blood samples were collected at the time of admission (before specific therapeutic measures), centrifuged and plasma was stored at −80 °C. Plasma CTRP1 concentrations

were determined using a quantitative sandwich enzyme immunoassay (ELISA), according to the manufacturer's instructions (Human CTRP1, #RD191153100R, BioVendor, Brno, Czech Republic).

A repeat measurement for CTRP1 concentrations outside the linearity was not performed due to the small sample volumes available. Pre-dilution was used instead (20-fold). Thus, only a few patients had CTRP1 concentrations (n=23) above linearity. CTRP1 concentrations above the linearity of the standard curve (1600 ng/mL) were set to 1600 ng/mL (corresponding to the highest CTRP1 concentration in the standard preparations) in order to minimize accidental overinterpretation of the data. All samples were included in the statistical analyses.

Owing to the skewed distribution of the parameters, data are given as median and range, and shown graphically by box-and-whiskers plots. They show a summary of the median, quartiles, range and extreme values. Their whiskers range from the minimum to the maximum value, excluding outliers displayed as separate points. An outlier was defined as a value that is smaller than the lower quartile minus 1.5-times interquartile range, or larger than the upper quartile plus 1.5-times the interquartile range.

A far out value was defined as a value that is smaller than the lower quartile minus three times the interquartile range, or larger than the upper quartile plus three times the interquartile range. The degree of association between two variables was assessed by the Spearman rank correlation test. Comparisons of parameters between two different groups were conducted with the Mann–Whitney U-test.

All values, including outside values as well as far out values, were included. p-values less than 0.05 were considered as statistically significant. All analyses were performed with IBM SPSS Statistics (SPSS; Chicago, IL, USA).

Results

CTRP1 plasma levels are significantly elevated in critically Ill patients as compared with healthy controls

Our study cohort comprised of 218 patients that had been admitted to the medical ICU. About two thirds of the patients were mechanically ventilated, the median Acute Physiology and Chronic Health Evaluation (APACHE II) score was 18 and 22.5% died during the course of ICU treatment (Table 6.1), thereby representing a cohort of critically ill patients with a high risk profile. CTRP1 plasma levels were significantly elevated in this cohort of critically ill patients (median 747.1 ng/mL, range 200.5–

1600.0 ng/mL; Table 6.1) at admission to the ICU, as compared with 66 healthy controls (median 316.3 ng/mL, range 171.1–1308.7 ng/mL, p<0.001; Figure 6.1A).

Table 6.1 Baseline patient characteristics and CTRP1 plasma measurements.

Parameter	All Patients	Non-Sepsis	Sepsis	* *p*
Number n	218	73	145	
Sex (male/female) n	133/85	48/25	85/60	n.s.
Age (years)	64 (18–90)	61 (18–85)	65 (20–90)	n.s.
APACHE-II score	18 (2–43)	13.5 (2–33)	19 (4–43)	<0.001
ICU days	7 (1–137)	6 (1–45)	9 (1–137)	0.004
Death during ICU n (%)	49 (22.5%)	9 (12.3%)	40 (27.6%)	0.010
Death during follow-up (total) n (%)	89 (40.8%)	22 (30.1%)	67 (46.2%)	0.026
Mechanical ventilation n (%)	143 (65.6%)	46 (63%)	97 (66.9%)	n.s.
Pre-existing diabetes n (%)	64 (29.4%)	22 (30.1%)	42 (29.0%)	n.s.
BMI (m^2/kg)	25.8 (14–86)	25.7 (15.9–40.5)	28.9 (14–86.5)	n.s.
WBC ($\times 10^3/\mu L$)	13.1 (0.1–208)	12.5 (1.8–29.6)	14.1 (0.1–208)	0.024
CRP (mg/dL)	100.5 (5–230)	17 (5–230)	164 (5–230)	<0.001
IL-6 (pg/mL)	150.0 (2–28000)	66.5 (1.5–5000)	250 (0.1–28000)	<0.001
Procalcitonin (ng/mL)	0.7 (0.03–207.5)	0.2 (0.03–100)	2.2 (0.1–207.5)	<0.001
Creatinine (mg/dL)	1.3 (0.1–15)	1.0 (0.2–15)	1.5 (0.1–10.7)	0.017
GFR-Cystatin C (mL/min)	34 (0–379)	59 (5–379)	21.5 (0–218)	<0.001
INR	1.16 (0.92–13)	1.17 (0.95–6.73)	1.16 (0.92–13)	n.s.
CTRP1 day 1 (ng/mL)	747.1 (200.5–1600)	574.2 (227.2–1600)	779.6 (200.5–1600)	0.006

For quantitative variables, median and range (in parenthesis) are given. Percentages in parenthesis refer to the total number of patients in the respective groups. * Significance between sepsis and non-sepsis patients was assessed using the Mann–Whitney-U-test (for quantitative variables) or the chi-square test (for categorical variables). Abbreviations: n.s. not significant: APACHE acute Physiology And Chronic Health Evaluation; BMI body mass index; CRP C-reactive protein; IL-6 interleukin 6; ICU intensive care unit; INR international normalized ratio; WBC white blood cell.

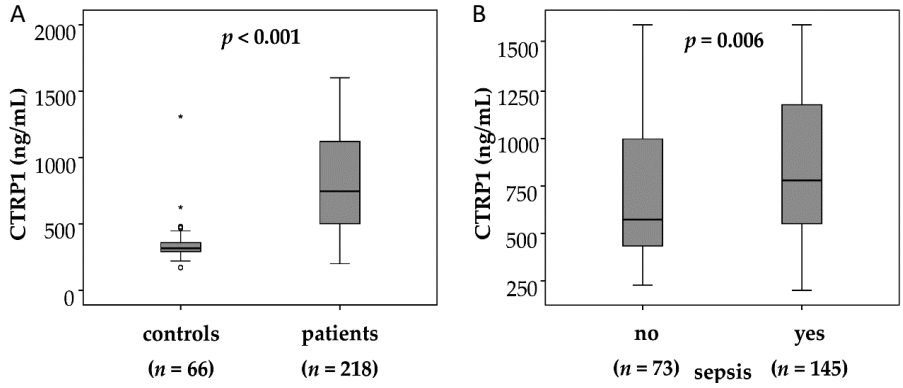


Figure 6.1 CTRP1 levels in critically ill patients and sepsis. (**A**) CTRP1 plasma concentrations, at time of admission to the ICU, were significantly elevated in critically ill patients (n=218) compared with healthy controls (n=66) (p<0.001; U-Test). (**B**) CTRP1 levels are associated with the presence of sepsis (sepsis, n=145; no sepsis, n=73) (p=0.006; U-Test). * - extreme outlier; Abbreviations: ICU; intensive care unit; CTRP1; C1q/TNF-related protein 1.

Elevated CTRP1 plasma levels in critically Ill patients are associated with the presence of sepsis

Within the cohort of ICU patients, plasma concentrations of CTRP1 were significantly increased in patients with sepsis (n=145, median 779.6 ng/mL, range 200.5-1600.0 ng/mL) as compared to patients without sepsis (n=73, median 574.2 ng/mL, range 227.2–1600.0 ng/mL, p=0.006; Figure 6.1B).

Typical sites of infection in sepsis were pneumonia, abdominal and urogenital tract, while non-sepsis causes of critical illness included, among others, cardiopulmonary diseases, acute pancreatitis and decompensated liver cirrhosis (Table 6.2). Among the critically ill patients, there was no association between CTRP1 plasma concentrations and these different disease aetiologies leading to ICU admission (all p>0.05).

Table 6.2 Disease aetiology of the study population leading to ICU admission.

	Sepsis *n*=145	Non-Sepsis *n*=73
Aetiology of sepsis critical illness		
Site of infection n (%)		
Pulmonary	72 (50%)	
Abdominal	28 (19%)	
Urogenital	11 (8%)	
Other	34 (23%)	
Aetiology of non-sepsis critical illness n (%)		
Cardio-pulmonary disorder		29 (40%)
Acute pancreatitis		10 (14%)
Acute liver failure		4 (5.5%)
Decompensated liver cirrhosis		9 (12%)
Severe gastrointestinal hemorrhage		4 (5.5%)
Non-sepsis other		17 (23%)

CTRP1 plasma levels in critically Ill patients are not associated with disease severity or mortality

Circulating CTRP1 has been previously suggested as a biomarker for disease severity in various clinical settings[24,25]. Plasma CTRP1 concentrations were not associated with disease severity, as determined by comparing ICU patients with a low (≤10) or high (>10) APACHE-II score. Patients with a high APACHE-II score showed an association between disease severity and elevated CTRP1, but weak evidence at ICU admission (median 768.3 ng/mL vs. median 663.5 ng/mL in APACHE-II ≤10, p=0.339) (Figure 6.2A).

In critically ill patients, who subsequently died during the ICU treatment (n=49), we did not find significantly altered CTRP1 levels at admission to the ICU, indicating that CTRP1 at ICU admission is not a prognostic biomarker in critical diseases. Nevertheless, we observed a tendency towards increased CTRP1 levels in the deceased patients

compared to the surviving patients (median 812.4 ng/mL vs. median 711.4 ng/mL in ICU survivors, p=0.166) (Figure 6.2B).

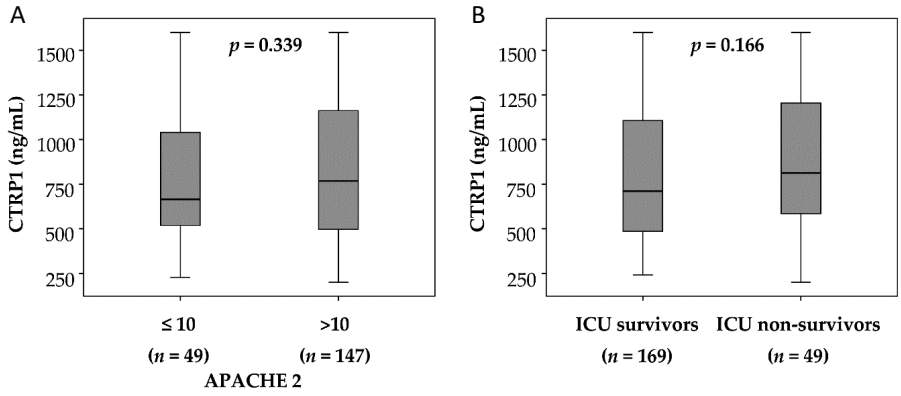


Figure 6.2 CTRP1 levels in critically ill patients are not associated with disease severity and short-term mortality. (**A**) Patients with high disease severity (n=147), as defined by an APACHE-II score above 10, are not associated with elevated plasma CTRP1, but show a tendency towards higher CTRP1 levels at ICU admission (p=0.339; U-Test). (**B**) Patients that died during the course of ICU treatment (n=49) are characterized by a tendency towards higher plasma CTRP1 concentrations already at ICU admission (p=0.166; U-Test). Abbreviations: APACHE: Acute Physiology And Chronic Health Evaluation; ICU: intensive care unit; CTRP1: C1q/TNF-related protein 1.

Elevated CTRP1 plasma levels in critically Ill patients are closely associated with diabetic comorbidity but not pre-existing obesity

Elevated CTRP1 was previously reported in patients with diabetes. CTRP1 has been associated with alterations in systemic energy metabolism in various conditions[1,12,26]. We therefore assessed whether metabolic comorbidities, including pre-existing obesity or diabetes, impacted CTRP1 levels at ICU admission.

Patients with pre-existing diabetes had the highest CTRP1 levels among all ICU patients (n=64, median 833.5 ng/mL vs. median 626.3 ng/mL in non-diabetic patients, n=150, p=0.004; Figure 6.3A). Moreover, we observed an association between plasma CTRP1 and chronic hyperglycemia. CTRP1 in critically ill patients displayed a positive correlation with glycated haemoglobin (HbA1c) at ICU admission (r=0.301, p=0.011) (Table 6.3), but did not correlate with insulin, glucose or measures of homeostasis model assessment-insulin resistance (HOMA-IR), its reciprocal insulin sensitivity and β-cell function (HOMA-β). In addition, ICU patients with pre-existing obesity, defined as a body mass index (BMI) above 30 kg/m², showed positive correlation between CTRP1 and BMI at ICU admission (r=0.189; p=0.007) (Table 6.3, Figure 6.3C).

Despite elevated CTRP1 levels in patients with BMI>30 kg/m², this correlation between BMI and CTRP1 is of weak evidence at ICU admission (n=35, median 798.7 ng/mL, range 286.2–1600.0 ng/mL vs. n=99, median 663.5 ng/mL, range 227.2–1600.0 ng/mL (BMI≤30 kg/m²), p=0.219; Figure 6.3B).

Interestingly, we could not find any correlations of CTRP1 to circulating levels of leptin, leptin receptor, adiponectin, ghrelin, resistin and retinol-binding protein 4 (RBP4).

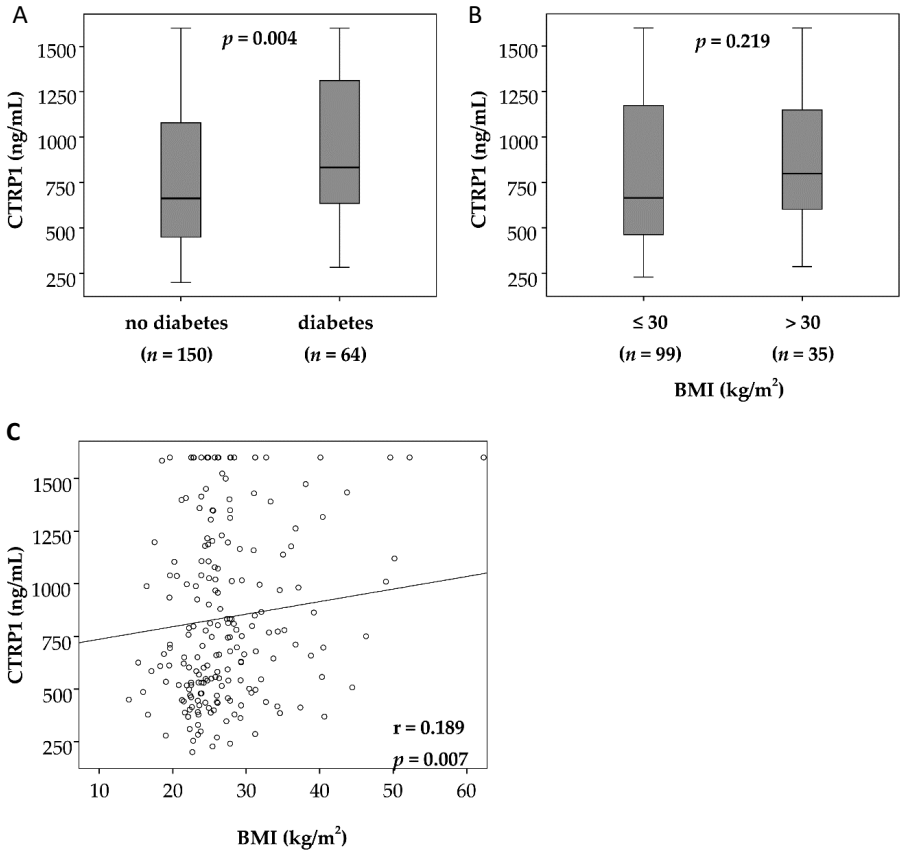


Figure 6.3 Impact of metabolic comorbidities on CTRP1 levels. CTRP1 plasma concentrations are significantly elevated in ICU patients with pre-existing type 2 diabetes (n=150) (p=0.004; U-test) (**A**). CTRP1 levels are not associated with obesity, as defined by a body-mass index (BMI) above 30 kg/m² (n=55) (p=0.219; U-Test (**B**) and r=0.189, p=0.007; Spearman rank correlation test (**C**). Abbreviations: BMI: body mass index; ICU: intensive care unit; CTRP1: C1q/TNF-related protein 1.

Table 6.3 Correlations with CTRP1 plasma concentrations at ICU admission day.

	ICU Patients	
Parameters	**r**	***p***
Obesity/diabetes		
BMI	0.189	0.007
HbA1c	0.301	0.011
Inflammatory response		
CRP	0.238	<0.001
IL-6	0.317	<0.001
PCT	0.414	<0.001
suPAR	0.279	0.001
Renal function		
Urea	0.324	<0.001
Creatinine	0.283	<0.001
Cystatin C	0.287	0.001
GFR Cystatin C	−0.291	0.001
Liver injury/cholestasis		
Bilirubin	0.422	*<0.001*
GLDH	0.154	*0.033*
γ-GT	0.243	*<0.001*
AP	0.211	*0.003*

Spearman rank correlation test, only statistically significant results are shown. The overall weak associations may not be clinically relevant. However, the purpose of the statistical correlation analysis is to descriptively discuss the clinical relevance of CTRP1. Understanding these aspects will help better utilize the evidence to improve clinical decision-making. BMI: body mass index; HbA1c: hemoglobin A1c; CRP: C-reactive protein; IL-6: interleukin 6; PCT: procalcitonin; suPAR: soluble urokinase-type plasminogen activator receptor; GFR: glomerular filtration rate; GLDH: glutamate dehydrogenase; γ-GT: gamma-glutamyltransferase; AP: alkaline phosphatase.

CTRP1 levels in critically Ill patients are correlated with biomarkers of inflammation, cholestasis and renal failure

We investigated the potential association between CTRP1 and inflammatory response. In agreement with the association between CTRP1 and sepsis, we observed a correlation between CTRP1 and classical markers of inflammation in our cohort, such as interleukin 6 ($r=0.317$, $p<0.001$), procalcitonin ($r=0.414$, $p<0.001$), C-reactive protein ($r=0.238$, $p<0.001$) and suPAR serum levels ($r=0.279$, $p=0.001$; Table 6.3), an experimental marker of systemic inflammation[27]. We found no correlation to the anti-inflammatory cytokine interleukin 10.

Moreover, we found correlations to markers of renal function including creatinine ($r=0.283$, $p<0.001$, Figure 6.4A), GFR-cystatin C ($r=-0.291$, $p=0.001$) (Figure 6.4B), urea ($r=0.324$, $p<0.001$) and cystatin C ($r=0.287$, $p=0.001$, Table 6.3), as well as to markers indicative of cholestasis such as bilirubin ($r=0.422$, $p<0.033$), γ-glutamyltransferase ($r=0.243$, $p<0.001$) and alkaline phosphatase ($r=0.211$, $p<0.003$, Table 6.3).

In a multivariate logistic regression analysis with CTRP1 and these parameters to test its association with sepsis, only CRP remained an independent and highly significant predictor of sepsis.

When we included only creatinine and CTRP1 in the regression model, CTRP1 (and not creatinine) remained independently associated with sepsis (p=0.039), indicating that CTRP1 is associated with sepsis, independent from renal function.

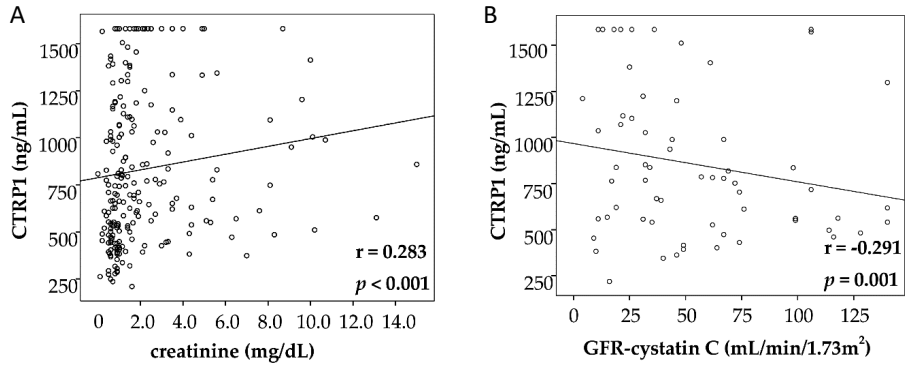


Figure 6.4 CTRP1 levels correlate with renal function in critically ill patients. In the ICU cohort, CTRP1 correlates with markers of excretory renal function such as creatinine (**A**) and GFR-cystatin C (**B**). Abbreviations: GFR: glomerular filtration rate.

Discussion

In our study, we demonstrate that CTRP1 plasma levels are significantly upregulated in critical illness as compared to healthy volunteers, displaying highest levels in patients admitted due to sepsis. Despite increased levels of CTRP1 in pre-existing type 2 diabetes and positive correlation with HbA1c in critically ill patients, we did not find an association with insulin resistance or glucose concentration in the blood.

CTRP1 levels in our ICU cohort did not correlate with disease severity or short-term ICU survival, but tend to higher CTRP1 levels. In critically ill patients, CTRP1 levels appeared particularly linked to systemic inflammation, metabolic disturbances and organ dysfunction. The potential pathogenic role of CTRP1 in critical illness and sepsis, however, requires further studies.

In general, CTRP1 has been associated with changes in systemic energy metabolism in different metabolic and dietary conditions[28,29]. In our cohort of critically ill patients, CTRP1 was indeed related to pre-existing diabetes as well as to long-term blood glucose

control reflected by HbA1c, similar to findings in non-ICU patients with diabetes and obesity[14].

Despite the positive correlation of CTRP1 with BMI, but weak evidence, we found that CTRP1 in critical illness is not associated with a variety of established biomarkers of energy substrate metabolism or diabetes-related cytokines such as insulin, leptin, leptin receptor, ghrelin, adiponectin, resistin or retinol-binding protein 4 (RBP4).

The explanation of these findings could potentially be assumed in experimental outcomes of animal models of normal and insulin resistant ob/ob mice. On the one hand, elevated CTRP1 levels lowers blood glucose levels without altering insulin or adiponectin levels[2,3], and on the other hand, high CTRP1 concentrations protect from diet-induced obesity and insulin-resistance, while CTRP1 knockout mice developed insulin resistance and hepatic steatosis[6,11].

A further key reason besides its protective role against insulin resistance may be the overlapping inflammatory activity in critically ill patients, which we discuss in the following paragraph. Impaired glucose metabolism in CTRP1 knock out-mice was associated with increased hepatic gluconeogenic gene expression, decreased muscle glucose transporter glucose transporter (GLUT) 4 levels and AMP-activated protein kinase activation[11,12].

Consistent with its effect in mice, CTRP1 was described to act directly and independently of insulin, and to regulate gluconeogenesis in cultured hepatocytes[2]. These expected associations between CTRP1 and metabolic parameters likely reflect the physiological, homeostatic functions of CTRP1 before the onset of critical illness.

Our study identified additional, unexpected correlations between elevated levels of CTRP1 and inflammation as well as organ failure in ICU patients. CTRP1 is inversely correlated with the glomerular filtration rate (GFR). In addition, we found further positive correlations with urea, creatinine and cystatin C. This association of CTRP1 to renal impairment might be a possible reason for elevated CTRP1 in circulation. This could be due to the retention of CTRP1 in patients with renal insufficiency. CTRP1 correlated significantly with indicative markers of liver damage and cholestasis. These results are presumably related to the corresponding correlation results with parameters of glucose and energy metabolism as well as inflammatory response.

Experimental studies indeed support that CTRP1 is involved in controlling systemic inflammatory responses. In lipopolysaccharide (LPS) stimulated rats, CTRP1 is expressed at high levels in adipose tissues[8]. The LPS-induced increase in CTRP1 gene expression was found to be mediated by inflammatory cytokines including tumor necrosis factor-α

and interleukin 1β[8,30]. CTRP1 itself causes a concentration-dependent expression of further inflammatory markers in endothelial and vascular smooth muscle cells[1,19,31], thereby serving as an amplifier of inflammation beyond its tissue of origin.

For instance, in heart failure CTRP1 levels in epicardial adipose tissue and blood circulation are elevated[8]. Elevated CTRP1 levels in circulation increased IL-6 mRNA levels in congestive heart failure and induced aldosterone release through JAK2-STAT3 signaling pathways[8,30,32]. Similar mechanisms may be present in patients with sepsis, where disturbed blood pressure, vascular tone and vascular barrier dysfunction are commonly observed[31,33]. On the other hand, CTRP1 administration in experimental CTRP1 knockout mice protected against hyper-inflammation through activation of the S1P/cAMP signalling pathways in cardiomyocytes[6,32].

We found a strong correlation of CTRP1 with classical inflammatory parameters such as CRP, procalcitonin and IL-6. Along with the positive correlation between CTRP1 and suPAR, CTRP1 might be induced by the activated immune system[27]. Despite the close correlation between CTRP1 and inflammatory markers in our study, elevated CTRP1 did not predict an adverse prognosis at the ICU.

Thus, further studies are warranted to clarify whether elevated CTRP1 reflect an either harmful or protective endogenous response to critical illness.

In summary, CTRP1 is correlated with several factors such as chronic hyperglycemia (HbA1c), inflammation (CRP), renal function (creatinine) and liver injury (bilirubin), making it challenging to dissect its clinical relevance from confounding factors.

However, although our study included 218 patients, the sample size of our study very likely does not allow to control for all potential confounders.

Conclusions

CTRP1 is a promising novel molecular mediator connecting inflammatory and metabolic diseases[3,4]. In our large, prospectively enrolled study, CTRP1 levels were significantly elevated in critically ill patients and were associated with inflammation and sepsis as well as diabetes and metabolic disturbances.

Our data support that CTRP1 is integrated in the complex network of adipokines in the pathogenesis of critical illness, sepsis and organ failure. However, CTRP1 does not yet have a clear clinical benefit, but the recognition of potential correlation between

metabolic changes and inflammation in intensive care patients could indicate a clinical relevance of this adipokine.

Mechanistic studies are needed to clarify its exact pathogenic role in this setting. A more detailed knowledge of the different roles of CTRP1 in metabolic and inflammatory pathways may result in a better understanding of critical disease conditions.

References

1. Bai B, Ban B, Liu Z, Zhang MM, Tan BK, Chen J. Circulating C1q complement/TNF-related protein (CTRP) 1, CTRP9, CTRP12 and CTRP13 concentrations in Type 2 diabetes mellitus: In vivo regulation by glucose. PLoS One 2017;12:e0172271.
2. Peterson JM, Wei Z, Wong GW. C1q/TNF-related protein-3 (CTRP3); a novel adipokine that regulates hepatic glucose output. J Biol Chem. 2010;285:39691–39701.
3. Schäffler A, Buechler C. CTRP family: Linking immunity to metabolism. Trends Endocrinol Metab. 2012;23:194–204.
4. Seldin MM, Tan SY, Wong GW. Metabolic function of the CTRP family of hormones. Rev Endocr Metab Disord. 2014;15:111–123.
5. Chen L, Wu F, Yuan S, Feng B. Identification and characteristic of three members of the C1q/TNF-related proteins (CTRPs) superfamily in Eudontomyzon morii. Fish Shellfish Immunol. 2016;59:233-240.
6. Shabani P, Emamgholipour S, Doosti M. CTRP1 in Liver Disease. Adv. Clin. Chem. 2017, 79, 1–23.
7. Kon, M.; Ebi, Y.; Nakagaki, K. Effects of a single bout of high-intensity interval exercise on C1q/TNF-related proteins. Appl Physiol Nutr Metab. 2019;44:47–51.
8. Yang Y, Liu S, Zhang RY, Luo H, Chen L, He WF, Lei R, Liu MR, Hu HX, Chen M. Association Between C1q/TNF-Related Protein-1 Levels in Human Plasma and Epicardial Adipose Tissues and Congestive Heart Failure. Cell Physiol Biochem. 2017;42:2130–2143.
9. Han S, Yang Y. A Novel Blood Pressure Modulator C1q/TNF-α-related Protein 1 (CTRP1). BMB Rep. 2018l51:611.
10. Chen H, Gao L, Huang Z, Liu Y, Guo S, Xing J, Meng Z, Liang C, Li Y, Yao R, et al. C1qTNF-related protein 1 attenuates doxorubicin-induced cardiac injury via activation of AKT. Life Sci. 2018;207:492–498.
11. Peterson JM, Aja S, Wei Z, Wong GW. CTRP1 protein enhances fatty acid oxidation via AMP-activated protein kinase (AMPK) activation and acetyl-CoA carboxylase (ACC) inhibition. J Biol Chem. 2012;287: 1576–1587.
12. Rodriguez S, Lei X, Petersen PS, Tan SY, Little HC, Wong GW. Loss of CTRP1 disrupts glucose and lipid homeostasis. Am J Physiol Endocrinol Metab. 2016;311:E678–E697.
13. Koch A, Weiskirchen R, Zimmermann HW, Sanson E, Trautwein C, Tacke F. Relevance of serum leptin and leptin-receptor concentrations in critically ill patients. Mediat Inflamm. 2010;2010:473540.
14. Chalupova L, Zakovska A, Adamcova K. Development of a novel enzyme-linked immunosorbent assay (ELISA) for measurement of serum CTRP1: A pilot study: Measurement of serum CTRP1 in healthy donors and patients with metabolic syndrome. Clin Biochem. 2013;46:73–78.
15. Koch A, Weiskirchen R, Krusch A, Bruensing J, Buendgens L, Herbers U, Yagmur E, Koek GH, Trautwein C, Tacke F. Visfatin Serum Levels Predict Mortality in Critically Ill Patients. Dis Markers 2018;2018: 7315356.
16. Koch A, Gressner OA, Sanson E, Tacke F, Trautwein C. Serum resistin levels in critically ill patients are associated with inflammation.; organ dysfunction and metabolism and may predict survival of non-septic patients. Crit Care. 2009;13:R95.
17. Luedde M, Benz F, Niedeggen J, Vucur M, Hippe HJ, Spehlmann ME, Schueller F, Loosen S, Frey N, Trautwein C; et al. Elevated Omentin Serum Levels Predict Long-Term Survival in Critically Ill Patients. Dis Markers. 2016;2016:3149243.
18. Koch A, Sanson E, Voigt S, Helm A, Trautwein C, Tacke F. Serum adiponectin upon admission to the intensive care unit may predict mortality in critically ill patients. J Crit Care. 2011;26:166–174.
19. Kim D, Park SY. C1q and TNF related protein 1 regulates expression of inflammatory genes in vascular smooth muscle cells. Genes Genomics. 2019;41:397-406.
20. Koch A, Weiskirchen R, Kunze J, Dückers H, Bruensing J, Buendgens L, Matthes M, Luedde T, Trautwein C, Tacke F. Elevated asymmetric dimethylarginine levels predict short- and long-term mortality risk in critically ill patients. J Crit Care. 2013;28:947–953.
21. Buendgens L, Yagmur E, Bruensing J, Herbers U, Baeck C, Trautwein C, Koch A, Tacke F. Growth Differentiation Factor-15 Is a Predictor of Mortality in Critically Ill Patients with Sepsis. Dis Markers. 2017;2017:5271203.

22. Rhodes A, Evans LE, Alhazzani W, Levy MM, Antonelli M, Ferrer R, Kumar A, Sevransky JE, Sprung CL, Nunnally ME, et al. Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock: 2016. Intensive Care Med. 2017;43:304–377.
23. Koch A, Voigt S, Kruschinski C, Sanson E, Dückers H, Horn A, Yagmur E, Zimmermann H, Trautwein C, Tacke F. Circulating soluble urokinase plasminogen activator receptor is stably elevated during the first week of treatment in the intensive care unit and predicts mortality in critically ill patients. Crit Care. 2011;15:R63.
24. Feng S, Su Y, Luo L, Jing F, Yi Q. Serum levels of C1q/tumor necrosis factor-related protein-1 in children with Kawasaki disease. Pediatr Res. 2018;83:999–1003.
25. Han S, Jeong AL, Lee S, Park JS, Buyanravjikh S, Kang W, Choi S, Park C, Han J, Son WC, et al. C1q/TNF-α-Related Protein 1 (CTRP1) Maintains Blood Pressure Under Dehydration Conditions. Circ Res. 2018; 123: e5–e19.
26. Han S, Kim JD, Lee S, Jeong AL, Park JS, Yong HJ, Boldbaatar A, Ka HI, Rhee EJ, Lee WY, et al. Circulating CTRP1 Levels in Type 2 Diabetes and Their Association with FGF21. Int J Endocrinol. 2016;2016: 5479627.
27. Backes Y, van der Sluijs KF, Mackie DP, Tacke F, Koch A, Tenhunen JJ, Schultz MJ. Usefulness of suPAR as a biological marker in patients with systemic inflammation or infection: A systematic review. Intensive Care Med. 2012;38:1418–1428.
28. Pan X, Lu T, Wu F, Jin L, Zhang Y, Shi L, Li X, Lin Z. Circulating complement-C1q TNF-related protein 1 levels are increased in patients with type 2 diabetes and are associated with insulin sensitivity in Chinese subjects. PLoS One. 2014;9:e94478.
29. Han S, Park JS, Lee S, Jeong AL, Oh KS, Ka HI, Choi HJ, Son WC, Lee WY, Oh SJ, et al. CTRP1 protects against diet-induced hyperglycemia by enhancing glycolysis and fatty acid oxidation. J Nutr Biochem. 2016;27:43–52.
30. Jeon JH, Kim KY, Kim JH, Baek A, Cho H, Lee YH, Kim JW, Kim D, Han SH, Lim JS, et al. A novel adipokine CTRP1 stimulates aldosterone production. FASEB J. 2008;22:1502–1511.
31. Lu L, Zhang RY, Wang XQ, Liu ZH, Shen Y, Ding FH, Meng H, Wang LJ, Yan XX, Yang K, et al. C1q/TNF-related protein-1: An adipokine marking and promoting atherosclerosis. Eur Heart J. 2016;37: 1762-1771.
32. Yuasa D, Ohashi K, Shibata R, Mizutani N, Kataoka Y, Kambara T, Uemura Y, Matsuo K, Kanemura N, Hayakawa S, et al. C1q/TNF-related protein-1 functions to protect against acute ischemic injury in the heart. FASEB J. 2016;30:1065–1075.
33. Liu ZH, Li C, Chen JW, Shen Y, Gao J, Shen WF, Zhang RY, Wang XQ, Lu L. C1q/TNF-related protein 1 promotes endothelial barrier dysfunction under disturbed flow. Biochem Biophys Res Commun. 2017; 490:580–586.

Chapter 7

Decreased CTRP3 Plasma Concentrations are Associated with Sepsis and Predict Mortality in Critically Ill Patients

Eray Yagmur
Simone Otto
Ger H. Koek
Ralf Weiskirchen
Christian Trautwein
Frank Tacke
Alexander Koch

Diagnostics (Basel). 2019:9(2):63

Abstract

C1q/ tumor necrosis factor (TNF)-like protein 3 (CTRP3) represents a novel member of the adipokine family that exerts favorable metabolic actions in humans. However, the role of CTRP3 in critical illness and sepsis is currently unknown.

Upon admission to the medical intensive care unit (ICU), we investigated CTRP3 plasma concentrations in 218 critically ill patients (145 with sepsis, 73 without sepsis). Results were compared with 66 healthy controls.

CTRP3 plasma levels were significantly decreased in critically ill patients, when compared to healthy controls. In particular, low CTRP3 levels were highly associated with the presence of sepsis. CTRP3 levels were neither associated with obesity nor diabetes. In critically ill patients, CTRP3 plasma concentrations were inversely correlated with inflammatory cytokines and classical sepsis markers.

Among a wide group of adipokines, CTRP3 only correlated with circulating resistin. Low CTRP3 plasma levels were associated with the overall mortality, and CTRP3 levels below 620.6 ng/mL indicated a particularly increased mortality risk in ICU patients.

Our study demonstrates for the first time the role of circulating CTRP3 as a biomarker in critically ill patients that might facilitate diagnosis of sepsis as well as prognosis prediction. The association between low CTRP3 and increased inflammation warrants further pathophysiological investigations.

Introduction

Critical illness is associated with alterations in secretory and metabolic functions of adipose tissue[1-4]. In principle, the endocrine productions of the adipose tissue (i.e., adipocytokines or adipokines), are involved in a wide range of processes including dietary intake and appetite regulation, energy expenditure, insulin resistance, lipid metabolism, immunity, inflammatory and acute phase responses, vascular homeostasis, endothelial function, and angiogenesis[5-9].

Based on this wide range of physiological functions, alterations of adipokines have been convincingly linked to inflammatory responses and metabolic alterations that occur during critical illness[2,3].

To date, more than 50 adipokines have been reported[10]. Adipokines with recognized functions in critical illness are leptin, retinol binding protein 4 (RBP4), adiponectin, ghrelin, resistin, and C1q/tumor necrosis factor (TNF) related protein 1 (CTRP1)[1,11-16]. Despite its potential involvement in the pathogenesis of inflammation and metabolism, some of these adipokines demonstrate interesting potential as prognostic biomarkers, as their circulating levels are associated with the short- or long-term survival in critically ill patients[4,11-16].

Nevertheless, a number of potential confounders must be considered for adipokines, including the etiology of critical illness (e.g., sepsis), pre-existing diabetes, obesity, and organ failure[4,11-16].

Complement C1q/tumor necrosis factor (TNF) related protein 3 (CTRP3) is a novel adipokine mainly expressed in subcutaneous and visceral adipose tissue[17-20]. Like other adipokines, the CTRP family is involved in a variety of clinical and pathophysiological processes including metabolism, food intake, inflammation, tumor metastasis, apoptosis, vascular disorders, and ischemic injury responses[21,22]. Similarly, CTRP3 is considered to have multiple, primarily “beneficial” effects, including lowering glucose levels, inhibiting gluconeogenesis in the liver, increasing angiogenesis, and anti-inflammation[23-25]. Its expression is upregulated by insulin and downregulated by chronic lipopolysaccharide exposure[26]. Circulatory CTRP3 levels are reduced in human and animal models of obesity and diabetes[2,17,21].

In this work, we investigated the clinical and prognostic relevance of CTRP3 plasma concentrations in a large cohort of critically ill patients (septic and non-septic patients) from a medical intensive care unit (ICU).

Materials and methods

The study included 218 critically ill patients, who were admitted to the medical ICU at the RWTH University Hospital Aachen, Germany. The current cohort of patients was collected from an ongoing, prospective observational trial in our unit, in which patients were included consecutively.

For current analysis, we therefore randomly enrolled 218 (n=218) patients that had been treated between 2006 and 2011 from the existing biobank. We excluded patients, who had an elective procedure or were admitted for post-interventional observational stay[27]. We used the Third International Consensus Definitions for Sepsis and Septic Shock as a post-hoc definition for sepsis patients; the others were categorized as non-sepsis patients[28]. The patients were treated following the current guidelines for treatment of sepsis (Surviving Sepsis Campaign)[29].

As a healthy control group, we analyzed 66 (n=66) blood donors (43 male, 23 female, median age 29.5 years, range 18-67 years, body mass index (BMI) median 25.4 kg/m², range 17.9-37 kg/m²). None of the investigated controls had apparent disturbed blood counts or elevated values of liver enzymes. All healthy controls had a negative serology for viral hepatitis and human immunodeficiency virus (HIV)[30].

Obesity was classified in agreement with the recommended World Health Organization (WHO) BMI value ≥30 kg/m^2 (obesity class I, BMI 30.0-34.99 kg/m^2) that is primarily based on the association between BMI and mortality[31].

To assess the patients' long-term outcome, we contacted the patients, their relatives and/or the primary care physician in approximately six-month intervals after discharge from the hospital for two years[30].

The study protocol was approved by the local ethics committee and conducted in accordance to the ethical standards laid down in the Declaration of Helsinki (ethics committee of the University Hospital Aachen, RWTH-University Aachen, Germany, reference number EK 150/06, day month year). All included participants provided written informed consent.

Blood samples were collected directly at the time of admission to the ICU (before specific therapeutic measures). After centrifugation at 4°C for 10 min, serum and plasma aliquots of 1 mL were frozen immediately at −80°C. Plasma CTRP3 concentrations were determined using a quantitative sandwich enzyme immunoassay (ELISA), according to the manufacturer's instructions (CTRP3 (human) competitive ELISA Kit, #AG-45A-0042TP-KI01, AdipoGen, Liestal, Switzerland). Measurements of the other

adipocytokines and related proteins CTRP1, leptin, RBP4, adiponectin, ghrelin, and resistin were included, as previously reported[11-16].

Data are given as median and range due to the skewed distribution of most of the parameters. Box plot graphics are used to illustrate differences between subgroups. Since most samples were not normally distributed, the Mann–Whitney U-test was applied to test for statistically significant differences between the two groups. Correlations were assessed by the Spearman's rank correlation method. All values, including outside values as well as far-out values, were included. P-values less than 0.05 were considered as statistically significant. The prognostic value of CTRP3 on the outcome was evaluated by Cox regression models and survival curves were generated by Kaplan–Meier analyses with a CTRP3 cut-off level calculated via the Youden index[32]. All analyses were conducted using IBM SPSS Statistics (SPSS; Chicago, IL, USA).

Results

CTRP3 plasma levels are significantly decreased in critically ill patients as compared with healthy controls

In critical illness, many adipokines are significantly elevated in the blood circulation, such as ghrelin, resistin, and CTRP1 blood concentrations[14-16]. On the contrary, we found that CTRP3 plasma levels were significantly decreased in a large cohort of 218 critically ill patients (median 545.1 ng/mL, range 82.9–2395.3 ng/mL; Table 7.1) at admission to the ICU as compared with 66 healthy controls (median 1088,4 ng/mL, range 539.2–2547.9 ng/mL, $p<0.001$; Figure 7.1A).

Reduced CTRP3 plasma levels in critically ill patients are associated with the presence of sepsis

CTRP3 specifically blocks the binding of lipopolysaccharide (LPS) to its receptor, the toll-like receptor 4 (TLR4)[33,34], thereby inhibiting inflammatory responses in innate immune cells[33]. Within the cohort of ICU patients, plasma concentrations of CTRP3 were significantly decreased in patients with sepsis (n=145, median 493.4 ng/mL, range 82.9–2395.3 ng/mL) as compared to patients without sepsis (n=73, median 758.8 ng/mL, range 260.4–2269.1 ng/mL, $p<0.001$; Figure 7.1A).

Typical sites of infection in sepsis are pneumonia and abdominal and urogenital tract infections, while non-sepsis causes of critical illness include, among others, cardiopulmonary diseases, acute pancreatitis, and decompensated liver cirrhosis (Table 7.2). Among the septic or non-septic critically ill patients, there was no association

between CTRP3 plasma concentrations and these different disease etiologies leading to ICU admission (data not shown).

Table 7.1 Baseline patient characteristics and C1q/ tumor necrosis factor (TNF)-like protein 3 (CTRP3) plasma measurements.

Parameter	All Patients	Non–Sepsis	Sepsis	* *p*
Number (*n*)	218	73	145	
Sex (male/female) (*n*)	133/85	48/25	85/60	n.s.
Age (years)	64 (18–90)	61 (18–85)	65 (20–90)	n.s.
APACHE-II score	18 (2–43)	13.5 (2–33)	19 (4–43)	<0.001
SOFA score	9 (0–19)	9.5 (2–19)	7 (0–17)	0.002
Intensive care unit (ICU) days	7 (1–137)	6 (1–45)	9 (1–137)	0.004
Death during ICU *n* (%)	49 (22.5)	9 (12.3)	40 (27.6)	0.010
Death during follow-up (total) *n* (%)	89 (40.8)	22 (30.1)	67 (46.2)	0.026
Mechanical ventilation *n* (%)	143 (65.6)	46 (63)	97 (66.9)	n.s.
Pre-existing diabetes *n* (%)	64 (29.4)	22 (30.1)	42 (29.0)	n.s.
BMI (m^2/kg)	25.8 (14–86)	25.7 (15.9–40.5)	25.9 (14–86.5)	n.s.
Glucose (mg/dL)	137 (1–663)	150 (49–663)	133 (1–476)	n.s.
WBC ($x10^3/\mu L$)	13.1 (0.1–208)	12.5 (1.8–29.6)	14 (0.1–208)	0.024
CRP (mg/dL)	100.5 (5–230)	17 (5–230)	164 (5–230)	<0.001
IL-6 (pg/mL)	150.0 (2–28000)	66.5 (1.5–5000)	250 (0.1–28000)	<0.001
Procalcitonin (ng/mL)	0.7 (0.03–207.5)	0.2 (0.03–100)	2.2 (0.1–207.5)	<0.001
Creatinine (mg/dL)	1.3 (0.1–15)	1.0 (0.2–15)	1.5 (0.1–10.7)	0.017
GFR-Cystatin C (mL/min)	34 (0–379)	59 (5–379)	21.5 (0–218)	<0.001
AST (U/L)	42 (5–20332)	47 (11–20332)	41 (5–7832)	n.s.
ALT (U/L)	30 (5–7867)	36.5 (7–7867)	25 (5–5890)	n.s.
γ-GT (U/L)	59 (5–1764)	56 (10–1764)	60 (5–5000)	n.s.
GLDH (U/L)	6 (1–5000)	8 (1–5000)	5 (1–686)	n.s.
AP (U/L)	82 (5–686)	77 (2–290)	86 (5–686)	n.s.
PCHE (U/L)	3997 (10–11001)	5189 (405–11001)	3698 (10–10896)	0.005
Bilirubin, total (mg/dL)	0.7 (0.1–20.8)	0.7 (0.1–20.8)	0.7 (0.1–18.9)	n.s.
Albumin (mg/dL)	28 (0.1–61.4)	29.1 (1.6–52.2)	27.1 (0.1–61.4)	n.s.
INR	1.16 (0.92–13)	1.17 (0.95–6.73)	1.16 (0.92–13)	n.s.
CTRP3 day 1 (ng/mL)	545.1 (82.9–2395.3)	758.8 (260.4–2269.1)	493.4 (82.9–2395.3)	<0.001

For quantitative variables, median and range (in parenthesis) are given. * Significance between sepsis and non-sepsis patients was assessed using the Mann–Whitney U-test (for quantitative variables) or chi-squared test (for categorical variables). APACHE: Acute Physiology And Chronic Health Evaluation; SOFA: sequential organ failure assessment; SAPS: simplified acute physiology score; BMI: body mass index; CRP: C-reactive protein; IL-6: interleukin 6; ICU: intensive care unit; INR: international normalized ration; WBC: white blood cell; GFR: glomerular filtration rate; AST: aspartate aminotransferase; ALT: alanine aminotransferase; γ-GT: gamma-glutamyltransferase; GLDH: glutamate dehydrogenase; AP: alkaline phosphatase; PCHE: cholinesterase; n. s.: not significant.

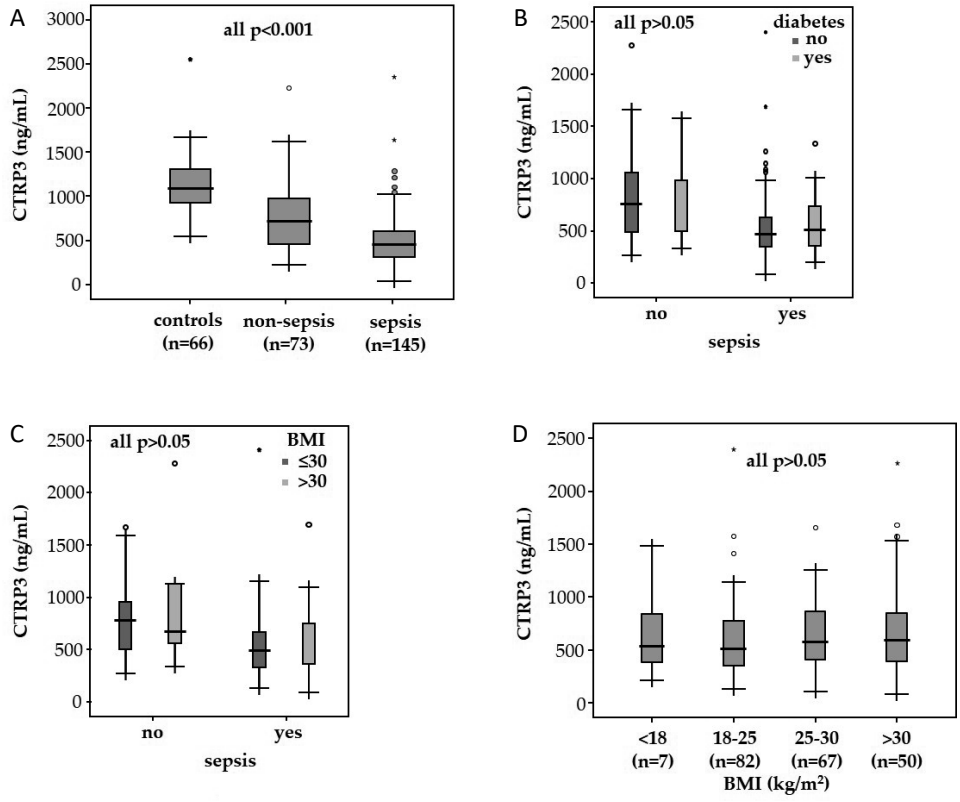


Figure 7.1 CTRP3 levels in critically ill patients. CTRP3 plasma concentrations are significantly decreased in critically ill patients compared with healthy controls (**A**). ICU patients with sepsis displayed significantly decreased CTRP3 levels compared to patients without sepsis (**A**). CTRP3 plasma concentrations in ICU patients are neither associated with pre-existing type 2 diabetes (**B**) nor obesity, as defined by a body mass index (BMI) above 30 kg/m^2 (**C**). ICU patients did not show significant differences in reference to BMI classification (**D**). P-values (U-test) are given in the figure.

Table 7.2 Disease etiology of the study population leading to ICU admission.

	Sepsis ***n*=145**	**Non-Sepsis** ***n*=73**
Bacterial etiology of critical illness (sepsis)		
Site of infection *n* (%)		
Pulmonary	72 (50)	
Abdominal	28 (19)	
Urogenital	11 (8)	
Other site of infection	34 (23)	
Non-bacterial etiology of critical illness (non-sepsis) *n* (%)		
Cardio-pulmonary disorder		29 (40)
Acute pancreatitis		10 (14)
Acute liver failure		4 (5.5)
Decompensated liver cirrhosis		9 (12)
Severe gastrointestinal hemorrhage		4 (5.5)
Other non-bacterial etiology		17 (23)

CTRP3 plasma levels in critically ill patients are not associated with diabetes and obesity

CTRP3 is primarily involved in glucose metabolism, supporting that its plasma levels are associated with type 2 diabetes and obesity[17]. We therefore assessed whether metabolic comorbidities, including pre-existing obesity or diabetes, impacted CTRP3 levels at ICU admission.

Surprisingly, neither pre-existing type 2 diabetes nor obesity, as defined by a body mass index (BMI) above 30 kg/m², were associated with CTRP3 plasma concentrations (Figure 1b,c). Moreover, serum glucose at ICU admission (r=−0.126, p=0.064) or glycosylated hemoglobin A1 (HbA1c) (r=−0.051, p=0.671) did not correlate with CTRP3 levels in critically ill patients (Table 7.3). CTRP3 did not show any correlations with other key markers of glucose metabolism such as insulin or the homeostasis model assessment-insulin resistance (HOMA-IR) in ICU patients (data not shown).

However, β-cell function (HOMA-β) (r=−0.371, p=0.002) and C-peptide (r=−0.281, p=0.020) correlated inversely with CTRP3 (Table 7.3).

CTRP3 levels in critically Ill patients are inversely correlated with biomarkers of inflammatory responses in critically ill patients

Adipose tissue inflammation attributes to dysregulated production and release of inflammatory cytokines and adipokines, including IL-6 and TNF-α as well as leptin, resistin, and adiponectin[35].

Table 7.3 Correlations with CTRP3 plasma concentrations at ICU admission (Spearman rank correlation test).

	ICU Patients	
Parameters	***r***	****p***
Disease severity		
APACHE-II score	−0.063	0.378
SOFA score	−0.237	0.007
SAPS2 score	−0.187	0.129
Diabetes/insulin resistance		
Glucose	0.126	0.064
Glycosylated hemoglobin A1	0.051	0.671
C-peptide	−0.281	0.020
HOMA-β	−0.371	0.002
Inflammatory response		
White blood cell count	−0.148	0.029
Lymphocyte count	0.081	0.339
C-reactive protein	−0.390	<0.001
Procalcitonin	−0.207	0.009
TNF-α	−0.399	0.018
Interleukin-6	−0.266	0.001
Interleukin 10	−0.085	0.382
suPAR	−0.251	0.003
NTproCNP	−0.352	<0.001
Renal function		
Urea	−0.216	0.001
Cardiac function		
NTproBNP	−0.278	0.001
Liver function		
Protein	0.221	0.003
Albumin	0.330	<0.001
AST	0.102	0.147
ALT	0.048	0.483
Pseudocholinesterase	0.326	<0.001
Alkaline phosphatase	−0.145	0.039
aPTT	−0.156	0.023
Antithrombin III	0.240	0.006
D-dimers	−0.316	0.008
Lipid metabolism		
Cholesterol	0.213	0.005
LDL-cholesterol	0.326	0.008
HDL-cholesterol	0.387	0.001
Adipokines		
Resistin	−0.397	0.002
Leptin	0.258	0.055
RBP4	0.072	0.565
Adiponectin	−0.196	0.147
Ghrelin	−0.090	0.500
CTRP1	−0.075	0.270

SOFA: sequential organ failure assessment; SAPS2: simplified acute physiology score 2; HOMA-β: homeostasis model assessment-beta cell function; TNF-α: tumor necrosis factor-α; suPAR: soluble urokinase-type plasminogen activator receptor; NTproBNP: N-terminal pro C-type natriuretic peptide; NTproCNP: N-terminal pro B-type natriuretic peptide; CTRP1: C1q/tumor necrosis factor (TNF) related protein 1; RBP4: retinol binding protein 4; aPTT: activated prothrombin time; LDL: low-density lipoprotein; HDL: high-density lipoprotein; * - P-values less than 0.05 were considered as statistically significant.

We investigated the potential association between CTRP3 and inflammatory responses in critically ill patients. In agreement with the proposed anti-inflammatory function of CTRP3, we observed an inverse correlation between CTRP3 and classical markers of inflammation or sepsis, such as interleukin 6 (r=−0.266, p=0.001, Figure 7.2A), procalcitonin (r=−0.207, p=0.009, Figure 7.2B), C-reactive protein (r=−0.390, p<0.001), TNF-α (r=−0.399, p=0.018), and soluble CD87 (soluble urokinase-type plasminogen activator receptor (suPAR), r=−0.251, p=0.003) (Table 7.3). We found no correlation to the anti-inflammatory interleukin 10 (r=−0.085, p=0.382).

Among a broad range of adipokines, including CTRP1, leptin, RBP4, adiponectin, ghrelin, and resistin[11-16], CTRP3 only correlated, inversely, with resistin (r=−0.397, p=0.002, Figure 7.2C).

Circulating CTRP3 displayed no association with markers of renal failure, such as creatinine, cystatin C, and the glomerular filtration rate (GFR), or markers reflecting liver damage and cholestasis like alanine aminotransferase (ALT) and total bilirubin. However, CTRP3 levels correlated with lipid metabolism as reflected by cholesterol, low-density lipoprotein (LDL) cholesterol and high-density lipoprotein (HDL) cholesterols (Table 7.3).

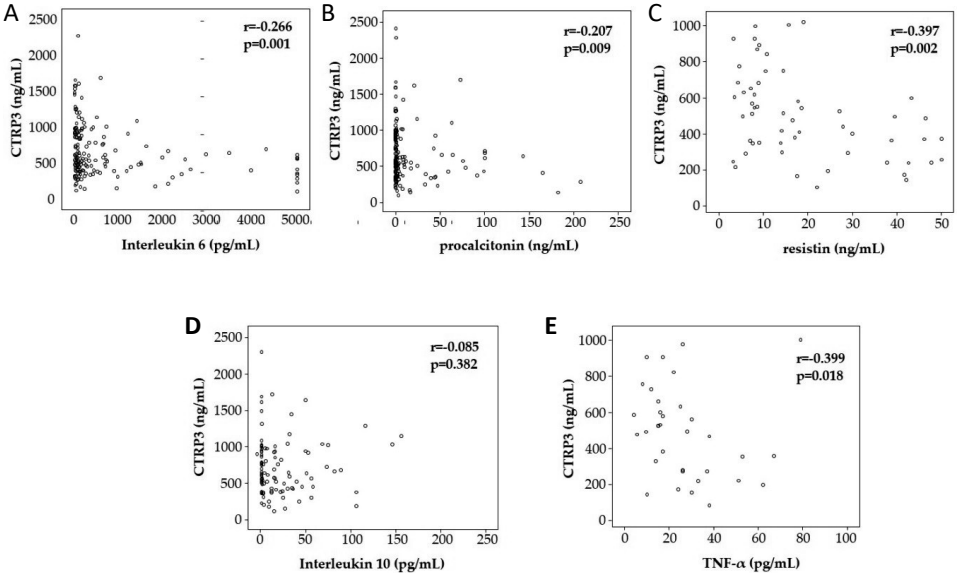


Figure 2. CTRP3 levels correlate inversely with inflammatory response (Spearman rank correlation test). CTRP3 plasma levels, at time of admission to the ICU, are correlated inversely with inflammatory biomarkers such as serum interleukin 6 (**A**), procalcitonin (**B**), and TNF-α (**E**), but not IL-10 (**D**). CTRP3 correlated inversely with serum resistin concentrations in ICU patients (**C**). TNF: tumor necrosis factor; IL-10: interleukin 10.

Low CTRP3 plasma levels in critically ill patients are associated with adverse prognosis

Circulating adipokines have been previously suggested as biomarkers for disease severity as well as short- and long-term survival in various critical illness conditions[2-4]. In fact, CTRP3 plasma levels correlated inversely with sequential organ failure assessment (SOFA; r=−0.237, p=0.007, Table 7.3), but not with other ICU scores like acute physiology and chronic health II (APACHE II; r=−0.063, p=0.378) or simplified acute physiology score 2 (SAPS2; r=−0.187, p=0.129) scores (Table 7.3).

Critically ill patients have a high risk of mortality, not only during the ICU treatment but also after successful discharge from the ICU[36].

We were able to assess the long-term survival in 207 out of 218 patients by contacting the patients or their relatives during the first three years after ICU discharge, thereby providing a comprehensive picture of overall mortality (during ICU and during follow-up). CTRP3 levels at ICU admission were significantly lower in patients that subsequently died (n=89) compared with survivors (n=118) (Figure 7.3A). Cox regression analysis revealed that CTRP3 levels at ICU admission were a significant predictor of overall mortality (p=0.020).

This finding was corroborated by Kaplan–Meier survival curves analyses, demonstrating that patients with CTRP3 plasma levels of the upper quartile (>75%, corresponding to >848 ng/mL) had the best survival rates, while patients with admission CTRP3 levels of the lower quartile (<25%, corresponding to <387.4 ng/mL) had an unfavorable outcome (Figure 7.3B). Using the calculated optimal cut-off for CTRP3 of 620.6 ng/mL, patients with low CTRP3 demonstrated a high mortality rate, as depicted by Kaplan–Meier survival curve analysis (Figure 7.3C).

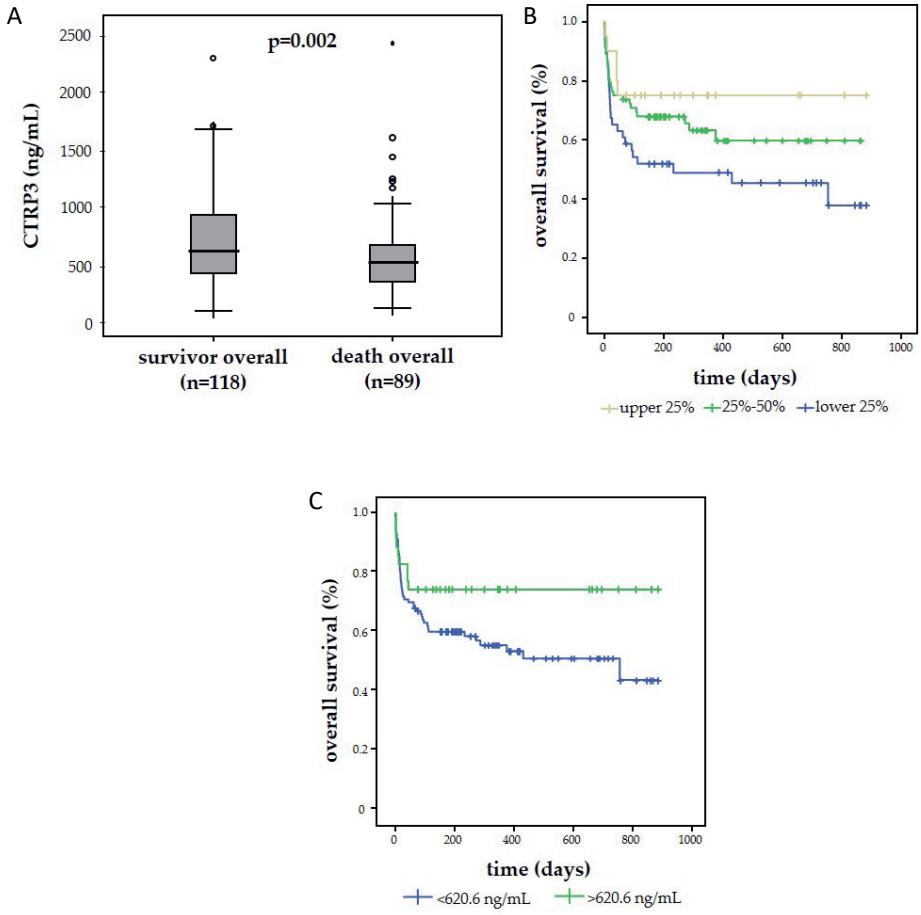


Figure 7.3 CTRP3 is a biomarker for mortality in critically ill patients. Patients that subsequently died displayed significantly lower CTRP3 levels at ICU admission compared to patients that survived in the long-term follow-up (**A**). High vs. low CTRP3 plasma concentrations discriminated survival of the critically ill patients, as displayed by Kaplan–Meier survival curve analysis for CTRP3 separated into quartiles (**B**). Low CTRP plasma concentrations at ICU admission (optimal cut-off: 620.6 ng/mL) predicted the overall mortality in critically ill patients (**C**).

Discussion

In this study, we demonstrate that circulating levels of the novel adipokine family member CTRP3 has potential as a biomarker in critically ill patients.

Critically ill patients had significantly reduced CTRP3 levels compared with healthy controls, and CTRP3 was particularly low in ICU patients with sepsis. Thus, CTRP3 is the only adipocytokine in septic intensive care patients that is significantly decreased compared to published adipokines and circulating soluble metabolic proteins cytokines such as CTRP1, ghrelin, resistin, RBP4, leptin, and ghrelin[11-16].

Values below 620.6 ng/mL might indicate a potentially severe progression of critical illness at the ICU. Metabolic comorbidities did not impact CTRP3 in the setting of critical illness. Moreover, low CTRP plasma concentrations predicted the overall mortality in critically ill patients.

Critically ill patients show dramatic metabolic and inflammatory derangements, which include dysregulated adipokines[1,2]. Several observations support that dysregulated adipokines are involved in the pathogenesis of critical illness and sepsis[3,4]. However, adipokine levels might be prone to confounding factors such as age, gender, disease severity, feeding protocols, baseline body fat mass, and nutritional status[2]. However, there is evidence that the presence of a systemic inflammatory response is associated with malnutrition such as increased weight loss, an elevated resting energy expenditure and loss of lean tissue and functional decline.

This malnutrition is related with hypoalbuminemia. Thus, in our ICU cohort the close association between albumin and CTRP3 reflects both the loss of the amount of lean tissue and systemic inflammatory response. CTRP3 might potentially be associated with overall nutritional status. Nonetheless, the adipose tissue secretes numerous adipokines that contribute to a wide array of biological and clinical processes[11-16]. Thus, adipose tissue might play a major role in metabolic alterations of critical illness. For instance, increased proinflammatory cytokines are expressed in hypertrophied adipocytes and adipose tissue resident immune cells[37].

CTRP3 is particular among adipokines, because it has been associated with manifold beneficial metabolic as well as anti-inflammatory functions. In an animal model of LPS-induced sepsis, CTRP3 overexpression protected against myocardial dysfunction[38,39]. Furthermore, LPS inhibits adipose tissue differentiation, induces insulin resistance, and prevents the expression of CTRP3[40]. CTRP3 specifically blocks the binding of LPS to its receptor TLR4[33,34] and inhibits proinflammatory responses[33].

However, neither chronic CTRP3 deficiency nor overexpression altered the inflammatory response to a sublethal challenge to LPS[25]. The anti-inflammatory effect of CTRP3 has been rather characterized by decreased mRNA levels of TNF-α and IL-6[2-4,17,21]. It is known that low-grade inflammation in adipose tissue attributes to dysregulated production and release of cytokines and adipokines, including IL-6, TNF-α,

monocyte chemotactic protein (MCP)-1, leptin, resistin, and adiponectin[35]. In adipose tissue, weight cycling leads to significantly decreased CTRP3 mRNA expression and impaired glucose metabolism and insulin sensitivity by decreasing CTRP3[26].

In our study, circulating CTRP3 correlated inversely with several inflammatory markers and cytokines, supporting a counter-regulatory mechanism in critical illness and sepsis. However, we cannot conclude from these associations whether low CTRP3 levels are an important physiological response to allow inflammation or whether inadequately low CTRP3 contributes to disease pathogenesis in ICU patients. The close association between low CTRP3 and adverse prognosis, however, supports the latter hypothesis. Basic research in experimental sepsis models should investigate the functional contribution of low CTRP3 to excessive inflammation as well as the potential to therapeutically target CTRP3 in this setting.

Another aspect of CTRP3 biology is the promotion of insulin resistance by activating inflammatory signaling pathways of c-Jun N-terminal kinase and inhibitor of kB kinase[41]. This attenuates insulin effectiveness by serine phosphorylation of the insulin receptor substrate protein-1[42]. Impaired glucose tolerance and insulin sensitivity leads to decreased CTRP3. In an experimental animal model, both CTRP3 overexpression and daily CTRP3 administration were effective in regulating high-fat diet-induced hepatic insulin resistance and hepatic steatosis[43], but not in mice fed a low-fat diet[43,44].

These data suggest that the metabolic effect of CTRP3 is specific to the liver, as no changes to metabolism is observed in skeletal muscle in any experimental model examined. CTRP3 may function specifically to regulate metabolism in response to elevated lipid consumption[26,43,44].

In our study, we found some correlations of CTRP3 with lipid metabolism, beta-cell function, and C-peptide, but no association between CTRP3 and diabetes or obesity. This is in principle unexpected, based on CTRP3's role in glucose homeostasis[17]. However, CTRP3 levels have been reported to be elevated[45], unaltered[46,47], or reduced[48-50] in individuals with obesity and/or diabetes.

While our study established a role of circulating CTRP3 as a biomarker in critical illness, several limitations need to be mentioned. Although our study comprises a heterogeneous cohort of "real-life" medical ICU patients, the single-center design and the limited number of study subjects may not allow to conduct multiple regression and very detailed subgroup analyses.

Thus, our findings need to be validated in larger, multi-center analyses. In addition, the close association between CTRP3 and inflammation, as well as prognosis, are at this point descriptive.

Nonetheless, our data provide a strong rationale for investigating the function involvement of CTRP3 in systemic inflammation and sepsis. Moreover, we found a broad variety of correlations with inflammation, coagulation and others. This is in line with the currently accepted characteristic of this member of the CTRP superfamily that has been documented to have a wide range of effects on metabolism, food intake, inflammation, vascular disorders. Thus, there are many aspects of CTRP3's regulation and function that have to be explored[17].

Conclusions

CTRP3 is a novel member of the adipokine family, linking inflammatory and metabolic diseases. Our comprehensive analysis of CTRP3 plasma concentrations in a large, prospectively enrolled cohort of critically ill medical patients support that CTRP3 is an interesting biomarker in this setting.

Low CTRP3 values may have diagnostic implications by pointing towards inflammatory and infectious diseases as well as prognostic implications by indicating a high risk of mortality. Thereby, CTRP3 appears to be integrated in the tightly regulated and complex network of adipose tissue-derived endocrine mediators during critical illness.

However, CTRP1 does not yet have a clear clinical benefit, but the recognition of potential correlation between metabolic changes and inflammation in intensive care patients could indicate a clinical relevance of this adipokine. Its functional contribution and validation as a prognostic biomarker warrant further investigation.

References

1. Van den Berghe G. Endocrine evaluation of patients with critical illness. Endocrinol Metab Clin. 2003; 32:385–410.
2. Alipoor E, Mohammad Hosseinzadeh F, Hosseinzadeh-Attar MJ. Adipokines in critical illness: A review of the evidence and knowledge gaps. Biomed Pharmacother. 2018;108:1739–1750.
3. Robinson K, Prins J, Venkatesh B. Clinical review: Adiponectin biology and its role in inflammation and critical illness. Crit Care. 2011;15:221.
4. Hillenbrand A, Knippschild U, Weiss M, Schrezenmeier H, Henne-Bruns D, Huber-Lang M, Wolf AM. Sepsis induced changes of adipokines and cytokines—Septic patients compared to morbidly obese patients. BMC Surg. 2010;10:26.
5. Trayhurn P, Wood IS. Adipokines: Inflammation and the pleiotropic role of white adipose tissue. Br J Nutr. 2004;92:347–355.
6. Lago F, Dieguez C, Gómez-Reino J, Gualillo O. The emerging role of adipokines as mediators of inflammation and immune responses. Cytokine Growth Factor Rev. 2007;18:313–325.
7. Frühbeck G, Gómez-Ambrosi J. Control of body weight: A physiologic and transgenic perspective. Diabetologia. 2003;46:143–172.
8. Rodríguez A, Ezquerro S, Méndez-Giménez L, Becerril S, Frühbeck G. Revisiting the adipocyte: A model for integration of cytokine signaling in the regulation of energy metabolism. Am J Physiol Endocrinol Metab. 2015;309:E691–E714.
9. Andrade-Oliveira V, Câmara NO, Moraes-Vieira PM. Adipokines as drug targets in diabetes and underlying disturbances. J Diabetes Res. 2015;2015:681612.
10. Azamar-Llamas D, Hernández-Molina G, Ramos-Ávalos B, Furuzawa-Carballeda J. Adipokine Contribution to the Pathogenesis of Osteoarthritis. Mediat Inflamm. 2017;2017:5468023.
11. Koch A, Weiskirchen R, Zimmermann HW, Sanson E, Trautwein C, Tacke F. Relevance of serum leptin and leptin-receptor concentrations in critically ill patients. Mediat Inflamm. 2010;2010:473540.
12. Koch A, Weiskirchen R, Sanson E, Zimmermann HW, Voigt S, Dückers H, Trautwein C, Tacke F. Circulating retinol binding protein 4 in critically ill patients before specific treatment: Prognostic impact and correlation with organ function, metabolism and inflammation. Crit Care. 2010;14:R179.
13. Koch A, Sanson E, Voigt S, Helm A, Trautwein C, Tacke F. Serum adiponectin upon admission to the intensive care unit may predict mortality in critically ill patients. J Crit Care. 2011;26:166–174.
14. Koch A, Sanson E, Helm A, Voigt S, Trautwein C, Tacke F. Regulation and prognostic relevance of serum ghrelin concentrations in critical illness and sepsis. Crit Care. 2010;14:R94.
15. Koch A, Gressner OA, Sanson E, Tacke F, Trautwein C. Serum resistin levels in critically ill patients are associated with inflammation, organ dysfunction and metabolism and may predict survival of non-septic patients. Crit Care. 2009;13:R95.
16. Yagmur E, Buergerhausen D, Koek GH, Weiskirchen R, Trautwein C, Koch A, Tacke F. Elevated CTRP1 Plasma Concentration is Associated with Sepsis and Pre-Existing Type 2 Diabetes Mellitus in Critically Ill Patients. J Clin Med. 2019;8:E611.
17. Li Y, Wright GL, Peterson JM. C1q/TNF-Related Protein 3 (CTRP3) Function and Regulation. Compr Physiol. 2017;7:863–878.
18. Schäffler A, Ehling A, Neumann E, Herfarth H, Tarner I, Gay S, Schölmerich J, Müller-Ladner U. Genomic organization, chromosomal localization and adipocytic expression of the murine gene for CORS-26 (collagenous repeat-containing sequence of 26 kDa protein). Biochim Biophys Acta. 2003;1628:64–70.
19. Schäffler A, Weigert J, Neumeier M, Schölmerich J, Buechler C. Regulation and function of collagenous repeat containing sequence of 26-kDa protein gene product “cartonectin”. Obesity 2007;15:303–313.
20. Wong GW, Krawczyk SA, Kitidis-Mitrokostas C, Revett T, Gimeno R, Lodish HF. Molecular, biochemical and functional characterizations of C1q/TNF family members: Adipose-tissue-selective expression patterns, regulation by PPAR-gamma agonist, cysteine-mediated oligomerizations, combinatorial associations and metabolic functions. Biochem J. 2008;416:161–177.
21. Seldin MM, Tan SY, Wong GW. Metabolic function of the CTRP family of hormones. Rev Endocr Metab Disord. 2014;15:111–123.

22. Chen L, Wu F, Yuan S, Feng B. Identification and characteristic of three members of the C1q/TNF-related proteins (CTRPs) superfamily in Eudontomyzon morii. Fish Shellfish Immunol. 2016;59: 233-240.
23. Peterson JM, Wei Z, Wong GW. C1q/TNF-related protein-3 (CTRP3), a novel adipokine that regulates hepatic glucose output. J Biol Chem. 2010;285:39691–39701.
24. Yi W, Sun Y, Yuan Y, Lau WB, Zheng Q, Wang X, Wang Y, Shang X, Gao E, Koch WJ, et al. C1q/tumor necrosis factor-related protein-3, a newly identified adipokine, is a novel antiapoptotic, proangiogenic, and cardioprotective molecule in the ischemic mouse heart. Circulation. 2012;125:3159–3169.
25. Petersen PS, Wolf RM, Lei X, Peterson JM, Wong GW. Immunomodulatory roles of CTRP3 in endotoxemia and metabolic stress. Physiol Rep. 2016;4:e12735.
26. Li X, Jiang L, Yang M, Wu YW, Sun JZ, Sun SX. CTRP3 improves the insulin sensitivity of 3T3-L1 adipocytes by inhibiting inflammation and ameliorating insulin signalling transduction. Endokrynol Pol. 2014;65:252–258.
27. Koch A, Weiskirchen R, Kunze J, Dückers H, Bruensing J, Buendgens L, Matthes M, Luedde T, Trautwein C, Tacke F. Elevated asymmetric dimethylarginine levels predict short- and long-term mortality risk in critically ill patients. J Crit Care. 2013;28:947–953.
28. Buendgens L, Yagmur E, Bruensing J, Herbers U, Baeck C, Trautwein C, Koch A, Tacke F. Growth Differentiation Factor-15 is a Predictor of Mortality in Critically Ill Patients with Sepsis. Dis Markers. 2017;2017:5271203.
29. Rhodes A, Evans LE, Alhazzani W, Levy MM, Antonelli M, Ferrer R, Kumar A, Sevransky JE, Sprung CL, Nunnally ME, et al. Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock: 2016. Intensive Care Med. 2017;43:304–377.
30. Koch A, Voigt S, Kruschinski C, Sanson E, Dückers H, Horn A, Yagmur E, Zimmermann H, Trautwein C, Tacke F. Circulating soluble urokinase plasminogen activator receptor is stably elevated during the first week of treatment in the intensive care unit and predicts mortality in critically ill patients. Crit Care. 2011;15:R63.
31. Branca F, Nikogosian H, Lobstein T. The Challenge of Obesity in the WHO European Region and the Strategies for Response: Summary. In Copenhagen: World Health Organization, Regional Office for Europe; World Health Organization: Geneva, Switzerland, 2007.
32. Buendgens L, Yagmur E, Bruensing J, Herbers U, Baeck C, Trautwein C, Koch A, Tacke F. C-terminal proendothelin-1 (CT-proET-1) is associated with organ failure and predicts mortality in critically ill patients. J Intensive Care. 2017;5:25.
33. Kopp A, Bala M, Buechler C, Falk W, Gross P, Neumeier M, Schölmerich J, Schäffler A. C1q/TNF-related protein-3 represents a novel and endogenous lipopolysaccharide antagonist of the adipose tissue. Endocrinology. 2010;151:5267–5278.
34. Compton SA, Cheatham B. CTRP-3: Blocking a toll booth to obesity-related inflammation. Endocrinology. 2010;151:5095–5097.
35. Tsai S, Clemente-Casares X, Revelo XS, Winer S, Winer DA. Are obesity-related insulin resistance and type 2 diabetes autoimmune diseases? Diabetes. 2015;64:1886–1897.
36. Wilson ME, Barwise A, Heise KJ, Loftsgard TO, Dziadzko M, Cheville A, Majzoub A, Novotny PJ, Gajic O, Biehl M. Long-Term Return to Functional Baseline After Mechanical Ventilation in the ICU. Crit Care Med. 2018;46:562–569.
37. Persson PB, Bondke Persson A. Metabolism, obesity and the metabolic syndrome. Acta Physiol. 2018; 223:e13096.
38. Rogero MM, Calder PC. Obesity, Inflammation, Toll-Like Receptor 4 and Fatty Acids. Nutrients. 2018; 10:E432.
39. Wei WY, Ma ZG, Zhang N, Xu SC, Yuan YP, Zeng XF, Tang QZ. Overexpression of CTRP3 protects against sepsis-induced myocardial dysfunction in mice. Mol Cell Endocrinol. 2018;476:27–36.
40. Schmid A, Kopp A, Hanses F, Bala M, Müller M, Schäffler A. The novel adipokine C1q/TNF-related protein-3 is expressed in human adipocytes and regulated by metabolic and infection-related parameters. Exp. Clin. Endocrinol. Diabetes. 2012;120:611–617.
41. Zand H, Morshedzadeh N, Naghashian F. Signaling pathways linking inflammation to insulin resistance. Diabetes Metab Syndr. 2017;11:S307–S309.

42. De Felice FG, Ferreira ST. Inflammation, defective insulin signaling, and mitochondrial dysfunction as common molecular denominators connecting type 2 diabetes to Alzheimer disease. Diabetes. 2014;63: 2262–2272.
43. Peterson JM, Seldin MM, Wei Z, Aja S, Wong GW. CTRP3 attenuates diet-induced hepatic steatosis by regulating triglyceride metabolism. Am J Physiol Gastrointest Liver Physiol. 2013;305:G214–G224.
44. Wolf RM, Lei X, Yang ZC, Nyandjo M, Tan SY, Wong GW. CTRP3 deficiency reduces liver size and alters IL-6 and TGFβ levels in obese mice. Am J Physiol Endocrinol Metab. 2016;310:E332–E345.
45. Choi KM, Hwang SY, Hong HC, Yang SJ, Choi HY, Yoo HJ, Lee KW, Nam MS, Park YS, Woo JT, et al. C1q/TNF-related protein-3 (CTRP-3) and pigment epithelium-derived factor (PEDF) concentrations in patients with type 2 diabetes and metabolic syndrome. Diabetes. 2012;61:2932–2936.
46. Flehmig G, Scholz M, Klöting N, Fasshauer M, Tönjes A, Stumvoll M, Youn BS, Blüher M. Identification of adipokine clusters related to parameters of fat mass, insulin sensitivity and inflammation. PLoS One. 2014;9:e99785.
47. Wagner RM, Sivagnanam K, Clark WA, Peterson JM. Divergent relationship of circulating CTRP3 levels between obesity and gender: A cross-sectional study. PeerJ. 2016;4:e2573.
48. Ban B, Bai B, Zhang M, Hu J, Ramanjaneya M, Tan BK, Chen J. Low serum cartonectin/CTRP3 concentrations in newly diagnosed type 2 diabetes mellitus: In vivo regulation of cartonectin by glucose. PLoS One. 2014;9:e112931.
49. Deng W, Li C, Zhang Y, Zhao J, Yang M, Tian M, Li L, Zheng Y, Chen B, Yang G. Serum C1q/TNF-related protein-3 (CTRP3) levels are decreased in obesity and hypertension and are negatively correlated with parameters of insulin resistance. Diabetol Metab Syndr. 2015;7:33.
50. Qu H, Deng M, Wang H, Wei H, Liu F, Wu J, Deng H. Plasma CTRP-3 concentrations in Chinese patients with obesity and type II diabetes negatively correlate with insulin resistance. J Clin Lipidol. 2015;9: 289-294.

Chapter 8

Serum Perilipin 2 (PLIN2) Predicts Multiple Organ Dysfunction in Critically Ill Patients

Berkan Kurt
Lukas Buendgens
Theresa H Wirtz
Sven H Loosen
Maximilian Schulze-Hagen
Daniel Truhn
Jonathan F Brozat
Samira Abu Jhaisha
Philipp Hohlstein
Ger Koek
Ralf Weiskirchen
Christian Trautwein
Frank Tacke
Karim Hamesch
Alexander Koch

Biomedicines. 2021:13;9(9):1210

Abstract

Perilipin 2 (PLIN2) is a lipid droplet protein with various metabolic functions. How-ever, studies investigating PLIN2 in the context of inflammation, especially in systemic and acute inflammation, are lacking.

Hence, we assessed the relevance of serum PLIN2 in critically ill pa-tients. We measured serum PLIN2 serum in 259 critically ill patients (166 with sepsis) upon admission to a medical intensive care unit (ICU) compared to 12 healthy controls. A subset of 36 patients underwent computed tomography to quantify body composition. Compared to controls, serum PLIN2 concentrations were elevated in critically ill patients at ICU admission.

Interestingly, PLIN2 independently indicated multiple organ dysfunction (MOD), defined as a SOFA score >9 points, at ICU admission, and was also able to independently predict MOD after 48 h. Moreover, serum PLIN2 levels were associated with severe respiratory failure potentially reflecting a moribund state. However, PLIN2 was neither a predictor of ICU mortality nor did it reflect metabolic dysregulation.

Conclusively, the first study assessing serum PLIN2 in critical illness proved that it may assist in risk stratification because it is capable of independently indicating MOD at admission and predicting MOD 48 h after PLIN2 measurement. Further evaluation regarding the underlying mechanisms is warranted.

Introduction

Perilipin 2 (PLIN2), also referred to as Adipose differentiation-related protein (ADRP) or Adipophilin, belongs to the PAT family of lipid droplet proteins, which is named after the first three proteins that were discovered in this group (Perilipin (PLIN), ADRP/PLIN2 and TIP47 (Tail-interacting protein of 47 kDa)/PLIN3). PLIN2 is not only expressed in adipocytes, but also ubiquitously in non-adipose tissue (e.g., cardiomyocytes and skeletal muscle cells, hepatocytes, intestinal cells and endothelial cells)[1-6].

Although PLIN2's main function is the regulation of lipid metabolism as the regulation of intracellular lipid storage and lipolysis[5,7-9], recent evidence uncovered various additional functions. A growing body of studies demonstrates the complex regulation and pathophysiological connections of PLIN2 in lipid metabolism and beyond[10,11]. For instance, experimental and clinical data suggest that PLIN2 is involved in the pathophysiology of insulin resistance and type 2 diabetes mellitus[12-14], dyslipidemia[5,7,15,16] and fatty liver disease[7,14,17-22].

Additionally, there has been evidence connecting PLIN2 to the development of age-related vascular disease, such as atherosclerosis[15,23-27]. Moreover, PLIN2 is also important in regulating lipid accumulation in cardiomyocytes[28]. In addition to metabolic and cardiovascular associations, PLIN2 has been described as a potential tumor marker in prevalent malignancies such as colorectal or lung carcinoma[29,30].

Furthermore, recent data suggested a possible connection between PLIN2, muscle weakness and age-related sarcopenia via a mechanism of excessive intramuscular triglyceride storage[31-34].

Disorders in lipid metabolism contribute to chronic inflammatory processes leading to metabolic diseases[35] and, in turn, metabolic comorbidities were shown to contribute to mortality and morbidity in critically ill patients on the intensive care unit (ICU)[36]. Hence, a biomarker reflecting these pathogenic processes may provide better prediction of critical illness outcomes.

Given the described associations with multiple systemic, age-related and metabolic diseases, it is tempting to speculate that PLIN2 may reflect metabolic dysregulation and a moribund state in critical illness. However, no assessment currently exists of PLIN2 in critically ill patients and sepsis. This prompted us to analyze the usefulness of serum PLIN2 in a large cohort of well-characterized critically ill patients admitted to a medical ICU.

Materials and methods

Study design and patient characteristics

The study was constructed as a prospective, explorative, observational cohort study aiming to evaluate the role of Perilipin 2 (PLIN2) serum levels in critically ill patients in the intensive care unit. In the period from 2006 to 2011, all patients were enrolled at ad-mission to a medical intensive care unit of the University Hospital RWTH Aachen. Study-specific exclusion criteria were (i) age below 18 years, (ii) length of stay less than 24 h, (iii) admission due to post-operative or post-interventional observation, (iv) pregnancy, (v) breastfeeding, and (vi) missing informed consent. An over-the-phone follow up with the patient or their relatives was conducted to collect information about outcomes until 2011. Serum PLIN2 was analyzed in 259 patients with available follow-up data. The reference group consisted of 12 healthy blood donors without a known acute or chronic illness, without any chronic medication, with negative serological testing for HIV and viral hepatitis, and with negative inflammatory serum markers and unremarkable biochemical workup.

Diagnosis of sepsis was determined retrospectively by use of "The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3)"[37] and the treatment was conducted according to current guidelines at that time. Blood biochemistry markers, clinical data (e.g., on ventilation and medication) and composite scores (e.g., "Sequential Organ Failure Assessment Score" (SOFA) and "Acute Physiology And Chronic Health Evaluation II" (APACHE-II))[37,38] were used to evaluate the presence of organ dysfunction.

Standard biochemistry blood markers were taken for clinical routine measurements and analyzed in the central laboratory at University Hospital RWTH Aachen with the use of Sysmex XN9000 (Sysmex GmbH, Norderstedt, Germany) and Cobas 8000 c701 (Hoffmann-La Roche AG, Basel, Switzerland).

Ethical approval was provided by the institutional review board of the University Hospital RWTH Aachen University (Aachen, Germany; reference number: EK 150/06; date of approval: 2 November 2006). Written informed consent was obtained by the patients or their legal representatives. The study was executed in accordance with the ethical guidelines of the Declaration of Helsinki (Hong Kong Amendment) and Good Clinical Practice (European guidelines).

PLIN2 measurements

Blood samples were collected at admission to the ICU after written informed consent was obtained. After centrifugation (at 4°C for 10 min) and aliquotation, the serum

samples were immediately transferred to a −80°C freezer. For the measurements of serum PLIN2, a commercial quantitative sandwich enzyme immunoassay (ELISA) was used according to the manufacturer's instructions (Uscn Life Science. Inc., Wuhan, China; No: E91350Hu). The minimum detectable dose of PLIN2 was less than 0.0053 µg/dL. The determined sensitivity of this assay (i.e., the lower limit of detection) as given by the manufacturer was determined by adding three standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration. The instructions of the manufacturer state high sensitivity and specificity for detection of human PLIN2 with no further quantification. No significant cross-reactivity or interference between human PLIN2 and analogues was observed. The successor test (No: SEB350Hu) has comparable specifications: the minimum detectable dose of PLIN2 is less than 0.0055 µg/dL. The determined sensitivity of this assay (i.e., the lower limit of detection) as given by the manufacturer was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration. The intra-assay and inter-assay precisions are lower than 10% and 12%, respectively. No significant cross-reactivity or interference between PLIN2 and analogue are observed in this double-antibody sandwich ELISA[39]. Measurements were performed by experienced laboratory personnel that was fully blinded to any clinical or other laboratory data of the patients or controls.

Assessment of computed tomography scan body composition markers

Data from patients who received a computed tomography (CT) scan at admission to our ICU were included in our analysis. CTs were performed on either a 40-row spiral CT scanner (Somatom Definition AS 40, Siemens Medical Systems, Erlangen, Germany) or 128-row spiral CT scanners (Somatom Definition Flash or Somatom Definition AS, Siemens Medical Systems, Germany).

The scans were acquired in the craniocaudal direction during a single breath-hold with a tube voltage of 120 kV and automated tube current modulation. Reconstructed slice thickness was 5 mm and only venous-phase scans were used for body composition calculations. According to the literature, there is a strong association between single-slice measurements and total compartment volumes[40-42].

Hence, total visceral and subcutaneous adipose tissue (VAT, SAT), skeletal muscle area and its mean attenuation given in Hounsfield units (HU) were segmented at the center plane of the third lumbar vertebra on axial CT scans. The semi-automatic segmentation tool "3D slicer", an open-source software application for medical image computing, was used to determine the given body composition markers[43]. The skeletal muscle index was calculated for standardization purposes via dividing the skeletal muscle area by the corresponding body height.

Statistical analysis

All categorical variables were described as absolute (n) and relative (%) frequencies and the corresponding contingency tables were analyzed with chi-square tests or Fisher's exact test if n≤5. Continuous variables were displayed as stated (mainly median and range) and were analyzed by the Mann–Whitney U test. The Kruskal–Wallis H test was used to analyze differences for multiple comparisons. Correlation analyses (e.g., between clinical variables and blood biomarkers) were assessed using Spearman's Rho correlation coefficient (r).

Graphical illustration of data was performed via box plots including median, quartiles and whiskers to indicate the range of values. The median PLIN2 serum level was used as a cut-off for subsequent analyses. Time-to-event variables were displayed using Kaplan-Meier curves and differences in survival were tested using the log-rank test.

To analyze the independent value of PLIN2 as a prognostic biomarker, univariable and multivariable analyses of median PLIN2 serum level for outcomes such as sepsis and multiple organ dysfunction (MOD) were conducted using logistic regression analyses to calculate odds ratios (OR).

Distributions among groups were assessed by univariable and forward-stepwise multiple logistic regression analyses to calculate OR. Multivariable logistic regression analyses were performed to test for independent associations. ORs were presented with their corresponding 95% confidence intervals (CI) given in brackets. p-values <0.05 were considered statistically significant whereas p-values >0.10 were given as "n.s." (not significant) to offer better readability. The data were analyzed and visualized using SPSS Statistics version 27 (IBM; Armonk, NY, USA).

Results

PLIN2 serum levels were significantly elevated in critically Ill patients

We measured PLIN2 serum concentrations in 259 patients on ICU admission. In comparison to a healthy control group consisting of 12 blood donors free from any chronic comorbidities or laboratory abnormalities, PLIN2 serum levels were markedly elevated in ICU patients (5.23 (0.48–59.5) µg/dL vs. 1.83 (1.36–2.07) µg/dL, p<0.001; Figure 8.1A).

Among ICU patients, no significant difference in PLIN2 serum concentrations was observed between male and female patients (5.27 (0.11–59.5) µg/dL vs. 5.21 (0.48-48.3) µg/dL, p=0.562; Figure 8.1B). PLIN2 serum levels neither showed a

significant difference between patients that were ≤65 years old versus patients >65 years (5.02 (0.11–59.5) µg/dL vs. 5.39 (0.48–48.3) µg/dL, p=0.535; Figure 8.1C) nor did it correlate directly with age (r=0.063, p=0.305; Table 8.1).

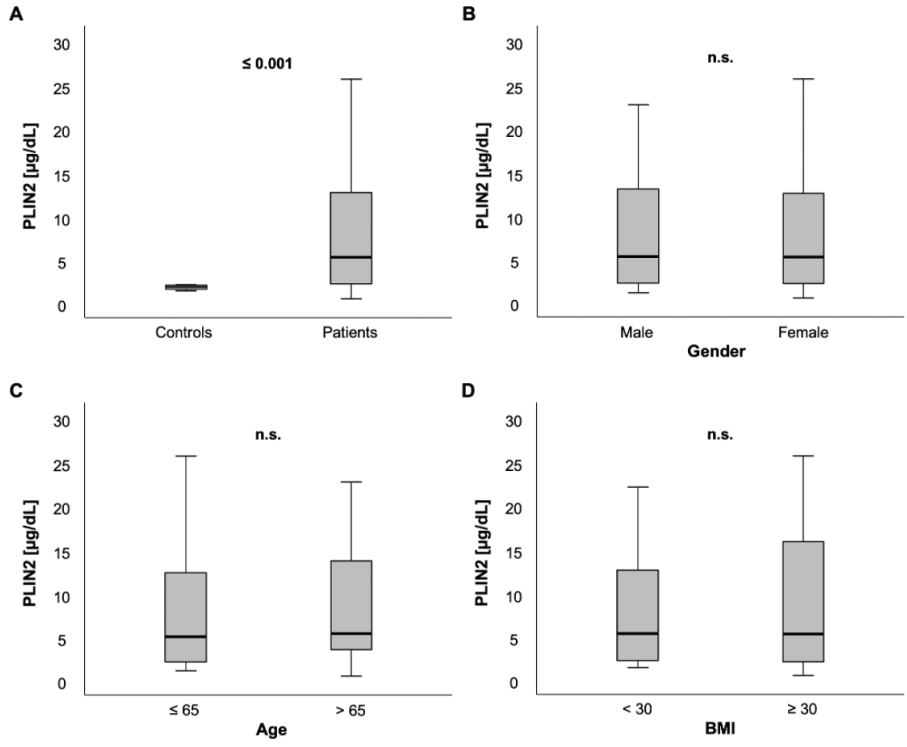


Figure 8.1 PLIN2 serum concentrations in critically ill patients at intensive care unit (ICU) admission. (**A**) Critically ill patients on the ICU had elevated PLIN2 serum levels compared to healthy controls. (**B–D**) No difference of PLIN2 serum concentrations was seen between male and female ICU patients (**B**), ICU patients ≤65 vs. >65 years (**C**) or ICU patients with a body mass index (BMI) <30 vs. ≥30 kg/m2 (**D**).

Table 8.1 Correlations of clinical and laboratory parameters with PLIN2 serum concentrations at ICU admission.

Parameters	**r**	***p***
Demographics		
Age	0.064	0.305
Body mass index	−0.099	0.129
FBC and markers of inflammation		
MCHC	0.166	0.007*
Platelets	−0.135	0.030*
WBC	−0.109	0.080
C-reactive protein	0.025	0.688
Procalcitonin	−0.023	0.751
Interleukin 6	0.050	0.486
Interleukin 10	0.092	0.303
TNF-α	−0.165	0.157
Electrolytes and renal system		
Sodium	−0.101	0.105
Potassium	−0.143	0.021*
Urea	0.013	0.839
Creatinine	−0.042	0.499
HPB system		
Albumin	−0.020	0.805
INR	0.004	0.955
Bilirubin, total	−0.056	0.369
γGT	−0.099	0.115
AST	−0.072	0.263
Lipase	−0.509	<0.001*
Cardiopulmonary system		
NTproBNP	0.024	0.785
Norepinephrine demand at day 1 (µg/kg/min)	0.066	0.322
Horovitz quotient (P_aO_2/F_iO_2)	0.202	0.052
Ventilatory F_iO_2 demand	−0.224	0.026*
Metabolism and endocrinology		
Glucose	0.066	0.292
HbA1c	−0.090	0.355
Insulin	−0.035	0.720
C-Peptide	−0.095	0.330
HOMA IR	−0.173	0.077
Cholesterol	−0.011	0.868
HDL-cholesterol	0.062	0.533
LDL-cholesterol	0.101	0.309
Triglycerides	−0.094	0.170
ICU parameters		
Days on ICU	0.132	0.034*
SOFA day 1	0.149	0.113
SOFA day 3	0.261	0.014*
APACHE-II day 1	−0.102	0.151
APACHE-II day 3	0.240	0.020*

Spearman rank correlation test was used to calculate significant correlations of positive and negative nature. p-values <0.05 were considered statistically significant and were highlighted ("*")Abbreviations: ICU: intensive care unit; FBC: Full blood count; MCHC: Mean corpuscular hemoglobin concentration; WBC: White blood cell count; TNF: Tumor necrosis factor; GFR: Glomerular filtration rate; HPB: Hepato-Pancreato-Biliary; INR: International normalized ratio; γGT: Gamma-glutamyl transpeptidase; AST: Aspartate aminotransferase; NTproBNP: N-terminal pro B-type natriuretic peptide; F_{iO2}: Fraction of inspired oxygen; HbA1c: Glycosylated hemoglobin A1; HOMA: Homeostatic model assessment; HDL: high-density lipoprotein; LDL: low-density lipoprotein; SOFA: Sequential organ failure assessment; APACHE-II: acute physiology and chronic health evaluation II.

Associations of PLIN2 serum concentrations with clinical data and blood-based parameters

Because PLIN2 is described to be essential in the formation of cytoplasmic lipid droplets[3,15,44], we hypothesized that PLIN2 concentrations may be associated with obesity and established lipid markers.

However, no significant difference was observed between ICU patients with a BMI≥30 kg/m^2, defining obesity, versus <30 kg/m^2 (5.3 (1.4–59.5) µg/dL vs. 5.27 (0.48-57.1) µg/dL, p=0.570; Figure 8.1D). In line with this, there was no correlation between serum PLIN2 and BMI (r=−0.099, p=0.129; Table 8.1) and between different BMI classes (<18.5, 18.5–24.9, 25–29.9, ≥30 kg/m^2, p=0.678, data not shown).

Furthermore, we compared serum PLIN2 with various parameters of lipid metabolism. Unexpectedly, we did not observe any associations between PLIN2 levels and total cholesterol (r=−0.011, p=0.868), high density lipoprotein (HDL) cholesterol (r=0.062, p=0.533) or low-density lipoprotein (LDL) cholesterol (r=0.101, p=0.309).

Next, we analyzed parameters of glucose metabolism. Serum PLIN2 levels were not elevated in patients with diabetes mellitus (5.13 (0.48–59.5) µg/dL vs. 5.32 (1.4-22.0) µg/dL, p=0.287; Figure 8.2A). Moreover, we did not observe correlations between PLIN2 serum levels and glycated hemoglobin (HbA1c, r=−0.090, p=0.355; Table 8.1) or homeo-stasis model assessment-insulin resistance (HOMA-IR, r=−0.173, p=0.077; Table 8.1).

While ICU patients with arterial hypertension (AH) showed a trend of having higher serum PLIN2 levels compared to their counterparts without AH (3.1 (1.1–39.3) µg/dL vs. 4 (4.8–48.3) µg/dL, p=0.072; Figure 8.2B), patients with coronary artery disease (CAD) showed no significant difference in PLIN2 concentrations compared to patients without CAD (3.5 (0.48–48.3) µg/dL vs. 4.02 (1.69–21.3) µg/dL, p=0.567; Figure 8.2C).

Furthermore, PLIN2 serum levels did not differ in patients with and without chronic obstructive pulmonary disease (COPD) (3.5 (0.48–48.3) µg/dL vs. 3.74 (1.68-21.3) µg/dL, p=0.974; Figure 8.2D).

ICU patients with or without liver cirrhosis did not have different serum PLIN2 levels (3.56 (0.48–48.3) µg/dL vs. 3.11 (1.78–6.07) µg/dL, p=0.595; Figure 8.2E). Similarly, a history of alcohol abuse did not show altered PLIN2 levels in ICU patients (3.52 (0.48-48.3) µg/dL vs. 3.61 (1.53–40) µg/dL, p=0.860; Figure 8.2F).

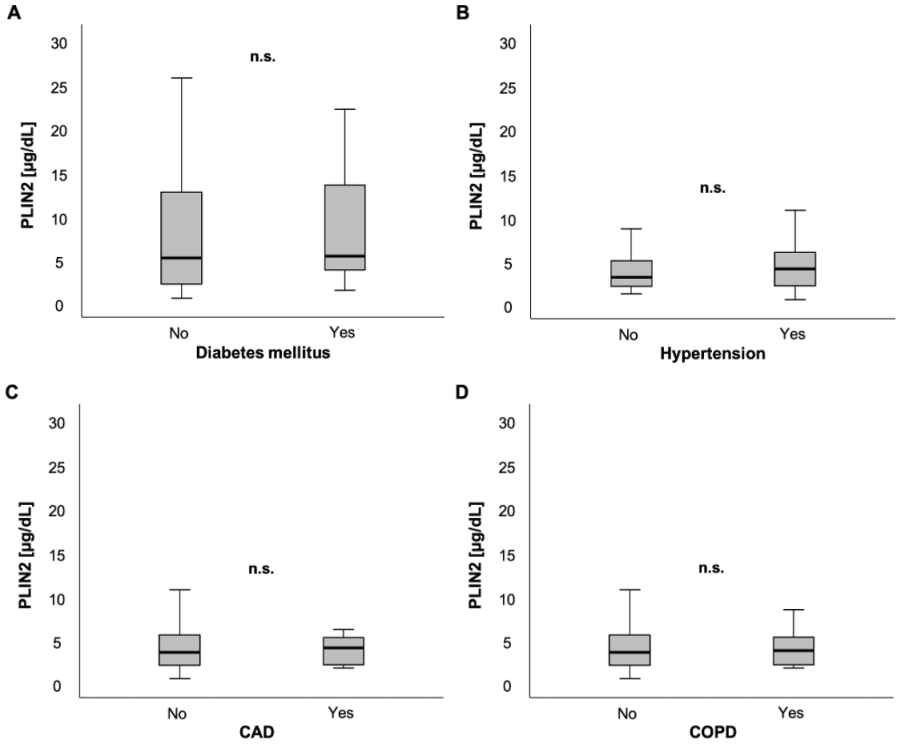
Surprisingly, we did not observe any significant correlations between traditional markers of inflammation and PLIN2 concentrations (WBC: r=−0.109, p=0.080; C-reactive

protein: r=0.025, p=0.688; Procalcitonin: r=−0.023, p=0.751; Interleukin 6: r=0.050, p=0.486; TNF-α: r=−0.165, p=0.157).

Serum PLIN2 had a strong and negative correlation with lipase (r=−0.509, p<0.001). Corresponding to that, compared to ICU patients without acute pancreatitis, PLIN2 concentrations were reduced in patients admitted due to acute pancreatitis (5.31 (0.48–59.5) µg/dL vs. 2.07 (1.59–32.4) µg/dL, p=0.042; Figure S8.1).

Interestingly, in contrast to ICU patients without active malignancy, ICU patients with active malignant disease had higher serum PLIN2 concentrations (2.45 (0.48–40) µg/dL vs. 5.02 (1.78–48.3) µg/dL, p=0.007; Figure 8.2G).

Finally, we compared the serum concentrations of PLIN2 with experimental biomarkers (Table S8.1). Adipokines such as Adiponectin emerged as a new and promising group of inflammatory biomarkers in intensive care medicine[45,46]. PLIN2 levels showed an inverse correlation with Adiponectin (r=−0.273, p=0.007) and a correlation with Myostatin (r=0.160, p=0.010). Furthermore, we detected a correlation of PLIN2 with Asymmetric dimethylarginine (ADMA) (r=−0.197, p=0.002) and Symmetric dimethylarginine (SDMA) (r=−0.154, p=0.017), two endogenous nitric oxide modulators.



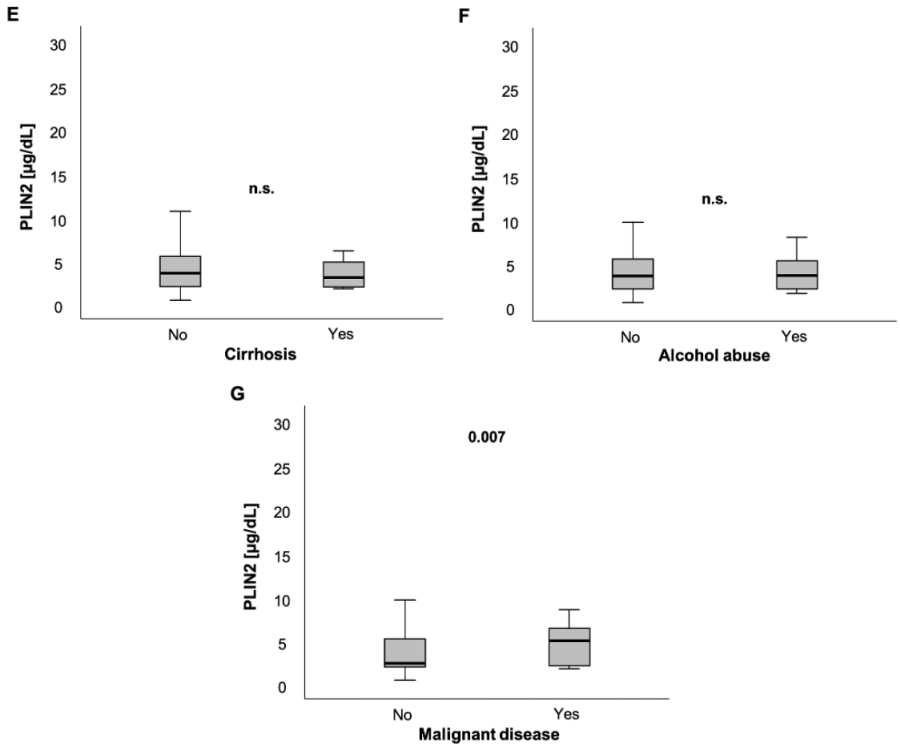


Figure 8.2 PLIN2 serum levels and preexisting chronic comorbidities. PLIN2 levels were not significantly different in ICU patients with preexisting diabetes mellitus, arterial hypertension, coronary artery disease, chronic obstructive pulmonary disease, liver cirrhosis and chronic alcohol abuse compared to their counterparts without these comorbidities (**A–F**). ICU patients with pre-diagnosed malignant disease had significantly elevated serum PLIN2 levels (**G**).

PLIN2 levels were associated with organ failure and disease severity

Because sepsis was a main cause for admission to our medical ICU (Table S8.2), we assessed whether PLIN2 levels were associated with sepsis occurrence. ICU patients with sepsis had comparable baseline characteristics and comorbidities as their counterparts without sepsis (Table 8.2). Septic ICU patients had a higher demand for mechanic ventilation, vasopressor therapy and renal replacement (Table 8.2).

In terms of laboratory parameters, septic patients had higher levels of inflammatory markers (e.g., white blood cell count, C-reactive protein and procalcitonin), elevation of kidney function parameters (e.g., creatinine), higher norepinephrine demand, and higher SOFA and APACHE-II scores on the day of ICU admission (Table 8.2).

Moreover, septic ICU patients had a longer ICU stay, higher ICU mortality and higher overall mortality (Table 8.2).

Table 8.2 Baseline patient characteristics at ICU admission divided by the presence of sepsis.

Parameters	**All Patients** ***n*=259**	**Non-Sepsis** ***n*=93**	**Sepsis** ***n*=166**	***p***
Female (%)	40.5%	39.8%	41%	n.s.
Age (years)	63 (18–89)	60 (18–85)	64 (21–89)	n.s.
Body mass index (kg/m^2)	26 (15.9–86.5)	25.8 (15.9–53.3)	26.1 (17.1–86.5)	n.s.
Comorbidities				
Arterial Hypertension (%)	23.9	26.9	22.3	n.s.
Diabetes mellitus (%)	31.3	32.3	30.7	n.s.
Coronary artery disease (%)	12.7	15.1	11.5	n.s.
COPD (%)	17.4	21.5	15.1	n.s.
Liver cirrhosis (%)	3.1	5.4	1.8	n.s.
Malignant disease (%)	11.2	7.5	13.3	n.s.
Clinical parameters				
Mechanical ventilation demand at day 1 (%)	72.2	64.5	76.5	0.039
Norepinephrine demand at day 1 (%)	59.1	46.2	66.3	<0.001
Norepinephrine demand at day 1 (µg/kg/min)	0 (0–2.4)	0 (0–2.4)	0.1 (0–1.5)	0.001
Renal replacement therapy demand at day 1 (%)	27.4	18.3	32.5	0.010
Renal replacement therapy (days)	0 (0–37)	0 (0–21)	0 (0–37)	0.006
APACHE-II score at day 1	17 (2–43)	14 (2–33)	19 (3–43)	<0.001
APACHE-II score at day 3	19 (0–36)	12 (0–28)	22 (6–36)	<0.001
SOFA score at day 1	9 (0–19)	7 (0–17)	10 (3–19)	<0.001
SOFA score at day 3	9 (0–18)	6 (0–15)	10 (1–18)	<0.001
Days on ICU	8 (2–137)	6 (2–45)	10 (2–137)	<0.001
Death on ICU (%)	24.7	17.2	28.9	0.036
180-day mortality (%)	20.8	17.2	22.9	n.s.
Observation period (days)	137 (1–884)	195.5 (1–883)	110 (1–884)	n.s.
Overall mortality (%)	47.5	34.0	54.8	0.002
Laboratory data at day 1:				
WBC [x10^3/µL]	12.7 (0–149)	11.4 (1.8–29.6)	13.1 (0–149)	0.011
C-reactive protein [mg/dL]	97 (5–230)	17 (5–230)	161.5 (5–230)	<0.001
Procalcitonin [ng/mL]	0.8 (0–248)	0.2 (0–100)	2.7 (0.1–248)	<0.001
Creatinine [mg/dL]	1.4 (0.2–21.6)	1 (0.2–15)	1.6 (0.2–21.6)	0.025
Creatinine GFR [mL/min]	54 (2–60)	60 (6–60)	38 (2–60)	0.004
INR [units]	1.2 (0.9–6.7)	1.2 (0.9–6.7)	1.2 (0.9–4.6)	n.s.
Albumin [mg/dL]	27 (1.6–61.4)	30.1 (1.6–48.5)	25.6 (5–61.4)	0.005
Lactate [mmol/l]	1.6 (0.4–21.9)	1.8 (0.6–18.1)	1.5 (0.4–21.9)	0.094
PLIN2 [µg/dL]	5.23 (0.48–59.5)	4.86 (1.4–32.4)	5.47 (0.48–59.5)	0.021

For quantitative values, median and range (in parenthesis) are given. Abbreviations: COPD: chronic obstructive pulmonary disease; APACHE-II: acute physiology and chronic health evaluation II; SOFA: sequential organ failure assessment; ICU: intensive care unit; WBC: white blood cell count; GFR: glomerular filtration rate; INR: international normalized ratio; PLIN2: Perilipin 2.

PLIN2 serum levels were slightly elevated in ICU patients with sepsis compared to ICU patients without sepsis (5.47 (0.48–595) µg/dL vs. 4.86 (1.4–32.4) µg/dL, p=0.021; Figure 8.3A). Moreover, patients with high sepsis disease severity and multiple organ dys-function (MOD), defined as a SOFA score >9 points on the day of ICU admission, had markedly higher PLIN2 concentrations at ICU admission than their counterparts

with SOFA ≤9 points (4.22 (0.48–22) µg/dL vs. 2.05 (1.1–48.3) µg/dL, p=0.013; Figure 8.3B). Of note, these findings were independent of the infectious site (Figure S8.2).

Next, we tested whether PLIN2 was able to predict MOD at farther time points during ICU stay. Interestingly, serum PLIN2 levels on the day of ICU admission were higher in those ICU patients who had a SOFA score >9 points on the third day of ICU stay, i.e., 48 h after the PLIN2 measurement, compared to ICU patients who had a SOFA score ≤9 points on ICU day 3 (5.2 (1.82–32.4) µg/dL vs. 2.02 (1.1–48.3) µg/dL, p=0.001; Figure 8.3C). Corresponding to that, PLIN2 levels correlated with clinical parameters such as the length of ICU stay (r=0.132, p=0.034), and SOFA and APACHE-II scores at day 3 (r=0.261, p=0.014; r=0.240, p=0.020; respectively).

To understand whether a specific organ dysfunction was associated with increased PLIN2 levels, we dissected the individual components incorporated into the SOFA score. ICU patients without or with norepinephrine demand at admission to the ICU did not show any difference in PLIN2 concentrations (5.47 (1.1–57.1) µg/dL vs. 5.47 (0.48-59.5) µg/dL, p=0.467; Figure 8.3D). Because norepinephrine-induced lipolysis may be a source for PLIN2, we also calculated the standardized norepinephrine demand over 24 h. The norepinephrine concentration (µg/kg/min) did not correlate with serum PLIN2 concentrations (r=0.066, p=0.322; Table 8.1).

Additionally, there was no difference between patients with and without requirement of renal replacement therapy (5.47 (0.48–59.5) µg/dL vs. 4.95 (1.4–57.1) µg/dL, p=0.447; Figure 8.3E). However, anuric patients presented with significantly decreased PLIN2 concentrations when compared to patients with urine output ≥100 mL per day (2.09 (1.4–20.5) µg/dL vs. 5.47 (0.48–59.5) µg/dL, p=0.013; Figure S8.3A). We observed that this difference was stronger at day 3 (2.01 (1.53–10.23) µg/dL vs. 5.87 (0.48-76.8) µg/dL, p<0.001; Figure S8.3B).

Another organ system reflected in the SOFA score, namely, coagulation (platelets), showed a weak correlation with serum PLIN2 levels (r=−0.135, p=0.030).

Notably, among critically ill patients presenting with a P_aO_2/F_iO_2 ratio (Horovitz index) ≤100 mmHg, PLIN2 serum levels were decreased compared to ICU patients with a P_aO_2/F_iO_2 ratio >100 mmHg (Horovitz index: >300 vs. ≤100: 4.02 (1.74–39.3) µg/dL vs. 2.01 (0.48–7.9) µg/dL; 201–300 vs. ≤100: 3.69 (1.78–13.5) µg/dL vs. 2.01 (0.48-7.9) µg/dL; 101–200 vs. ≤100: 4.23 (1.69–17.2) µg/dL vs. 2.01 (0.48–7.9) µg/dL; p=0.016, p=0.033, p=0.005, respectively; Figure 8.3F).

Further analyses revealed that demand of F_iO_2 levels during ventilation correlated with PLIN2 concentrations (r=−0.224, p=0.026). Additionally, there was a trend towards a

weak but insignificant correlation between PLIN2 levels and the Horovitz quotient (r=0.202, p=0.052).

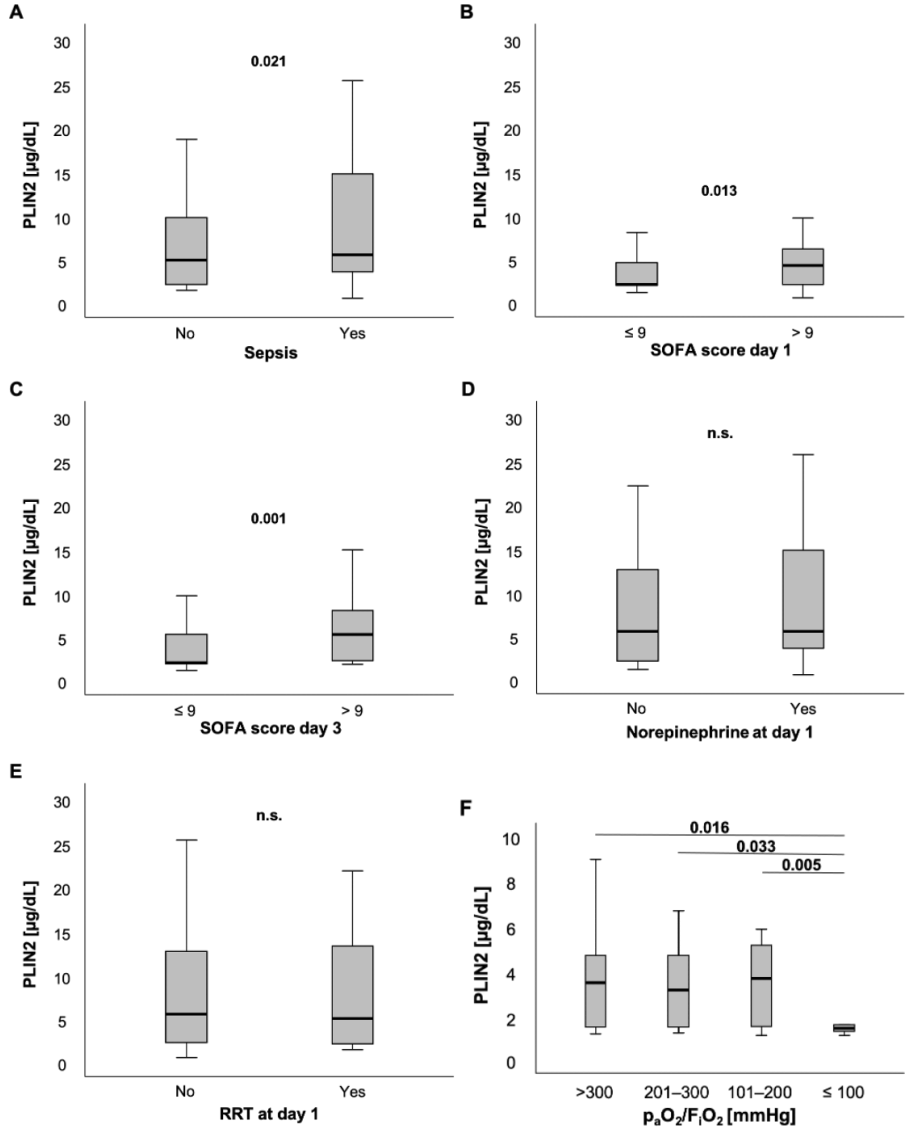


Figure 8.3 PLIN2 levels in different subsets of ICU patients. (**A**) PLIN2 serum concentrations were elevated in ICU patients with sepsis. (**B**) ICU patients with a SOFA score >9 points had higher serum PLIN2 levels. (**C**) Serum PLIN2 on the day of ICU admission was higher in ICU patients with a SOFA score >9 points at ICU day 3. (**D–E**) Neither patients with norepinephrine (**D**) nor renal replacement therapy demand showed differences in PLIN2 levels (**E**). (**F**) Serum PLIN2 levels in the different P_aO_2/F_iO_2 (Horovitz index) subgroups are shown.

We performed uni- and multivariable logistic regression analyses to evaluate whether PLIN2 acts as an independent marker for occurrence of sepsis and disease severity (i.e., multiple organ dysfunction (MOD)) (Table 8.3). To test for independent association of serum PLIN2, we performed various multivariable logistic regression analyses accounting for different parameters that were either associated with PLIN2 serum levels in the literature and/or are associated with sepsis[6,18,32,47,48], i.e., diabetes mellitus (DM) occurrence and age, BMI, norepinephrine demand and established laboratory markers for inflammation, such as C-reactive protein (CRP) and procalcitonin (PCT).

For indicating sepsis, PLIN2 serum levels above the median had a sensitivity of 55.4%, a specificity of 58.1%, a positive predictive value (PPV) of 70.2% and a negative predictive value (NPV) of 42.2%. Sepsis occurrence at ICU admission was weakly associated with elevated PLIN2 levels above the median concentration in the unadjusted analysis (OR = 1.72 (1.03–2.88), p=0.038; Table 8.3, upper panel). Presence of sepsis at ICU ad-mission was still associated with serum PLIN2 above the median concentration after multivariable adjustment for age and DM (OR=1.71 (1.02–2.87), p=0.042; Table 8.3, upper panel).

The significance of association was lost after adjustment for CRP (OR=1.75 (0.94–3.28), p=0.079; Table 3, upper panel) and after adjustment for CRP and PCT (OR=2.07 (0.98–4.38), p=0.058; Table 8.3, upper panel). However, serum PLIN2 was significantly associated with sepsis occurrence at ICU admission after adjustment for norepinephrine demand (OR=1.73 (1.01–2.98), p=0.047; Table 8.3, upper panel).

Next, we analyzed whether serum PLIN2 was associated with MOD, defined as SO-FA score >9 points, at the time of ICU admission. For indicating SOFA >9 points, serum PLIN2 above the median had a sensitivity of 43.4%, a specificity of 80.3%, a PPV of 65.7% and a NPV of 62%. In the unadjusted analysis, serum PLIN2 levels above the median concentration were clearly associated with MOD (OR=3.13 (1.36−7.20); p=0.007; Table 8.3, middle panel). MOD was still associated with serum PLIN2 concentrations above the median after adjustment for age and DM (OR=2.96 (1.27-6.88), p=0.012; Table 8.3, middle panel). PLIN2's association with MOD at ICU admission remained significant after adjustment for CRP (OR=3.07 (1.33–7.09), p=0.009; Table 8.3, middle panel); however, this was lost after further adjustment for CRP and PCT (OR=2.62 (0.67–10.24), p=0.166; Table 8.3, middle panel).

Moreover, the association between PLIN2 and MOD at ICU admission remained significant after adjustment for norepinephrine demand (OR=2.72 (1.08–6.89), p=0.035; Table 8.3, middle panel).

Finally, we evaluated whether serum PLIN2 concentrations above the median at the time of ICU admission were associated with SOFA score >9 points, indicating MOD, on day 3 after admission (i.e., 48 h after PLIN2 measurement). Serum PLIN2 above the median had a sensitivity of 50%, a specificity of 75.5%, a PPV of 58.1% and a NPV of 69.0%. In the unadjusted analysis, there was a strong association between serum PLIN2 above the median concentration at ICU admission and SOFA >9 points at day 3 (OR=3.08 (1.25–7.60), p=0.015; Table 8.3, lower panel). This association remained significant after multi-variable adjustment for age and DM (OR=2.91 (1.16–7.29), p=0.023; Table 8.3, lower panel).

Furthermore, the association between PLIN2 and MOD on day 3 remained significant after adjustment for CRP (OR=2.93 (1.17–7.32), p=0.021; Table 8.3, lower panel) and after adjustment for CRP and PCT (15.93 (2.85–88.93), p=0.002; Table 8.3, lower panel). Moreover, the association between serum PLIN2 above median concentrations at ICU admission and SOFA score >9 points on day 3 remained significant after adjustment for norepinephrine demand (OR=2.79 (1.05–7.42), p=0.040; Table 8.3, lower panel).

Table 8.3 Logistic regression analyses for serum PLIN2 concentrations above the median concentration and clinically relevant outcome parameters.

Sepsis occurrence at ICU admission	**OR (95% CI)**	***p***
Unadjusted	1.72 (1.03–2.88)	0.038
Adjusted for age and DM	1.71 (1.02–2.87)	0.042
Adjusted for CRP	1.75 (0.94–3.28)	0.079
Adjusted for CRP and PCT	2.07 (0.98–4.38)	0.058
Adjusted for norepinephrine demand	1.73 (1.01–2.98)	0.047
SOFA >9 points at ICU admission	**OR (95% CI)**	***p***
Unadjusted	3.13 (1.36–7.20)	0.007
Adjusted for age and DM	2.96 (1.27–6.88)	0.012
Adjusted for CRP	3.07 (1.33–7.09)	0.009
Adjusted for CRP and PCT	2.62 (0.67–10.24)	0.166
Adjusted for norepinephrine demand	2.72 (1.08–6.89)	0.035
SOFA >9 points at day 3	**OR (95% CI)**	***p***
Unadjusted	3.08 (1.25–7.60)	0.015
Adjusted for age and DM	2.91 (1.16–7.29)	0.023
Adjusted for CRP	2.93 (1.17–7.32)	0.021
Adjusted for CRP and PCT	15.93 (2.85–88.93)	0.002
Adjusted for norepinephrine demand	2.79 (1.05–7.42)	0.040

Association between serum PLIN2 concentrations above the median of 5.23 µg/dL at the day of admission and sepsis occurrence at ICU admission (upper panel), SOFA score >9 points at ICU admission (middle panel) and SOFA score >9 points at ICU day 3 (i.e., 48 h after PLIN2 measurement; lower panel). The covariates (laboratory data and norepinephrine demand in µg/kg/min) are from the day of admission (same timepoint as PLIN2 measurement). Abbreviations: ICU: intensive care unit; OR: odds ratio; CI: confidence interval; DM: Diabetes mellitus; CRP: C-reactive protein; PCT: Procalcitonin; SOFA: sequential organ failure assessment.

PLIN2 serum levels and association with CT scan body composition markers

A subset of 36 patients, who underwent computed tomography scans, was used to assess body composition (Table 8.4).

While the mean skeletal muscle attenuation given in Hounsfield units was used as a surrogate parameter for myosteatosis, the normalized skeletal muscle index at L3 (L3SMI) served to evaluate sarcopenia. In the small subset of patients with a BMI ≥30 kg/m^2 (n=9), we detected an association of serum PLIN2 with visceral adipose tissue in (r=−0.750, p=0.020; Table 8.4).

Besides that, we did not observe any significant correlations between CT scan body composition markers and PLIN2 serum concentrations.

Table 8.4 Correlations between PLIN2 serum levels and CT-scan body composition markers.

r | p	VAT [mm^2]	SAT [mm^2]	Skeletal muscle [mm^2]	Skeletalmuscle Mean HU	L3SMI
All patients	−0.004 | 0.983	−0.160 | 0.353	−0.152 | 0.377	−0.213 | 0.213	−0.095 | 0.592
BMI <30 kg/m^2	−0.015 | 0.942	−0.214 | 0.305	−0.002 | 0.991	−0.261 | 0.208	0.033 | 0.875
BMI ≥30 kg/m^2	−0.750 | 0.020*	−0.517 | 0.154	−0.567 | 0.112	0.259 | 0.500	−0.533 | 0.139
Age <65 years	−0.030 | 0.898	−0.156 | 0.500	−0.138 | 0.552	−0.152 | 0.510	−0.072 | 0.770
Age ≥65 years	0.061 | 0.830	−0.036 | 0.899	−0.146 | 0.603	−0.152 | 0.589	−0.079 | 0.781

Spearman rank correlation test was used to calculate significant correlations of positive and negative nature. Abbreviations: CT: computed tomography; VAT: visceral adipose tissue; SAT: subcutaneous adipose tissue HU: Hounsfield unit; L3SMI: skeletal muscle index at third lumbar vertebra; BMI: body mass index.

Serum PLIN2 concentrations may predict ICU mortality in critically ill patients older than 65 years

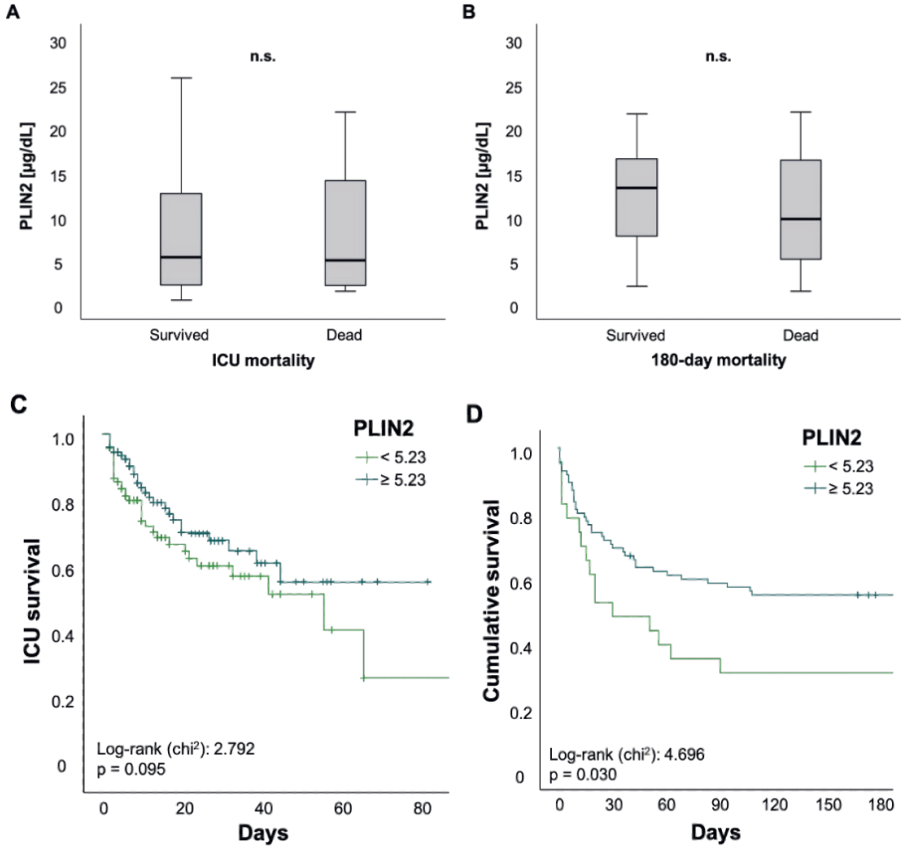
Finally, we investigated the value of PLIN2 as a biomarker for predicting mortality in critically ill patients. There was no difference in PLIN2 serum levels among ICU patients who survived or those who died during their ICU stay (5.36 (0.48–59.5) µg/dL vs. 5.01 (1.49–48.3) µg/dL, p=0.674; Figure 8.4A).

The same applied to ICU patients who were alive 180 days after ICU admission or their counterparts who died within 180 days after initial ICU admission (13.21 (2.05-59.5) µg/dL vs. 9.66 (1.49–21.71) µg/dL, p=0.117; Figure 8.4B).

To further evaluate the predictive capability of PLIN2, we performed Kaplan–Meier curve analyses. These revealed that PLIN2 serum levels below the median PLIN2 concentrations of all patients (5.23 µg/dL) tended to indicate poorer ICU survival (log-

rank p=0.095; Figure 8.4C) and were displaying higher 180 day mortality rates (log-rank p=0.03; Figure 8.4D).

Encouraged by data highlighting the role of PLIN2 in the pathophysiology of age-related and metabolic diseases[6], we performed subgroup survival time analyses. Patients >65 years with PLIN2 levels below median showed impaired ICU survival when compared to patients above the cut-off (log-rank p=0.026; Figure 8.4E). Moreover, Kaplan-Meier curve analysis demonstrated a trend towards impaired ICU survival in diabetic patients with lower PLIN2 serum levels (log-rank p=0.067; Figure 8.4F), whereas no such effect was seen in the subgroup of non-diabetics (data not shown) or patients with a body mass index ≥30 kg/m^2 (log-rank p=0.486; Figure 8.4G). Because serum PLIN2 was strongly negatively correlated with serum lipase and the presence of acute pancreatitis, we investigated whether in the subgroup of patients with acute pancreatitis serum PLIN2 was associated with increased ICU mortality. However, Kaplan–Meier curve analysis did not show a difference between pancreatitis patients with serum PLIN2 levels below or above the median concentration (log-rank p=0.617; Figure S8.4A).



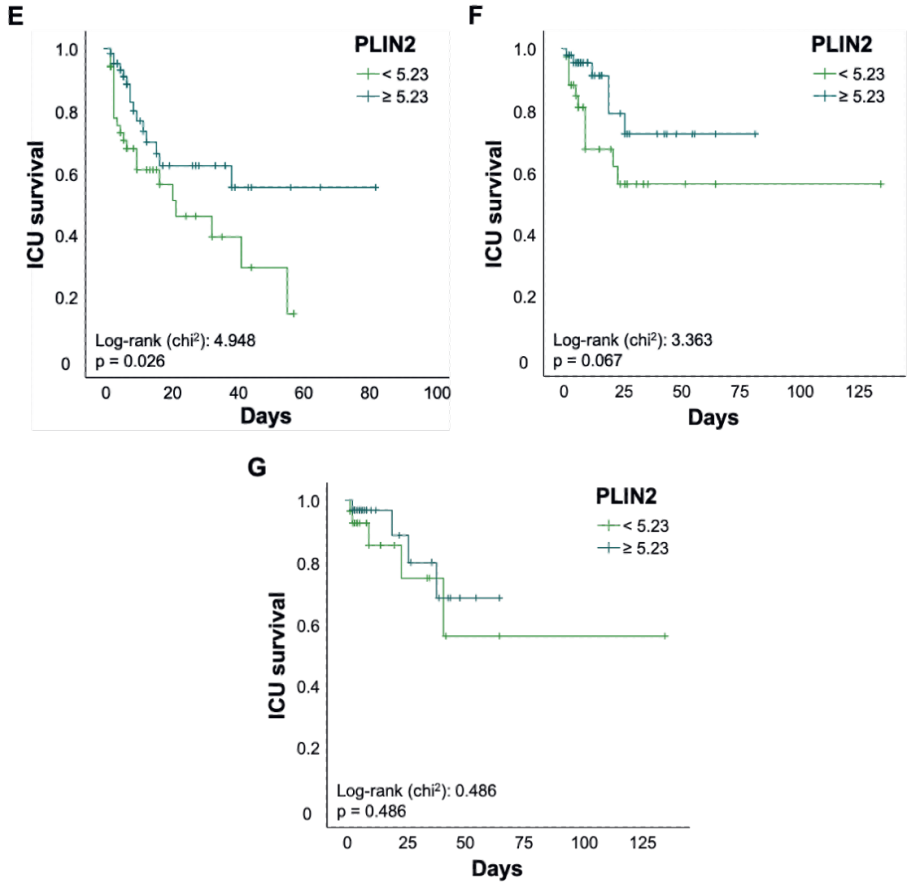


Figure 8.4 Serum concentrations of PLIN2 may predict ICU mortality in patients older than 65 years. (A–G) Neither total ICU- nor 180-day mortality was associated with significant changes in PLIN2 levels (A, B). Kaplan–Meier curve analysis showed a trend towards significancy for ICU mortality (C), whereas 180-day mortality was significant (D). Subgroup analysis revealed that reduced PLIN2 levels in patients older than 65 years may predict ICU mortality (E), whereas diabetic patients showed a trend towards significancy (F). PLIN2 was not able to predict mortality in patients with a body mass index above 30 (G).

Because serum PLIN2 was diminished in patients with severe respiratory failure, defined as P_aO_2/F_iO_2 <100, we analyzed if among this subgroup serum PLIN2 concentrations below or above the median were associated with ICU mortality. However, Kaplan–Meier curves analysis did not show a significant difference (log-rank p=0.206; Figure S8.4B).

Discussion

This is, to the best of our knowledge, the first study assessing serum PLIN2 in a representative cohort of critical illness at admission to a medical ICU. This study showed that PLIN2 is elevated in critically ill patients compared to controls. Most importantly, PLIN2 is capable of both independently indicating multiple organ dysfunction (MOD), defined as an increased SOFA score >9 points, at ICU admission, and independently predicting MOD 48 h after PLIN2 measurement.

To evaluate whether PLIN2 may serve as a biomarker for critically ill patients we performed comprehensive analyses to rule out confounding effects. In terms of patient characteristics, we evaluated the interdependency of PLIN2 with (i) sex, (ii) age, (iii) BMI, (iv) dyslipidemia, and (v) insulin resistance and diabetes mellitus. Taken together, these sensitivity analyses indicate that serum PLIN2 concentrations are independent of various patient characteristics and comorbidities, indicating this biomarker is independent of relevant confounding factors. At the same time, serum PLIN2 does not appear to reflect metabolic dysregulation in critically ill patients.

(i) Because of growing evidence suggesting sex differences in the pathophysiology of critical illness[49-51], we investigated whether PLIN2 concentrations show a different distribution based on sex. In contrast to recent data stating that PLIN2 levels were higher in women, especially in patients below 79 years[32], we did not detect any difference of serum PLIN2 in both sexes.
(ii) Previous work speculated on age-dependent changes of PLIN2 expression and link-age to sarcopenia[33,34]. To assess that, we analyzed serum PLIN2 concentrations in various age groups and did not detect significant differences.
(iii) As BMI and mortality act in a “J-shaped” dependence and, because overweight and moderate obesity appear to be protective factors in critically ill patients, called the “obesity paradox”[36,52,53], we correlated serum PLIN2 with BMI and analyzed PLIN2 in different BMI subgroups (underweight vs. normal vs. overweight vs. obese; data not shown). Serum PLIN2 did not show any significant differences in these analyses, which is in contrast to previously published data[32].
(iv) PLIN2 acts as a regulator in lipid metabolism[5,7,15,16] and previous murine and human studies have shown that PLIN2 dysregulation can be associated with lipid storage malfunction diseases[7,14,17-22]. Surprisingly and despite PLIN’s function as a lipid droplet protein, we did not detect an association of serum PLIN2 with classical markers of lipid metabolism such as cholesterol, LDL or triglycerides. These results are not in line with experimental studies, which showed reduction of hepatic steatosis or lower triglycerides in serum and liver after knock-out or downregulation of PLIN2 expression[6,7,9,19,54].

(v) Insulin resistance and diabetes mellitus are important modulators of mortality and morbidity in critically ill patients[36]. Experimental research suggests that PLIN2 is involved in the pathophysiology of insulin resistance[12-14]. This encouraged us to analyze the relationship between pre-existent diabetes and PLIN2 serum levels. Of note, serum PLIN2 concentrations were independent of the presence of diabetes. Moreover, we did not observe a correlation between PLIN2 levels and HbA1c levels, but a non-significant trend towards a rather weak correlation with HOMA-IR. Interestingly, a recently published study reports similar results, with no observed association with DM, but a significant correlation between PLIN2 and HOMA-IR[32]. An-other group described higher PLIN2 levels in diabetic patients with NAFLD com-pared to patients without NAFLD, and correlations with age, waist circumference, triglycerides and HOMA-IR. However, these results are difficult to compare to our study because we did not assess our patients for the presence of NAFLD.

PLIN2 concentrations were not only independent of the above-mentioned patient characteristics, but also independent of various established inflammatory markers (e.g., CRP and PCT). Of note, the missing association of PLIN2 with CRP was recently confirmed in an Italian cohort[32].

Additionally, PLIN2 levels were not influenced by the infectious source (Figure S8.2). Collectively, our multivariable analyses (Table 8.3) suggest a promising potential of PLIN2 as a biomarker for multiple organ dysfunction, irrespective of relevant confounders and commonly used biomarkers. Most excitingly, the predictive potential of MOD after 48 h could aid the intensivist in risk stratification of ICU patients at the time of PLIN2 measurement (Table 8.3).

To further understand the applicability of PLIN2, we performed a comprehensive analysis of various components of ICU parameters and organ dysfunction. Most components of the SOFA score, i.e., defining cardiovascular, hepatic, renal and coagulation failure, did not show consistent correlations with serum PLIN2. By comparison, we were surprised about the finding that patients with a Horovitz quotient ≤100 presented with decreased PLIN2 levels.

We suspect that during a state of severe critical illness and sub-sequent intense ventilation and medication support, compared to mild or moderate critical illness, PLIN2 expression could be downregulated due to multiple reasons that are difficult to dissect in such a setting. Moreover, this may also be a phenomenon of serious dysregulation of metabolism in moribund patients. Taken together, we were not able to define a relevant driving force associated with the observed changes in PLIN2 serum concentrations.

Therefore, we next investigated whether changes in serum PLIN2 were associated with the presence of various comorbidities: (a) cardiovascular disease, (b) hepatic disease, (c) pancreatic disease, and (d) malignant disease.

(a) There has been evidence of PLIN2 involvement in the development of age-related vascular disease, such as atherosclerosis[6,15,23-27]. Recent studies highlighted the importance of PLIN2 in cardiomyocyte lipid accumulation[28] and were able to connect PLIN2 to coronary microvascular obstruction and infarct size in patients with ST-elevation myocardial infarction and major adverse cardiovascular events during follow-up[55].
Intrigued by these findings, we investigated whether arterial hypertension or coronary artery disease are associated with altered PLIN2 serum levels. However, our results did not prove any obvious association between vascular diseases and PLIN2 levels.

(b) Prompted by experimental research connecting changes in PLIN2 serum levels with hepatic diseases and alcohol consumption[20,21,54,56], we analyzed serum PLIN2 concentrations in ICU patients suffering from liver cirrhosis and patients with a history of alcohol abuse. However, we did not observe any connections between cirrhosis or alcohol abuse and PLIN2 levels.
However, this may be due to missing statistical power because only 3.1% of our cohort had cirrhosis. Additionally, the majority of the mentioned studies assessed PLIN2 in NAFLD or non-alcoholic steatohepatitis (NASH) instead of cirrhosis, further reducing the comparability of the results.

(c) PLIN2's activation state is regulated by pancreatic hormones. While catecholamines permit lipolysis via phosphorylation and dissociation of PLIN2, insulin inhibits lipolysis via dephosphorylation of PLIN2, hindering hormone-sensitive lipases in accessing the lipid droplets[21]. Of note, we observed a strong negative correlation be-tween PLIN2 and lipase.
Correspondingly, patients admitted due to acute pancreatitis presented with decreased PLIN2 concentrations. However, serum PLIN2 was not able to discriminate a mortality difference in the small subgroup of patients with acute pancreatitis (n=13; Figure S8.4a). To the best of our knowledge, associations with pancreatic markers or disease have not been previously described.
Taking physiological mechanisms into consideration, the inverse association of PLIN2 with lipase raises the question of whether this is due to pancreatitis and its associated multiple organ dysfunction or rather an effect of higher PLIN2 metabolization of serum lipases. Importantly, serum PLIN2 levels were not associated with norepinephrine demand. Moreover, the logistic regression analyses of PLIN2 and SOFA score >9 points remained significant after adjustment for the norepinephrine demand (Table 8.3).

(d) Previous studies have demonstrated the role of PLIN2 as a tumor marker in different body fluids or in tumor tissue[6,57,58] for several malignant diseases, such as renal cell carcinoma[57,59-61], colorectal carcinoma[29] or lung adenocarcinoma[30]. In our ICU cohort, PLIN2 serum concentrations were elevated in patients with preexistent malignant disease. These consistent associations of serum PLIN2 with malignancy even during critical illness underline its potential capacity as a tumor marker for routine diagnostics.

Muscle weakness in ventilated patients with respiratory failure has been associated with poor outcome[62]. Different mechanisms leading to muscle weakness are, among others, infiltration of skeletal muscle tissue with adipose tissue (myosteatosis) or age-related muscle atrophy (sarcopenia), although both factors should be understood as complementary and interacting[62-64].

However, assessing muscle weakness may be challenging in the ICU setting. Using whole-body dual-energy X-ray absorptiometry (DXA) scans, a recent study observed associations between PLIN2 and fat mass parameters. While they did not observe an association between PLIN2 and visceral adipose tissue, there was a strong correlation between PLIN2 and subcutaneous adipose tissue. However, body composition analyses via DXA-scans are prone to both over- and underestimation, especially in non-healthy individuals or markedly obese women[65,66].

Computed tomography (CT) scan body composition analyses emerge as an attractive and potentially more reliable alternative in the ICU setting[41,42,64,67-69]. Subsequently, a well-characterized subset of our ICU cohort underwent CT-scan body composition analyses. Taken together, we observed no clinically meaningful associations in this rather small subgroup (n=36).

Although this is contrary to previous data[6,32], the missing association of PLIN2 with body composition markers is consistent with the missing association with markers of metabolic dysfunction. Of note, previous studies assessed Plin2 expression in skeletal muscle whereas, to the best of our knowledge, PLIN2 was not assessed as a serum biomarker in the context of muscle weakness or sarcopenia yet[31,33,34]. Although our hypothesis that PLIN2 associates with sarcopenia was not proven, this independency might further strengthen PLIN2's role as a potential biomarker for multiple organ dysfunction.

With ICU mortality being the most substantial endpoint, we did not observe significant differences in serum PLIN2 levels in patients surviving versus deceasing on the ICU. Additionally, analyses of 180 days post-discharge from the ICU did also not reveal significant changes. However, Kaplan-Meier curve analyses revealed that PLIN2 may

have predictive value for ICU mortality in patients >65 years. Although this is consistent with studies previously reporting about the relevance of PLIN2 in age-related diseases[6], those patients with serum PLIN2 levels above the median had worse outcomes, which may reflect the detrimental outcome of patients with severe respiratory failure. Although we discovered significant survival differences for PLIN2 and 180-day mortality, this finding should be interpreted with caution. Survival post discharge at 180 days is prone to multi-factorial confounders which cannot be accounted for in this type of study.

Moreover, those patients with serum PLIN2 levels above the median had a better survival, again, reflecting the role of severe respiratory failure that was associated with reduced serum PLIN2. Collectively, PLIN2 does not appear to be a useful mortality marker.

To further evaluate the diagnostic role of serum PLIN2 in critically ill patients, we compared its performance with other experimental biomarkers that were previously described in our ICU cohort. Such cross-validation might help to further characterize the usefulness of PLIN2 as an adjunct to currently used biomarkers as the latter still lack in predictive power, specificity and sensitivity when it comes to differentiating the various etiologies of critical illness[70,71].

First, we observed a correlation between PLIN2 and Myostatin, a protein negatively regulating skeletal muscle growth, which was reduced in ICU patients and mechanically ventilated patients, and was also an independent prognostic marker for overall survival[47]. Interestingly, this is consistent with the finding that decreased PLIN2 levels in critically ill patients were associated with severe oxygenation failure. Second, we observed a negative correlation between PLIN2 and Adiponectin, an adipokine[45,46]. This is coherent with previous literature, describing decreased Adiponectin levels in critically ill patients[72-75].

Moreover, Adiponectin is an independent positive predictor for short-term and overall survival, although the current literature is still ambiguous and controversial as to whether decreased or increased Adiponectin levels are predictors of sepsis and outcome[45,46,72,75]. Third, we observed an association between PLIN2 and Symmetric and Asymmetric dimethylarginine (SDMA/ADMA), which were previously described reflecting the vascular tone and endothelial dysfunction, being elevated in critically ill patients, and predicting short-term and long-term survival[76,77]. Interestingly, ADMA has additionally been reported in various contexts, such as chronic kidney disease[78], cardiovascular disease (e.g., hypertension, atherosclerosis and coronary artery disease) and diabetes mellitus[79,80].

Future studies are warranted to assess whether PLIN2 measurements can enhance the diagnostic and prognostic value of ADMA, especially in the context of the observed changes in PLIN2 levels in severe respiratory failure.

An important limitation is the cross-sectional nature of our study design. Although our cohort is well characterized with adequate statistical power for multiple clinical parameters and endpoints, and offering clinical and laboratory data from two time points (ICU admission and ICU day 3), our cohort cannot replicate the complete ICU course (e.g., data from farther time points) which, due to its nature, is rather complex. Another weakness of our study, given its exploratory approach, is that the data were gathered from a single ICU, which does not consider external influencing factors and does not allow for generalizability.

However, our study population includes heterogeneous disease etiologies over a recruitment period of five years, further increasing the representative capacity of our cohort. Although this may conceal disease-specific confounders, at the same time it also provides generalizability of the results. Another limitation is, due to the retrospective nature of our study, that our cohort stems from the previous era of sepsis definition. To reduce this limitation, we retrospectively applied the current Sepsis-3 criteria whereas, however, the treatment was conducted according to current guidelines at that time. A further major weakness is the small sample size of our control group. However, our focus was the comprehensive evaluation in our well-characterized cohort of critically ill patients.

Another restriction is that the observed association of serum PLIN2 with sepsis was rather weak and does not appear to be clinically meaningful. Moreover, the small but statistically significant difference of serum PLIN2 concentrations between septic and non-septic ICU patients is also clinically not meaningful and may be within the measurement error range of the used ELISA assay.

Furthermore, elevated PLIN2 levels were consistently associated with MOD whereas decreased PLIN2 levels were associated with increased mortality in subgroups. This contradiction is partly explained by the marked decrease of PLIN2 concentrations in severe respiratory failure, which in turn results in increased mortality. However, critically ill patients have multiple dysfunctions of various systems (e.g., MOD, dysregulated metabolism, dysregulated hormonal balance and immune system dysfunction) that are interconnected with each other and at the same time poorly understood at a mechanistic level.

Moreover, this dyshomeostasis is further affected by use of multiple medications and assist devices (e.g., respirator and renal replacement). In addition, due to the nature of

a biomarker study, the underlying mechanisms of the observed associations remain unknown. Finally, validation of our findings in an in-dependent cohort and different disease settings may be warranted to also investigate an optimal PLIN2 cut-off value.

Conclusions

Serum PLIN2 may be a useful marker of MOD both at ICU admission and after 48 h with potential for clinical risk stratification.

Although these independent and predictive associations are intriguing, the pathomechanisms leading to the observed changes remain to be elucidated.

Supplementary data

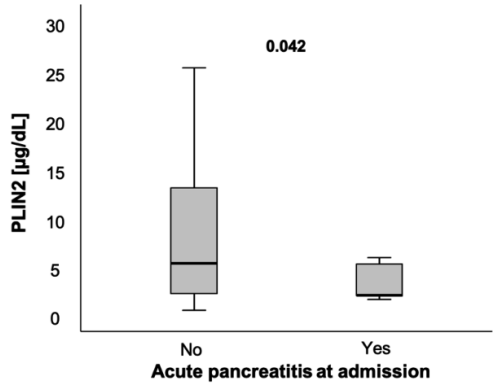


Figure S8.1 Patients admitted to the ICU due to acute pancreatitis showed decreased PLIN2 serum levels compared to patients admitted due to other reasons.

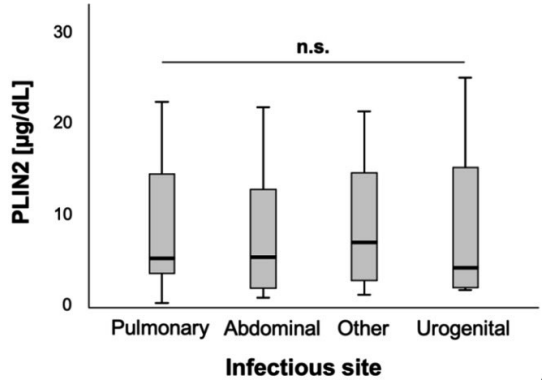


Figure S8.2 Serum PLIN2 levels at ICU admission in septic patients. PLIN2 serum concentrations were not different in patients with differing infectious sources.

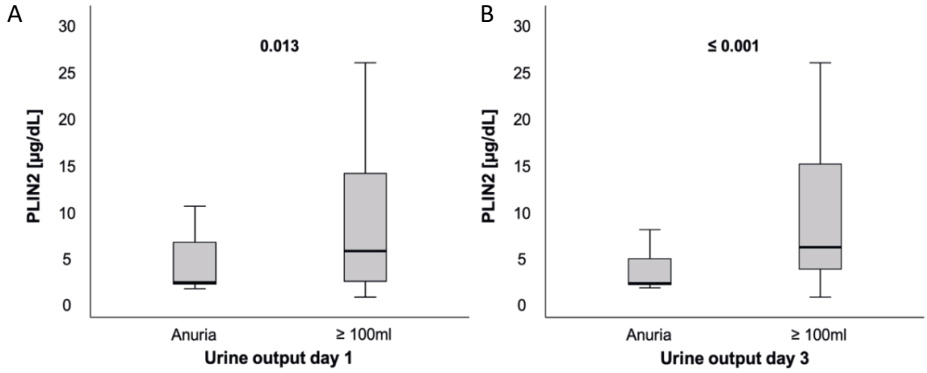


Figure S8.3 PLIN2 serum levels and urine output. (**A**) PLIN2 serum concentrations at day 1 were decreased in patients that were anuric at day of ICU admission. (**B**) Patients that were anuric at day 3 showed even lower PLIN2 levels at ICU admission.

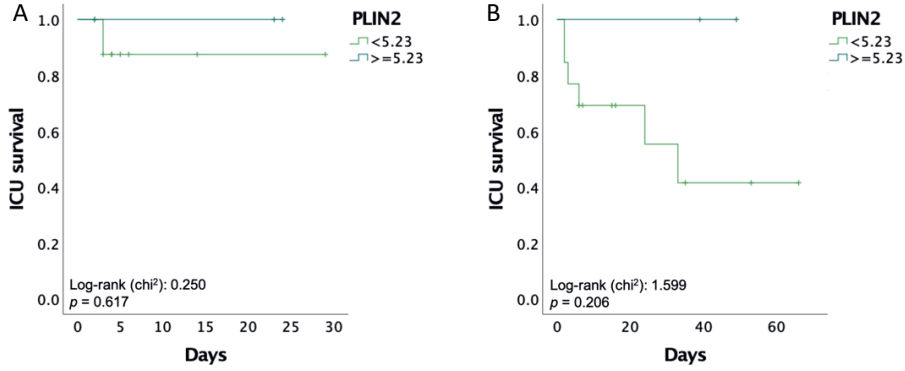


Figure S8.4 PLIN2 serum concentrations and ICU mortality. (**A**) Serum PLIN2 levels did not dis-criminate an ICU mortality difference in patients admitted due to acute pancreatitis. (**B**) Serum PLIN2 did not predict ICU mortality in patients with severe respiratory failure, defined as P_aO_2/F_iO_2 <100.

Table S8.1 Correlations of experimental biomarkers with PLIN2 serum concentrations at ICU admission.

Biomarker	**r**	***p***
Adiponectin	-0.273	0.007*
RBP4	0.202	0.065
APRIL	0.135	0.056
sFRP5	-0.175	0.007*
Calprotectin	0.097	0.125
Myostatin	0.160	0.010*
ADMA	-0.197	0.002*
SDMA	-0.154	0.017*
suPAR	-0.049	0.499

Spearman rank correlation test was used. Abbreviations: RBP4: Retinol binding protein 4; APRIL: A proliferation-inducing ligand; sFRP5: Secreted frizzled-related protein 5; ADMA: Asymmetric dimethylarginine; SDMA: Symmetric dimethylarginine; suPAR: Soluble urokinase-type plasminogen activator receptor.

Table S8.2 Main cause for admission to the intensive care unit.

Critical illness other than sepsis n (%)	**93 (35.9%)**
Etiology n (relative /absolute):	
Cardiopulmonary disease	32 (34.4% / 12.4%)
Decompensated liver cirrhosis	16 (17.2% / 6.2%)
Acute pancreatitis	13 (14% / 5%)
Severe gastrointestinal bleeding	6 (6.5% / 2.3%)
Acute liver failure	4 (4.3% / 1.5%)
Other	22 (23.7% / 8.5%)
Sepsis n (%)	166 (64.1%)
Infectious site n (relative / absolute):	
Pulmonary	92 (55.4% / 35.5%)
Abdominal	32 (19.3% / 12.4%)
Urogenital	7 (4.2% / 2.7%)
Other	35 (21.1% / 13.5%)

References

1. Jiang HP, Serrero G. Isolation and characterization of a full-length cDNA coding for an adipose differentiation-related protein. Proc Natl Acad Sci USA, 1992;89:7856–7860.
2. Jiang HP, Harris SE, Serrero G. Molecular cloning of a differentiation-related mRNA in the adipogenic cell line 1246. Cell Growth Differ. 1992;3:21–30.
3. Brasaemle DL, Barber T, Wolins NE, Serrero G, Blanchette-Mackie EJ, Londos C. Adipose differentiation-related protein is an ubiquitously expressed lipid storage droplet-associated protein. J Lipid Res. 1997; 38:2249–2263.
4. Heid HW, Moll R, Schwetlick I, Rackwitz HR, Keenan TW. Adipophilin is a specific marker of lipid accumulation in diverse cell types and diseases. Cell Tissue Res. 1998;294:309–321.
5. Imamura M, Inoguchi T, Ikuyama S, Taniguchi S, Kobayashi K, Nakashima N, Nawata H. ADRP stimulates lipid accumulation and lipid droplet formation in murine fibroblasts. Am J Physiol Endocrinol Metab. 2002;283:E775–E783.
6. Conte M, Franceschi C, Sandri M, Salvioli S. Perilipin 2 and Age-Related Metabolic Diseases: A New Perspective. Trends Endocrinol Metab. 2016;27:893–903.
7. Imai Y, Varela GM, Jackson MB, Graham MJ, Crooke RM, Ahima RS. Reduction of hepatosteatosis and lipid levels by an adipose differentiation-related protein antisense oligonucleotide. Gastroenterology 2007;132:1947–1954.
8. Listenberger LL, Ostermeyer-Fay AG, Goldberg EB, Brown WJ, Brown DA. Adipocyte differentiation-related protein reduces the lipid droplet association of adipose triglyceride lipase and slows triacylglycerol turnover. J Lipid Res. 2007;48:2751–2761.
9. Imai Y, Boyle S, Varela GM, Caron E, Yin X, Dhir R, Dhir R, Graham MJ, Ahima RS. Effects of perilipin 2 antisense oligonucleotide treatment on hepatic lipid metabolism and gene expression. Physiol Genom. 2012;44:1125–1131.
10. Sztalryd C, Brasaemle DL. The perilipin family of lipid droplet proteins: Gatekeepers of intracellular lipolysis. Biochim Biophys Acta Mol Cell Biol Lipids. 2017;1862:1221–1232.
11. Sztalryd C, Kimmel AR. Perilipins: Lipid droplet coat proteins adapted for tissue-specific energy storage and utilization, and lipid cytoprotection. Biochimie. 2014;96:96–101.
12. Varela GM, Antwi DA, Dhir R, Yin X, Singhal NS, Graham MJ, Crooke RM, Ahima RS. Inhibition of ADRP prevents diet-induced insulin resistance. Am J Physiol-Gastrointest Liver Physiol. 2008;295:G621–G628.
13. Bosma M, Hesselink MK, Sparks LM, Timmers S, Ferraz MJ, Mattijssen F, van Beurden D, Schaart G, de Baets MH, Verheyen FK, et al. Perilipin 2 improves insulin sensitivity in skeletal muscle despite elevated intramuscular lipid levels. Diabetes. 2012;61:2679–2690.
14. Chang BH, Li L, Saha P, Chan L. Absence of adipose differentiation related protein upregulates hepatic VLDL secretion, relieves hepatosteatosis, and improves whole body insulin resistance in leptin-deficient mice. J Lipid Res. 2010;51:2132–2142.
15. Larigauderie G, Cuaz-Pérolin C, Younes AB, Furman C, Lasselin C, Copin C, Jaye M, Fruchart JC, Rouis M. Adipophilin increases triglyceride storage in human macrophages by stimulation of biosynthesis and inhibition of beta-oxidation. FEBS J. 2006;273:3498–3510.
16. Ducharme NA, Bickel PE. Lipid droplets in lipogenesis and lipolysis. Endocrinology. 2008;149:942–949.
17. Chang BH, Li L, Paul A, Taniguchi S, Nannegari V, Heird WC, Chan L. Protection against fatty liver but normal adipogenesis in mice lacking adipose differentiation-related protein. Mol Cell Biol. 2006;26: 1063–1076.
18. Fan H, Diao H, Lu Y, Xie J, Cheng X. The relation between serum adipose differentiation-related protein and non-alcoholic fatty liver disease in type 2 diabetes mellitus. Ther Adv Endocrinol Metab. 2020;11: 2042018820969025.
19. McManaman JL, Bales ES, Orlicky DJ, Jackman M, MacLean PS, Cain S, Crunk AE, Mansur A, Graham CE, Bowman TA, et al. Perilipin-2-null mice are protected against diet-induced obesity, adipose inflammation, and fatty liver disease. J Lipid Res. 2013;54:1346–1359.
20. Motomura W, Inoue M, Ohtake T, Takahashi N, Nagamine M, Tanno S, Kohgo Y, Okumura T. Up-regulation of ADRP in fatty liver in human and liver steatosis in mice fed with high fat diet. Biochem Biophys Res Commun. 2006;340:1111–1118.

21. Straub BK, Stoeffel P, Heid H, Zimbelmann R, Schirmacher P. Differential pattern of lipid droplet-associated proteins and de novo perilipin expression in hepatocyte steatogenesis. Hepatology. 2008; 47:1936–1946.
22. Liu F, Wang C, Zhang L, Xu Y, Jang L, Gu Y, Cao X, Zhao X, Ye J, Li Q. Metformin prevents hepatic steatosis by regulating the expression of adipose differentiation-related protein. Int J Mol Med. 2014; 33:51–58.
23. Larigauderie G, Furman C, Jaye M, Lasselin C, Copin C, Fruchart JC, Castro G, Rouis M. Adipophilin enhances lipid accumulation and prevents lipid efflux from THP-1 macrophages: Potential role in atherogenesis. Arterioscler Thromb Vasc Biol. 2004;24:504–510.
24. Paul A, Chan L, Bickel PE. The PAT family of lipid droplet proteins in heart and vascular cells. Curr Hypertens Rep. 2008;10:461–466.
25. Paul A, Chang BH, Li L, Yechoor VK, Chan L. Deficiency of adipose differentiation-related protein impairs foam cell formation and protects against atherosclerosis. Circ Res. 2008;102:1492–1501.
26. Son SH, Goo YH, Choi M, Saha PK, Oka K, Chan LC, Paul A. Enhanced atheroprotection and lesion remodelling by targeting the foam cell and increasing plasma cholesterol acceptors. Cardiovasc Res. 2016;109:294–304.
27. Xu B, Zhao H, Wang S, Sun X, Qin X. Increased ADRP expression in human atherosclerotic lesions correlates with plaque instability. Int J Clin Exp Med. 2015;8:5414–5421.
28. Mardani I, Tomas Dalen K, Drevinge C, Miljanovic A, Ståhlman M, Klevstig M, Scharin Täng M, Fogelstrand P, Levin M, Ekstrand M, et al. Plin2-deficiency reduces lipophagy and results in increased lipid accumulation in the heart. Sci Rep. 2019;9:6909.
29. Matsubara J, Honda K, Ono M, Sekine S, Tanaka Y, Kobayashi M, Jung G, Sakuma T, Nakamori S, Sata N, et al. Identification of adipophilin as a potential plasma biomarker for colorectal cancer using label-free quantitative mass spectrometry and protein microarray. Cancer Epidemiol Prev Biomark. 2011;20: 2195–2203.
30. Zhang XD, Li W, Zhang N, Hou YL, Niu ZQ, Zhong YJ, Zhang YP, Yang SY. Identification of adipophilin as a potential diagnostic tumor marker for lung adenocarcinoma. Int J Clin Exp Med. 2014;7:1190–1196.
31. Conte M, Armani A, Conte G, Serra A, Franceschi C, Mele M, Sandri M, Salvioli S. Muscle-specific Perilipin2 down-regulation affects lipid metabolism and induces myofiber hypertrophy. J Cachexia Sarcopenia Muscle 2019;10:95–110.
32. Conte M, Santoro A, Collura S, Martucci M, Battista G, Bazzocchi A, Morsiani C, Sevini F, Capri M, Monti D, et al. Circulating perilipin 2 levels are associated with fat mass, inflammatory and metabolic markers and are higher in women than men. Aging 2021;13:7931–7942.
33. Conte M, Vasuri F, Bertaggia E, Armani A, Santoro A, Bellavista E, Degiovanni A, D'Errico-Grigioni A, Trisolino G, Capri M, et al. Differential expression of perilipin 2 and 5 in human skeletal muscle during aging and their association with atrophy-related genes. Biogerontology. 2015;16:329–340.
34. Conte M, Vasuri F, Trisolino G, Bellavista E, Santoro A, Degiovanni A, Martucci E, D'Errico-Grigioni A, Caporossi D, Capri M, et al. Increased Plin2 Expression in Human Skeletal Muscle Is Associated with Sarcopenia and Muscle Weakness. PLoS One. 2013;8:e73709.
35. Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. Nat Rev Immunol. 2011;11:85–97.
36. Schetz M, De Jong A, Deane AM, Druml W, Hemelaar P, Pelosi P, Pickkers P, Reintam-Blaser A, Roberts J, Sakr Y, et al. Obesity in the critically ill: A narrative review. Intensive Care Med. 2019;45:757–769.
37. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, Bellomo R, Bernard GR, Chiche JD, Coopersmith CM, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA. 2016;315:801–810.
38. Knaus W, Draper EA, Wagner DP, Zimmerman JE. APACHE II: A severity of disease classification system. Crit Care Med. 1985;13:818–829.
39. Cloud-Clone Corp: ELISA Kit for Adipose Differentiation Related Protein (ADRP). Availabe online: http://www.cloud-clone.com/products/SEB350Hu.html (accessed on 28 August 2021).
40. Faron A, Luetkens JA, Schmeel FC, Kuetting DLR, Thomas D, Sprinkart AM. Quantification of fat and skeletal muscle tissue at abdominal computed tomography: Associations between single-slice measurements and total compartment volumes. Abdom Radiol. 2019;44:1907–1916.

41. Wirtz TH, Loosen SH, Schulze-Hagen M, Weiskirchen R, Buendgens L, Abu Jhaisha S, Brozat JF, Puengel T, Vucur M, Paffenholz P, et al. CT-based determination of excessive visceral adipose tissue is associated with an impaired survival in critically ill patients. PLoS One. 2021;16:e0250321.
42. Loosen SH, Schulze-Hagen M, Püngel T, Bündgens L, Wirtz T, Kather JN, Vucur M, Paffenholz P, Demir M; Bruners P, et al. Skeletal Muscle Composition Predicts Outcome in Critically Ill Patients. Crit Care Explor. 2020;2:e0171.
43. Fedorov A, Beichel R, Kalpathy-Cramer J, Finet J, Fillion-Robin JC, Pujol S, Bauer C, Jennings D, Fennessy F, Sonka M, et al. 3D Slicer as an image computing platform for the Quantitative Imaging Network. Magn Reson Imaging. 2012;30:1323–1341.
44. Bickel PE, Tansey JT, Welte MA. PAT proteins, an ancient family of lipid droplet proteins that regulate cellular lipid stores. Biochim Biophys Acta. 2009;1791:419–440.
45. Loosen SH, Koch A, Tacke F, Roderburg C, Luedde T. The Role of Adipokines as Circulating Biomarkers in Critical Illness and Sepsis. Int J Mol Sci. 2019;20:4820.
46. Alipoor E, Mohammad Hosseinzadeh F, Hosseinzadeh-Attar MJ. Adipokines in critical illness: A review of the evidence and knowledge gaps. Biomed Pharmacother. 2018;108:1739–1750.
47. Wirtz TH, Loosen SH, Buendgens L, Kurt B, Abu Jhaisha S, Hohlstein P, Brozat JF, Weiskirchen R, Luedde T, Tacke F, et al. Low Myostatin Serum Levels Are Associated with Poor Outcome in Critically Ill Patients. Diagnostics. 2020;10:574.
48. Rittig N, Bach E, Thomsen HH, Pedersen SB, Nielsen TS, Jørgensen JO, Jessen N, Møller N. Regulation of Lipolysis and Adipose Tissue Signaling during Acute Endotoxin-Induced Inflammation: A Human Randomized Crossover Trial. PLoS One. 2016;11:e0162167.
49. Angele MK, Pratschke S, Hubbard WJ, Chaudry IH. Gender differences in sepsis: Cardiovascular and immunological aspects. Virulence. 2014;5:12–19.
50. Failla KR, Connelly CD. Systematic Review of Gender Differences in Sepsis Management and Outcomes. J Nurs Scholarsh. 2017;49:312–324.
51. Nachtigall I, Tafelski S, Rothbart A, Kaufner L, Schmidt M, Tamarkin A, Kartachov M, Zebedies D, Trefzer T, Wernecke KD, et al. Gender-related outcome difference is related to course of sepsis on mixed ICUs: A prospective, observational clinical study. Crit Care. 2011;15:R151.
52. De Jong A, Wrigge H, Hedenstierna G, Gattinoni L, Chiumello D, Frat JP, Ball L, Schetz M, Pickkers P, Jaber S. How to ventilate obese patients in the ICU. Intensive Care Med. 2020;46:2423–2435.
53. Pepper DJ, Sun J, Welsh J, Cui X, Suffredini AF, Eichacker PQ. Increased body mass index and adjusted mortality in ICU patients with sepsis or septic shock: A systematic review and meta-analysis. Crit Care. 2016;20:181.
54. Carr RM, Peralta G, Yin X, Ahima RS. Absence of perilipin 2 prevents hepatic steatosis, glucose intolerance and ceramide accumulation in alcohol-fed mice. PLoS One. 2014;9:e97118.
55. Russo M, Montone RA, D'Amario D, Camilli M, Canonico F, Santamaria C, Iannaccone G, Pedicino D, Pidone C, Galli M, et al. Role of perilipin 2 in microvascular obstruction in patients with ST-elevation myocardial infarction. Eur Heart J Acute Cardiovasc Care. 2020;10:633–342.
56. Najt CP, Senthivinayagam S, Aljazi MB, Fader KA, Olenic SD, Brock JR, Lydic TA, Jones AD, Atshaves BP. Liver-specific loss of Perilipin 2 alleviates diet-induced hepatic steatosis, inflammation, and fibrosis. Am J Physiol Gastrointest Liver Physiol. 2016;310:G726–G738.
57. Morrissey JJ, Mellnick VM, Luo J, Siegel MJ, Figenshau RS, Bhayani S, Kharasch ED. Evaluation of Urine Aquaporin-1 and Perilipin-2 Concentrations as Biomarkers to Screen for Renal Cell Carcinoma: A Prospective Cohort Study. JAMA Oncol. 2015;1:204–212.
58. Morrissey JJ, Kharasch ED. The specificity of urinary aquaporin 1 and perilipin 2 to screen for renal cell carcinoma. J Urol. 2013;189:1913–1920.
59. Morrissey JJ, Mobley J, Figenshau RS, Vetter J, Bhayani S, Kharasch ED. Urine aquaporin 1 and perilipin 2 differentiate renal carcinomas from other imaged renal masses and bladder and prostate cancer. Mayo Clin Proc. 2015;90:35–42.
60. Morrissey JJ, Mobley J, Song J, Vetter J, Luo J, Bhayani S, Figenshau RS, Kharasch ED. Urinary concentrations of aquaporin-1 and perilipin-2 in patients with renal cell carcinoma correlate with tumor size and stage but not grade. Urology. 2014;83:256.e9–256.e14.

61. Cao Q, Ruan H, Wang K, Song Z, Bao L, Xu T, Xiao H, Wang C, Cheng G, Tong J, et al. Overexpression of PLIN2 is a prognostic marker and attenuates tumor progression in clear cell renal cell carcinoma. Int J Oncol. 2018;53:137–147.
62. Files DC, Sanchez MA, Morris PE. A conceptual framework: The early and late phases of skeletal muscle dysfunction in the acute respiratory distress syndrome. Crit Care. 2015;19:266.
63. Muscaritoli M, Anker SD, Argilés J, Aversa Z, Bauer JM, Biolo G, Boirie Y, Bosaeus I, Cederholm T, Costelli P, et al. Consensus definition of sarcopenia, cachexia and pre-cachexia: Joint document elaborated by Special Interest Groups (SIG) "cachexia-anorexia in chronic wasting diseases" and "nutrition in geriatrics". Clin Nutr. 2010;29:154–159.
64. Aro R, Mäkäräinen-Uhlbäck E, Ämmälä N, Rautio T, Ohtonen P, Saarnio J, Meriläinen S. The impact of sarcopenia and myosteatosis on postoperative outcomes and 5-year survival in curatively operated colorectal cancer patients—A retrospective register study. Eur J Surg Oncol. 2020;46:1656–1662.
65. Bredella MA, Ghomi RH, Thomas BJ, Torriani M, Brick DJ, Gerweck AV, Misra M, Klibanski A, Miller KK. Comparison of DXA and CT in the assessment of body composition in premenopausal women with obesity and anorexia nervosa. Obesity. 2010;18:2227–2233.
66. Coletta AM, Klopp AH, Fogelman D, Parikh AM, Li Y, Garg N, Basen-Engquist K. Dual-Energy X-Ray Absorptiometry Compared to Computed Tomography for Visceral Adiposity Assessment Among Gastrointestinal and Pancreatic Cancer Survivors. Sci Rep. 2019;9:11500.
67. Ninomiya G, Fujii T, Yamada S, Yabusaki N, Suzuki K, Iwata N, Kanda M, Hayashi M, Tanaka C, Nakayama G, et al. Clinical impact of sarcopenia on prognosis in pancreatic ductal adenocarcinoma: A retrospective cohort study. Int J Surg. 2017;39:45–51.
68. Stretch C, Aubin JM, Mickiewicz B, Leugner D, Al-Manasra T, Tobola E, Salazar S, Sutherland FR, Ball CG, Dixon E, et al. Sarcopenia and myosteatosis are accompanied by distinct biological profiles in patients with pancreatic and periampullary adenocarcinomas. PLoS One. 2018;13:e0196235.
69. van Dijk DP, Bakens MJ, Coolsen MM, Rensen SS, van Dam RM, Bours MJ, Weijenberg MP, Dejong CH, Olde Damink SW. Low skeletal muscle radiation attenuation and visceral adiposity are associated with overall survival and surgical site infections in patients with pancreatic cancer. J Cachexia Sarcopenia Muscle. 2017;8:317–326.
70. Tacke F, Spehlmann ME, Vucur M, Benz F, Luedde M, Cardenas DV, Roy S, Loosen S, Hippe HJ, Frey N, et al. miR-155 Predicts Long-Term Mortality in Critically Ill Patients Younger than 65 Years. Mediators Inflamm. 2019;2019:6714080.
71. van Engelen TSR, Wiersinga WJ, Scicluna BP, van der Poll T. Biomarkers in Sepsis. Crit Care Clin. 2018; 34:139–152.
72. Langouche L, Vander PerreS, Frystyk J, Flyvbjerg A, Hansen TK, Van den Berghe G. Adiponectin, retinol-binding protein 4, and leptin in protracted critical illness of pulmonary origin. Crit Care. 2009;13:R112.
73. Marques MB, Langouche L. Endocrine, metabolic, and morphologic alterations of adipose tissue during critical illness. Crit Care Med. 2013;41:317–325.
74. Venkatesh B, Hickman I, Nisbet J, Cohen J, Prins J. Changes in serum adiponectin concentrations in critical illness: A preliminary investigation. Crit Care. 2009;13:R105.
75. Koch A, Sanson E, Voigt S, Helm,A, Trautwein C, Tacke F. Serum adiponectin upon admission to the intensive care unit may predict mortality in critically ill patients. J Crit Care. 2011;26:166–174.
76. Koch A, Weiskirchen R, Kunze J, Dückers H, Bruensing J, Buendgens L, Matthes M, Luedde T, Trautwein C, Tacke F. Elevated asymmetric dimethylarginine levels predict short- and long-term mortality risk in critically ill patients. J Crit Care. 2013;28:947–953.
77. Koch A, Weiskirchen R, Bruensing J, Dückers H, Buendgens L, Kunze J, Matthes M, Luedde T, Trautwein C, Tacke F. Regulation and prognostic relevance of symmetric dimethylarginine serum concentrations in critical illness and sepsis. Mediators Inflamm. 2013;2013:413826.
78. Matsuguma K, Ueda S, Yamagishi S, Matsumoto Y, Kaneyuki U, Shibata R, Fujimura T, Matsuoka H, Kimoto M, Kato S, et al. Molecular mechanism for elevation of asymmetric dimethylarginine and its role for hypertension in chronic kidney disease. J Am Soc Nephrol. 2006;17:2176–2183.
79. Böger RH, Maas R, Schulze F, Schwedhelm E. Asymmetric dimethylarginine (ADMA) as a prospective marker of cardiovascular disease and mortality--an update on patient populations with a wide range of cardiovascular risk. Pharmacol Res. 2009;60:481–487.

80. Sibal L, Agarwal SC, Home PD, Boger RH. The Role of Asymmetric Dimethylarginine (ADMA) in Endothelial Dysfunction and Cardiovascular Disease. Curr Cardiol Rev. 2010;6:82–90.

Part III

Biomarkers of Biological Stress in Critical Illness

Chapter 9

Clinical Relevance of Copeptin Plasma Levels as a Biomarker of Disease Severity and Mortality in Critically Ill Patients

Alexander Koch
Eray Yagmur
Alexander Hoss
Lukas Buendgens
Ulf Herbers
Ralf Weiskirchen
Ger H. Koek
Christian Trautwein
Frank Tacke

J Clin Lab Anal. 2018:32(9):e22614

Abstract

Background
Copeptin, also termed C-terminal pre-pro-vasopressin or CTproAVP, mirrors endogenous vasopressin (anti-diuretic hormone, ADH) activity and might thereby serve as a biomarker reflecting the biological stress level.
We therefore hypothesized that copeptin plasma concentrations are associated with disease severity in critically ill patients and could predict mortality.

Methods
We analyzed plasma copeptin levels in a prospective, single-center, observational study comprising 218 critically ill patients at admission to the medical intensive care unit (ICU). Mortality was assessed during a two-year observational follow-up period.

Results
Copeptin plasma levels were significantly elevated in critically ill patients (n=218) at ICU admission, as compared with 66 healthy controls. Neither sepsis as the cause of critical illness nor pre-existing metabolic disorders (type 2 diabetes, obesity) were found to influence copeptin levels.
On the contrary, plasma copeptin was closely associated with disease severity (e.g., APACHE-II score) and correlated with biomarkers of inflammation, renal failure, metabolism, vascular tone and tissue perfusion. Elevated copeptin levels at ICU admission predicted short-term and long-term mortality.

Conclusions
Copeptin plasma concentrations are significantly elevated in critically ill patients, correlate with disease severity and predict ICU and long-term outcome. Thus, copeptin could be a promising tool for prognostication and management of critically ill patients.

Introduction

Critical illness is a disease condition comprising a remarkable heterogeneity of underlying insults such as shock, infections, metabolic derangements, burns, trauma or severe blood loss.

Despite these broad range of different initial causes, the body's response to such threats is relatively uniform, and severity of critical illness is determined by the degree of systemic inflammation and subsequent hemodynamic changes, the extent of biological stress, the resultant organ failure(s) and, ultimately, death[1]. Circulating mediators of such core pathways could serve as prognostic biomarkers in critical care medicine[2].

The activation of neuroendocrine pathways, in particular the hypothalamic-pituitary-adrenal axis (e.g., corticotropin-releasing hormone [CRH] – adrenocorticotropic hormone [ACTH] – cortisol) and vasopressin release, is a characteristic response to biological stress[3]. Vasopressin, also known as arginine vasopressin (AVP) or anti-diuretic hormone (ADH), is secreted from the pituitary gland in response to hypovolemia, hypoxia, acidosis and changes in plasma osmolality[4,5]. Vasopressin is co-released with neurophysin II and the C-terminal part of the precursor pre-pro-vasopressin, termed CTproAVP or copeptin.

In contrast to vasopressin, which has a short half-life in blood, is bound to a great extent to platelets and is biochemically instable, copeptin is a stable protein in the circulation and reliably mirrors biologically functional vasopressin in both healthy and acutely ill patients[5-8].

Due to its biochemical properties as a potential biomarker, copeptin has been investigated in different acute and chronic diseases. In line with the biological functions of vasopressin such as water reabsorption, regulating osmolality, vasoconstriction and central nervous effects, copeptin levels were found elevated in patients with diabetes insipidus, metabolic diseases, chronic kidney diseases and cardiovascular disorders[4,9,10].

In cohorts of patients with acute illnesses, copeptin levels were increased, especially in patients with sepsis, shock, heart failure and respiratory distress[6,10-13]. Importantly, some of these studies suggested that elevated copeptin plasma concentrations might indicate an increased short-term mortality risk[6,11-13]. The favourable biochemical properties of copeptin, its implication in pathways of biological stress and the reported association with short-term outcome in acute illness prompted us to investigate plasma copeptin concentrations in a large, prospectively enrolled cohort of critically ill medical patients.

Materials and methods

Study design and patient characteristics

Critically ill patients were included at admission to the medical intensive care unit (ICU) at the University Hospital Aachen, Germany. Patients, who were admitted for post-interventional observational stay or underwent an elective procedure, were excluded[14]. The local ethics committee approved our study in accordance to the ethical standards laid down in the Declaration of Helsinki (reference number EK 150/06). Informed consent was obtained from each participant or their spouse.

The patients were categorized as sepsis and non-sepsis according to the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3)[15], and were treated following the current guidelines for treatment of sepsis (Surviving Sepsis Campaign)[16]. As a healthy control group, we analyzed blood donors with normal blood counts, normal values of liver enzymes and a negative serology for viral hepatitis and HIV[17].

In order to determine long-term outcome, we contacted the patients, their relatives and/or the general practitioner in approximately 6-months intervals after discharge from the hospital for two years[17].

Measurements of copeptin and other experimental markers

Blood samples were collected at the time of admission (before specific therapeutic measures), centrifuged, and serum and plasma samples were stored at -80°C. Copeptin was measured from EDTA plasma using a commercially available fluorescent immunoassay (BRAHMS GmbH/ThermoFischer Scientific, Henningsdorf, Germany) following the manufacturer's protocol.

The adipocytokines resistin, adiponectin, leptin and retinol-binding protein 4 (RBP4) have been quantified from serum of the same patients, as previously described[18-21].

Statistical analysis

All copeptin plasma concentrations are presented as median and range. The Mann-Whitney U-test was used to test differences between two groups, correlations were tested according to the Spearman's rank correlation method. All values, including outside values as well as far out values, were included. P-values less than 0.05 were considered as statistically significant.

The prognostic value of copeptin on the outcome was evaluated by Cox regression models. Survival curves were generated by Kaplan-Meier analyses with a copeptin cut-off level calculated via the Youden-Index[22]. All analyses were performed with IBM SPSS Statistics (SPSS; Chicago, Illinois).

Results

Copeptin plasma levels are significantly elevated in critically ill patients as compared with healthy controls, independent of sepsis

Copeptin plasma levels were significantly elevated in a large cohort of 218 critically ill medical patients (median 46.4 pmol/L, range 4.8-791.4, Table 9.1) at admission to the ICU, as compared with 66 healthy controls (median 4.7 pmol/L, range 4.6-8.5, $p<0.001$; Figure 9.1A).

Within the cohort of ICU patients, copeptin levels did not differ between patients with sepsis (n=145, median copeptin 45.9 pmol/L, range 4.8-791.4) and patients without sepsis (n=73, median 46.8 pmol/L, range 4.8-496.7; Figure 9.1B). Typical sites of infection in sepsis were pneumonia, abdominal and urogenital tract, while non-sepsis causes of critical illness included, among others, cardiopulmonary diseases, acute pancreatitis and decompensated liver cirrhosis (not shown).

Table 9.1 Baseline patient characteristics and copeptin plasma measurements.

Parameter	All Patients
Number	218
Sex (male/female)	133 / 85
Age median (range) [years]	64 (18-90)
APACHE-II score median (range)	18 (2-43)
ICU days median (range)	7 (1-137)
Death during ICU n(%)	48 (22%)
Death during follow-up (total) n(%)	87 (40%)
Mechanical ventilation n(%)	145 (67%)
pre-existing diabetes n(%)	66 (30%)
BMI median (range) [m^2/kg]	26 (15.3-86.5)
WBC median (range) [$x10^3/\mu L$]	13.0 (0-208)
CRP median (range) [mg/dL]	100.5 (0.2-230)
Procalcitonin median (range) [µg/L]	0.7 (0-207.5)
Creatinine median (range) [mg/dL]	1.3 (0.1-15)
INR median (range)	1.16 (0-13)
Copeptin median (range) [pmol/L]	46.4 (4.8-791.4)

For quantitative variables, median and range (in parenthesis) are given. Abbreviations: APACHE: Acute Physiology And Chronic Health Evaluation; BMI: body mass index; CRP: C-reactive protein; ICU: intensive care unit; INR: international normalized ration; WBC: white blood cell.

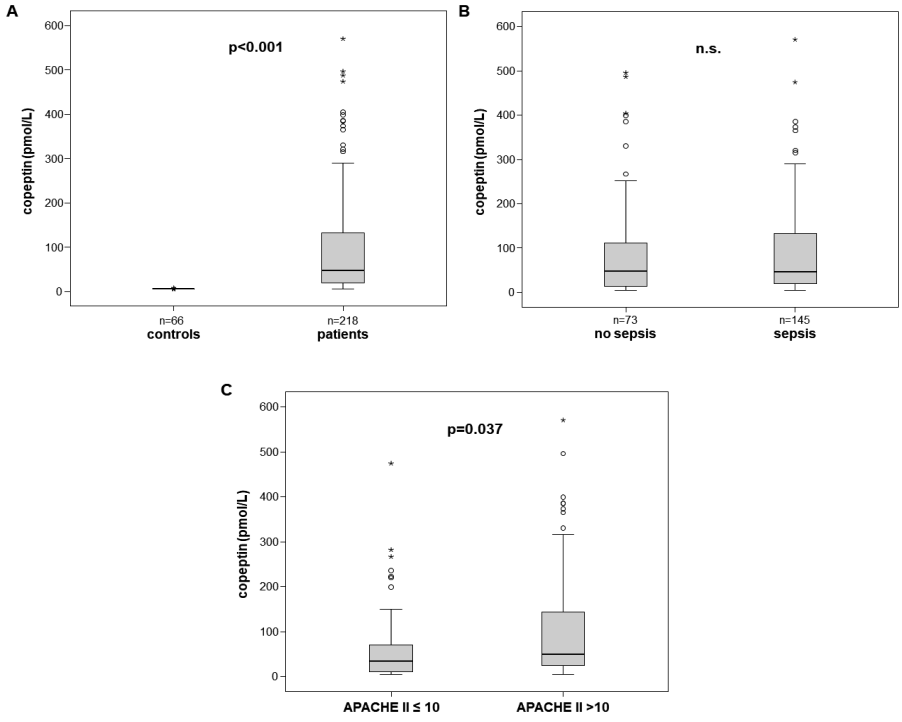


Figure 9.1 Copeptin levels in critically ill patients. (**A**) Copeptin plasma concentrations are significantly elevated in critically ill patients compared with healthy controls. (B) Copeptin levels do not differ between ICU patients with or without sepsis. (**C**) High disease severity, as defined by an APACHE II score above 10, is associated with elevated plasma copeptin. p-values (U-test) are given in the figure.

Notably, plasma copeptin concentrations were associated with disease severity, as expressed by the correlations between copeptin and ICU scores such as the Acute Physiology And Chronic Health Evaluation-II (APACHE-II) and the Simplified Acute Physiology Score 2 (SAPS2) (Table 9.2). Patients with a high APACHE-II score (above 10) had significantly higher copeptin levels than patients with an APACHE-II score below or equal to 10 (Figure 9.1C).

Table 9.2 Correlations with copeptin plasma concentrations at ICU admission (Spearman rank correlation test, only significant results are shown).

	ICU patients	
Parameters	**r**	**p**
Disease severity		
APACHE II score	0.218	0.002
SAPS2 score	0.450	<0.001
Inflammation		
Procalcitonin	0.164	0.039
suPAR	0.200	0.018
Interleukin-10	0.276	0.004
Renal function		
Creatinine	0.631	<0.001
GFR (creatinine)	-0.664	<0.001
Cystatin C	0.473	<0.001
GFR (cystatin C)	-0.518	<0.001
Urea	0.566	<0.001
Uric acid	0.320	<0.001
Vascular tone & Perfusion		
CT-proET-1	0.419	<0.001
ADMA	0.250	0.001
SDMA	0.538	<0.001
NT-proCNP	0.575	<0.001
NT-proBNP	0.492	<0.001
Lactate	0.157	0.021
pH	-0.301	<0.001
Metabolism		
Blood glucose	0.241	<0.001
Resistin	0.286	0.031
RBP4	0.485	<0.001
Visfatin	0.482	<0.001

Abbreviations: ADMA: asymmetric dimethylarginine; APACHE: Acute Physiology And Chronic Health Evaluation; CT-proET-1: C-terminal proendothelin-1; GFR: glomerular filtration rate; NT-proBNP: amino-terminal pro-B-type natriuretic peptide; NT-proCNP: amino-terminal pro-C-type natriuretic peptide; RBP4: retinol binding protein 4; SAPS: Simplified Acute Physiology Score; SDMA: symmetric dimethylarginine; suPAR: soluble urokinase plasminogen activator receptor.

Diabetes or obesity did not impact copeptin levels at admission to the ICU

Copeptin has been associated with glucose abnormalities, insulin resistance, type 2 diabetes and the metabolic syndrome[9,10]. We therefore assessed whether these comorbidities impacted copeptin levels at ICU admission.

Interestingly, patients with or without concomitant type 2 diabetes did not differ regarding their copeptin levels, although diabetic patients showed a tendency towards higher copeptin plasma concentrations (median 54.6 pmol/L in diabetics vs. median 44.3 pmol/L in non-diabetics, not significant; Figure 9.2A). However, we observed

significant correlations (Table 9.2) between copeptin and blood glucose as well as retinol-binding protein 4 (RBP4), a marker of insulin resistance[20].

A similar observation was found regarding obesity. Patients with pre-existing obesity, defined as a body mass index above 30 kg/m², showed a slight, but insignificant trend towards higher copeptin levels at ICU admission (median 57.5 pmol/L vs. median 43.7 pmol/L in non-obese patients, not significant; Figure 9.2B).

However, copeptin plasma concentrations correlated with the adipose tissue related factors ("adipocytokines") resistin and visfatin (Table 9.2).

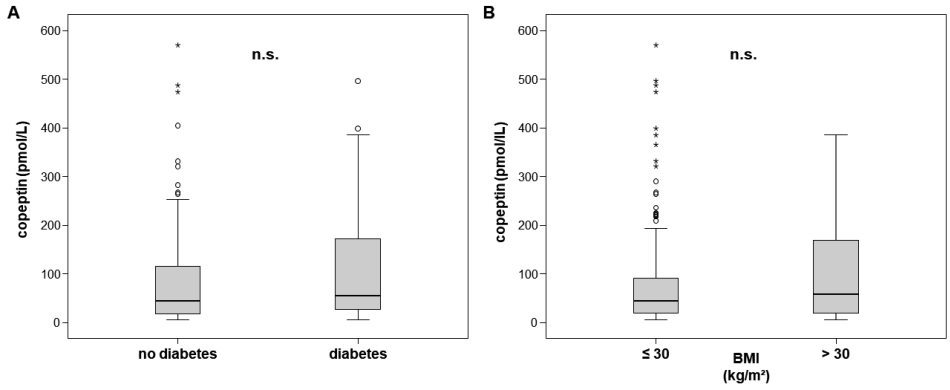


Figure 9.2 Impact of metabolic comorbidities on copeptin levels. Copeptin plasma concentrations did not differ between ICU patients with or without pre-existing type 2 diabetes (**A**) or obesity, as defined by a body-mass index (BMI) above 30 kg/m² (**B**).

Copeptin levels are correlated with biomarkers of renal failure and metabolic disturbances in critically ill patients

As copeptin mirrors endogenous vasopressin levels and thereby its hemodynamic and osmoregulatory functions[23], we hypothesized that copeptin plasma concentrations might be associated with biomarkers of organ function, tissue perfusion and metabolism.

In fact, copeptin was closely correlated with biomarkers reflecting renal function, inflammation, vascular tone and tissue perfusion (Table 9.2). The association between copeptin levels and renal failure is well established[4]. While copeptin concentrations were closely correlated with creatinine, cystatin C or urea, patients that developed subsequent renal failure and required renal replacement therapy at the ICU had significantly elevated circulating copeptin levels at ICU admission already (median

copeptin 33.9 pmol/L in patients without vs. median 91.2 pmol/L in patients with subsequent renal replacement therapy, p<0.001).

Regarding vasculature and tissue perfusion, copeptin levels correlated with C-terminal proEndothelin-1 (CT-proET-1), the circulating precursor protein of the vasoconstrictor Endothelin 1[22], asymmetric and symmetric dimethylarginine, two regulators of the endothelial nitric oxide pathways[14,24], and N-terminal pro-C-type natriuretic peptide (NT-proCNP, Table 9.2), a paracrine molecule synthesized in the vasculature with vasorelaxant effects[25].

High copeptin plasma concentrations at ICU admission are associated with adverse prognosis

By categorizing our critically ill patients into patients that survived ICU treatment (170/218, 78%) and patients that died during critical illness (48/218, 22%), we found significantly elevated copeptin plasma levels at the time-point of ICU admission in the patients that subsequently passed away at the ICU (median copeptin 39.6 pmol/L in ICU survivors vs. median 75.8 pmol/L in ICU deaths, p=0.007; Figure 9.3A).

We next calculated the optimal cut-off value of copeptin for predicting survival using the Youden index[26]. In fact, patients with copeptin levels above 65 pmol/L showed significantly decreased survival rates (p=0.009), as displayed by Kaplan-Meier curve analysis (Figure 9.3B).

In 207 out of our 218 patients, follow-up data on long-term survival for up to three years were available. Strikingly, copeptin levels obtained at ICU admission were significantly elevated in the 87 patients that died overall (median copeptin 38.1 pmol/L in survivors vs. 70.3 pmol/L in deaths, p=0.003; Figure 9.3C). Also, for the overall survival rate, patients with copeptin plasma levels above 65 pmol/L showed an increased mortality by Kaplan-Meier curve analysis (Figure 9.3D).

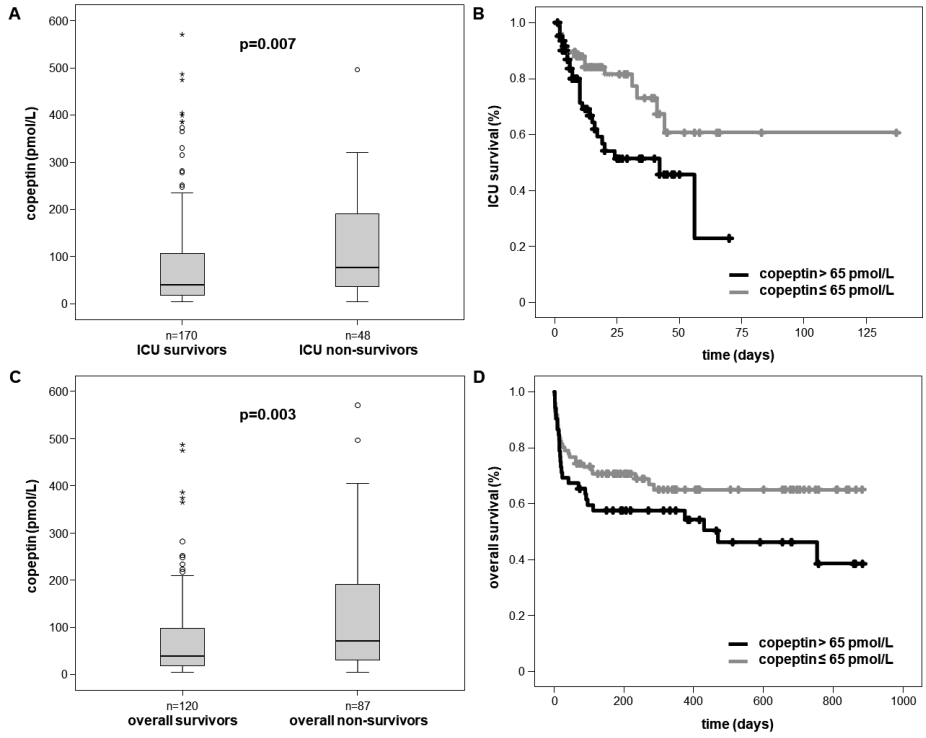


Figure 9.3 Copeptin is a prognostic biomarker for mortality in critically ill patients. (**A**) Patients that died during the course of ICU treatment are characterized by significantly higher plasma copeptin concentrations already at ICU admission. (**B**) Using a calculated cut-off of 65 pmol/L, high copeptin levels are associated with decreased ICU survival, as depicted by Kaplan-Meier survival curve analysis. (**C**) Patients that died either at the ICU or during the observation follow-up period of approximately two years have higher copeptin levels at ICU admission compared to overall survivors. (**D**) Elevated copeptin concentrations (>65 pmol/L) at ICU admission indicate overall mortality during long-term follow-up, as depicted by Kaplan-Meier survival curve analysis.

Discussion

We conducted a large observational study, in which we prospectively enrolled 218 critically ill patients from our medical ICU, to determine the value of copeptin as a biomarker in the intensive care setting.

In line with previous reports of patients with acute illnesses[6,10-13], critically ill patients had significantly higher circulating copeptin levels as compared to healthy controls. However, the disease entity (e.g., sepsis) or metabolic comorbidities that would affect copeptin levels in the chronic setting[4,9] did not determine copeptin concentrations in

critical illness. On the contrary, copeptin levels closely correlated with disease severity as well as biomarkers of renal failure, liver failure, altered metabolism and tissue perfusion.

As a consequence, high copeptin plasma concentrations at ICU admission reliably predicted short-term and long-term mortality in our cohort.

In contrast to our study, a large observational single-center study from Beijing that included 461 patients admitted to the Emergency Department reported that copeptin levels might serve as an early diagnostic indicator of sepsis[13]. Importantly, this study also noted an association between copeptin and disease severity as well as short-term mortality in the Emergency Department setting[13].

Our study that focused on critically ill patients at the ICU revealed that not the presence of sepsis, but the severity of critical illness as reflected by organ failure(s) and hemodynamic alterations was the main determinant for elevated copeptin. Possibly, sepsis is simply a characteristic condition, in which neuroendocrine pathway activation and subsequent vasopressin release reflect the stress level of critically ill patients. Of note, in the ICU setting, two smaller observational studies reported a stepwise increase in circulating copeptin levels from sepsis to severe sepsis to septic shock[12,27], which particularly mirrors the extent of cardiovascular instability.

The close association between copeptin and renal failure in ICU patients is not surprising, given the important role of vasopressin in water and sodium resorption, vascular tone of renal vessels and kidney perfusion[5].

In our critically ill patients, however, there was also a strong correlation between copeptin and biomarkers of endothelial dysfunction and systemic regulators of vascular tone such as CT-pro-ET1, ADMA, SDMA or NT-proCNP[14,22,24,25]. These data indicate that copeptin contributes to hemodynamic alterations resulting in tissue hypoperfusion. This assumption is corroborated by the correlations between copeptin and lactate as well as systemic acidosis in our study.

From our correlative analysis, it is impossible to dissect whether high copeptin in critical illness is cause or consequence of biological stress, vascular dysregulation and tissue hypoperfusion.

Biological stress activates key neuroendocrine pathways[3], namely the hypothalamic-pituitary-adrenal axis (e.g., CRH – ACTH – cortisol) as well as vasopressin release, which is derived from the same precursor protein as copeptin. Hypotension further stimulates vasopressin secretion in order to counteract shock by volume retention and

vasoconstriction[4]. Nonetheless, the activation of this pathway, as reflected by copeptin, is closely related to the patients' prognosis at and beyond the ICU. In agreement with prior studies in patients with acute illnesses that noted an association with short-term mortality[6,11-13], our study confirmed the value of copeptin levels as a prognostic biomarker for the ICU mortality.

More strikingly, copeptin plasma concentrations at the admission to the ICU even predicted the long-term, overall mortality of critically ill patients in our study. The long-term outcome of critically ill patients after discharge from the ICU depends on manifold clinical and biological factors, including age, comorbidity and length of stay at the ICU[28]. The association between copeptin plasma levels and overall mortality suggests that copeptin could be a sensitive biomarker for the accurate assessment of the initial disease severity, supporting that the copeptin-related pathologies as biological stress and hypoperfusion are major determinants of the severity of critical illness.

Future studies should prospectively evaluate, whether the implementation of copeptin measurements into multifactorial risk models could improve prognostication and thereby management of critically ill patients.

References

1. Martin-Loeches I, Wunderink RG, Nanchal R, et al. Determinants of time to death in hospital in critically ill patients around the world. Intensive Care Med. 2016;42:1454-1460.
2. Hotchkiss RS, Moldawer LL, Opal SM, Reinhart K, Turnbull IR, Vincent JL. Sepsis and septic shock. Nat Rev Dis Primers. 2016;2:16045.
3. McEwen BS. Physiology and neurobiology of stress and adaptation: central role of the brain. Physiol Rev. 2007;87:873-904.
4. Morgenthaler NG. Copeptin: a biomarker of cardiovascular and renal function. Congest Heart Fail. 2010;16(Suppl 1):S37-S44.
5. Bolignano D, Cabassi A, Fiaccadori E, et al. Copeptin (CTproAVP), a new tool for understanding the role of vasopressin in pathophysiology. Clin Chem Lab Med. 2014;52:1447-1456.
6. Jochberger S, Morgenthaler NG, Mayr VD, et al. Copeptin and arginine vasopressin concentrations in critically ill patients. J Clin Endocrinol Metab. 2006;91:4381-4386.
7. Morgenthaler NG, Struck J, Alonso C, Bergmann A. Assay for the measurement of copeptin, a stable peptide derived from the precursor of vasopressin. Clin Chem. 2006;52:112-119.
8. Szinnai G, Morgenthaler NG, Berneis K, et al. Changes in plasma copeptin, the c-terminal portion of arginine vasopressin during water deprivation and excess in healthy subjects. J Clin Endocrinol Metab. 2007;92:3973-3978.
9. Saleem U, Khaleghi M, Morgenthaler NG, et al. Plasma carboxy-terminal provasopressin (copeptin): a novel marker of insulin resistance and metabolic syndrome. J Clin Endocrinol Metab. 2009;94: 2558-2564.
10. Smaradottir MI, Ritsinger V, Gyberg V, Norhammar A, Nasman P, Mellbin LG. Copeptin in patients with acute myocardial infarction and newly detected glucose abnormalities -A marker of increased stress susceptibility? A report from the glucose in acute myocardial infarction cohort. Diab Vasc Dis Res. 2017; 14:69-76.
11. Krychtiuk KA, Honeder MC, Lenz M, et al. Copeptin predicts mortality in critically ill patients. PLoS One. 2017;12:e0170436.
12. Seligman R, Papassotiriou J, Morgenthaler NG, Meisner M, Teixeira PJ. Copeptin, a novel prognostic biomarker in ventilator-associated pneumonia. Crit Care. 2008;12:R11.
13. Zhang Q, Dong G, Zhao X, Wang M, Li CS. Prognostic significance of hypothalamic-pituitary-adrenal axis hormones in early sepsis: a study performed in the emergency department. Intensive Care Med. 2014;40:1499-1508.
14. Koch A, Weiskirchen R, Kunze J, et al. Elevated asymmetric dimethylarginine levels predict short-and long-term mortality risk in critically ill patients. J Crit Care. 2013;28:947-953.
15. Buendgens L, Yagmur E, Bruensing J, et al. Growth differentiation factor-15 is a predictor of mortality in critically ill patients with sepsis. Dis Markers. 2017;2017:5271203.
16. Rhodes A, Evans LE, Alhazzani W, et al. Surviving sepsis Campaign: International guidelines for management of sepsis and septic shock: 2016. Intensive Care Med. 2017;43:304-377.
17. Koch A, Voigt S, Kruschinski C, et al. Circulating soluble urokinase plasminogen activator receptor is stably elevated during the first week of treatment in the intensive care unit and predicts mortality in critically ill patients. Crit Care. 2011;15:R63.
18. Koch A, Gressner OA, Sanson E, Tacke F, Trautwein C. Serum resistin levels in critically ill patients are associated with inflammation, organ dysfunction and metabolism and may predict survival of non-septic patients. Crit Care. 2009;13:R95.
19. Koch A, Sanson E, Voigt S, Helm A, Trautwein C, Tacke F. Serum adiponectin upon admission to the intensive care unit may predict mortality in critically ill patients. J Crit Care. 2011;26:166-174.
20. Koch A, Weiskirchen R, Sanson E, et al. Circulating retinol binding protein 4 in critically ill patients before specific treatment: prognostic impact and correlation with organ function, metabolism and inflammation. Crit Care. 2010;14:R179.
21. Koch A, Weiskirchen R, Zimmermann HW, Sanson E, Trautwein C, Tacke F. Relevance of serum leptin and leptin-receptor concentrations in critically ill patients. Mediators Inflamm. 2010;2010:1-9.

22. Buendgens L, Yagmur E, Bruensing J, et al. C-terminal proendothelin-1 (CT-proET-1) is associated with organ failure and predicts mortality in critically ill patients. J Intensive Care. 2017;5:25.
23. Katan M, Muller B, Christ-Crain M. Copeptin: a new and promising diagnostic and prognostic marker. Crit Care. 2008;12:117.
24. Koch A, Weiskirchen R, Bruensing J, et al. Regulation and prognostic relevance of symmetric dimethylarginine serum concentrations in critical illness and sepsis. Mediators Inflamm. 2013;2013:413826.
25. Koch A, Voigt S, Sanson E, et al. Prognostic value of circulating amino-terminal pro-C-type natriuretic peptide in critically ill patients. Crit Care. 2011;15:R45.
26. Koch A, Weiskirchen R, Ludwig S, et al. Relevance of serum sclerostin concentrations in critically ill patients. J Crit Care. 2017;37:38-44.
27. Laribi S, Lienart D, Castanares-Zapatero D, Collienne C, Wittebole X, Laterre PF. NT-proAVP is not a good predictor of vasopressor need in septic shock. Shock. 2015;44:330-335.
28. Gayat E, Cariou A, Deye N, et al. Determinants of long-term outcome in ICU survivors: results from the FROG-ICU study. Crit Care. 2018;22:8.

Chapter 10

Elevated MR-proANP Plasma Concentrations are Associated with Sepsis and Predict Mortality in Critically Ill Patients

Eray Yagmur
Johanna Hermine Sckaer
Ger H. Koek
Ralf Weiskirchen
Christian Trautwein
Frank Tacke
Alexander Koch

J Transl Med. 2019:17(1):415

Abstract

Background and aims
Mid-regional pro atrial natriuretic peptide (MR-proANP) is an established biomarker for heart failure, based on its key role in regulating homeostasis of water balance and blood pressure.

The aim of the study was to determine the value of MR-proANP as a clinical biomarker in critical illness and/or sepsis. Upon admission to the medical intensive care unit (ICU), we investigated MR-proANP plasma concentrations in 217 critically ill patients (144 with sepsis, 73 without sepsis). Results were compared with 65 healthy controls.

Results
MR-proANP plasma levels were significantly elevated in critically ill patients, when compared to healthy controls. Notably, MR-proANP levels were significantly higher in ICU patients with sepsis. MR-proANP levels were not associated with metabolic comorbidities like diabetes or obesity.

In critically ill patients, MR-proANP plasma concentrations correlated with inflammatory cytokines, markers of organ dysfunction and several adipocytokines, such as resistin, retinol-binding protein 4 (RBP4) and adiponectin. Importantly, high MR-proANP plasma levels were associated with mortality, as MR-proANP levels above 227.0 pmol/l indicated a particularly increased mortality risk in ICU patients.

Conclusion
Our study emphasizes the role of circulating MR-proANP as a biomarker in critically ill patients, in which high MR-proANP indicates organ dysfunction, sepsis and mortality risk. The association between high MR-proANP and inflammatory as well as adipose tissue-derived endocrine mediators warrants further pathophysiological investigations.

Background

The natriuretic peptides of type A, B and C (ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; CNP, C-type natriuretic peptide) belong to a family of cardiac- and vascular-derived hormones. They exert diuretic, natriuretic and hypotensive actions and protect the organism from excessive fluid and high blood pressure.

Through a variety of effects on vascular tone, intravascular volume and redistribution, cardiovascular remodelling and energy metabolism, natriuretic peptides play a key role in maintaining cardiovascular homeostasis, water balance and blood pressure[1-4]. In this context, atrial natriuretic peptides (ANP) are predominantly expressed in the right atrium of the heart and secreted during an atrial distension such as in cardiac dysfunction or heart failure[1,5,6]. ANP represents more than 95% of natriuretic peptides in the blood circulation[7].

It is synthesized and stored in the atrial cardiomyocyte granules as a prohormone with 126 amino acids (proANP 1-126)[8]. The prohormone consists of several peptides with antihypertensive, natriuretic, diuretic and kaliuretic properties (e.g. proANP1-30 - long-acting natriuretic peptide; proANP31-67 - vasodilator; proANP79-98 - kaliuretic peptide)[9]. ANP prohormone processing differs within the kidney leading to an additional addition of four amino acids to the N-terminus of ANP (e.g. proANP95-126). The mature atrial natriuretic peptide consists of amino acids 99-126 and comprises 98% of the circulating natriuretic peptides[10].

In response to increased tension of the atrial wall, the active hormone ANP is secreted by splitting of its precursor into an amino-terminal (NT-proANP 1-98) and an active hormone (ANP 99-126)[7,11-14]. As the active ANP has a very short half-life of less than 5 minutes and the NT-proANP is released in the same molar ratio as ANP with significantly longer half-life (60-120 minutes), NT-proANP is considered a more reliable biomarker than ANP itself[8,15].

However, since NT-proANP can be further cleaved into smaller amino acid fragments in vivo, the detection of mid-regional proANP (amino acids 53-90; MR-proANP) is the preferred detection site of this natriuretic peptide[10,16-18].

ANP disrupts both the network of mitogen-activated protein kinase (MAPK) and transcription factors, mainly NF-κB[5]. Atrial dilatation leads to the expression and secretion of preproatrial or A-type natriuretic peptide and finally atrial natriuretic peptide (ANP), which has similar biological properties to B-type natriuretic peptide (BNP). However, the formation of the pre-produced BNP is induced by sodium and water retention and vasoconstrictions caused by the activation of RAAS and the

sympathetic nervous system, as well as the action of vasopressin. These factors lead to increased ventricular pre- and post-stress and increased wall stress and BNP release. Furthermore, the BNP prohormone is cleaved to BNP and N-terminal proBNP (NT-proBNP). NT-proBNP is biologically inactive and does not bind to the pGC-A receptor. However, the biological effects of ANP and BNP substrates are similar: induction of natriuresis, diuresis, vasodilatation, antifibrosis and anti-RAAS[5,9].

ANP acts through the natriuretic peptide receptor A (NPR-A) and is removed from the bloodstream by the natriuretic peptide receptor C (NPR-C)[7,19]. Binding to NPR-A activates the cyclic guanylyl monophosphate (cGMP) as second messenger in the target cells to mediate a variety of systemic effects as previously described[19]. Interestingly, in knock-out mice lacking the NPR 1 gene coding for NPR-A, not only high ANP concentrations, hypertension and cardiac hypertrophy, but also expression of pro-inflammatory markers are observed.

In line, recent data suggest that ANP regulates inflammatory processes such as macrophage function, priming of neutrophils and the expression of pro-inflammatory markers[20,21]. Thus, ANP also participates in innate immune reactions[2,22]. Moreover, activation of intracellular cGMP induces lipolysis and mobilization of free fatty acids in human adipocytes[23,24]. This demonstrates the interaction of ANP with white and brown adipose tissue.

Specifically, ANP increases the expression and secretion of adiponectin, an adipocytokine with insulin-sensitizing properties, as observed in primary human adipocyte cultures, healthy subjects and patients with congestive heart failure[23,25,26]. In addition, the ANP/cGMP signalling pathway increases β-cell mass and insulin secretion in the pancreas[23]. With regard to ANP removal, it has been clearly shown that upregulation of NPR-C is associated with metabolic alterations such as obesity and obesity-related metabolic disorders like type 2 diabetes and metabolic syndrome[23,27].

Based on this wide range of physiological functions of ANP and its associated alterations, ANP has been linked to inflammatory responses and metabolic alterations that occur during critical illness[9,28,29]. Critical illness and MR-proANP are associated with and affected by alterations in secretory and metabolic functions of adipose tissue[30,31]. In different cohorts of ICU patients, high MR-proANP plasma levels have been associated with disease severity and outcome of critical illness[8,11]. In addition, elevated MR-proANP levels are described to be diagnostic for sepsis after burn injury[32].

In this study, we investigated the clinical and prognostic relevance of MR-proANP plasma concentrations in a large cohort of critically ill patients from a medical ICU including sepsis, pre-existing diabetes, obesity and organ dysfunction.

Methods

Study design and patient characteristics

Critically ill patients were included at admission to the medical ICU at the RWTH University Hospital Aachen, Germany. Patients, who were admitted for post-interventional observational stay or underwent an elective procedure, were excluded[33]. The cohort consisted of 217 critically ill patients (144 with sepsis, 73 without sepsis). Patients' characteristics are shown in Table 10.1.

The patients were categorized as sepsis and non-sepsis according to the Third International Consensus Definitions for Sepsis and Septic Shock (sepsis-3)[34], and were treated following the current guidelines for treatment of sepsis (Surviving Sepsis Campaign)[35]. Underlying disease etiologies of sepsis and non-sepsis patients are shown in Table 10.2.

As a control group, we analysed healthy blood donors with normal blood counts, normal values of liver enzymes, glomerular filtration rates, serum creatinine and C-reactive protein (CRP) concentration[36]. All healthy subjects had a negative serology for human immunodeficiency virus (HIV)[36].

In order to determine long-term outcome, we contacted the patients, their relatives and/or the general practitioner in approximately 6-months intervals after discharge from the hospital over a period of three years[36].

Measurements of MR-proANP plasma levels

Before therapeutic interventions, blood samples were collected upon admission to the ICU, centrifuged, and plasma was stored at -80°C. Plasma MR-proANP concentrations (epitopes covering amino acids 53-90, equivalent to NT-proANP and active ANP[10] were determined using an automated immunofluorescent assay based on TRACE technology (Time-resolved Amplified Cryptate Emission, B.R.A.H.M.S Kryptor compact, Hennigsdorf, Germany), according to manufacturer`s instructions (MR-proANP Kryptor, #819.050, B.R.A.H.M.S, Hennigsdorf, Germany).

Measurements of the adipocytokines and related proteins leptin, retinol-binding protein 4 (RBP4), adiponectin, ghrelin, and resistin were included, as previously reported[37-41].

In addition, soluble urokinase-type plasminogen activator receptor (suPAR) and amino-terminal pro C-type natriuretic peptide (NT-proCNP) concentrations as markers of

disease severity and inflammatory response were also investigated as described previously[36,42].

Statistical analysis

Owing to the skewed distribution of the parameters, data are given as median and range, and shown graphically by box-and-whiskers plots. The degree of association between two variables was assessed by the Spearman rank correlation test. Comparisons of parameters between two different groups were conducted with the Mann-Whitney U-test. All values, including outside values as well as far out values, were included.

P-values less than 0.05 were considered as statistically significant. Receiver operating characteristic (ROC) curve analysis was carried out to determine the diagnostic sensitivity and specificity of MR-proANP in critically ill patients. The ROC curve analysis and the derived area under the curve (AUC) statistic provide a global and standardized appreciation of the accuracy of a marker or a composite score for predicting an event. ROC curves were generated by plotting sensitivity against 1-specificity[42].

The prognostic value of the variables was tested by univariate and multivariate analyses in the Cox regression model. Survival curves were generated by Kaplan-Meier analyses with a MR-proANP cut-off level calculated via the Youden Index[42]. All analyses were performed with IBM SPSS Statistics (SPSS; Chicago, IL, USA).

Results

MR-proANP plasma levels are significantly elevated in critically ill patients as compared with healthy controls

Based on the wide range of physiological functions of ANP and its associated alterations, ANP has been linked to both inflammatory and metabolic responses that typically occur during critical illness[9,28,29].

In our study, we found that MR-proANP plasma levels were significantly elevated in a large cohort of 217 critically ill patients (median 214.0 pmol/l, range 2.1-3417.0 pmol/l; Table 10.1) at admission to the ICU as compared with 65 healthy controls (median 18.5 pmol/l, range 3.5-61.7 pmol/l, $p<0.001$; Figure 10.1A).

Table 10.1 Baseline patient characteristics and MR-proANP plasma concentrations.

Parameter	All Patients	Non-Sepsis	Sepsis
Number	217	73	144
Sex (male/female)	133 / 84	48 / 25	85 / 59
Age median (range) [years]	64 (18-90)	61 (18-85)	65 (20-90)
APACHE-II score median (range)	18 (2-43)	13.5 (2-33)	19 (4-43)
SOFA score median (range)	9 (0-19)	7.0 (0-17)	9.5 (2-19)
SAPS2 score median (range)	41 (0-73)	41.0 (13-72)	40.5 (0-73)
ICU days median (range)	7 (1-137)	6 (1-45)	9 (1-137)
Death during ICU n(%)	46 (21.2%)	9 (12.3%)	37 (25.7%)
Death overall (total) n(%)	86 (39.6%)	22 (30.1%)	64 (44.4%)
Mechanical ventilation n(%)	144 (66.4%)	46 (63%)	98 (67%)
Preexisting diabetes n(%)	65 (30.0%)	22 (30.1%)	43 (29.9%)
BMI median (range) [m²/kg]	26.0 (15.3-86.5)	25.7 (15.9-40.5)	26.0 (15.3-86.5)
WBC median (range) [x10³/µl]	12.9 (0.1-208)	12.5 (1.8-29.6)	13.8 (0.1-208)
CRP median (range) [mg/dl]	103.0 (5-230)	17 (5-230)	163.5 (5-230)
IL-6 median (range) [pg/ml]	145.0 (2-28000)	66.5 (1.5-5000)	240 (2-28000)
Procalcitonin median (range) [pmol/l]	0.7 (0.03-207.5)	0.2 (0.03-100)	1.8 (0.03-207.5)
Creatinine median (range) [mg/dl]	1.3 (0.1-15)	1.0 (0.2-15)	1.6 (0.1-10.7)
INR median (range)	1.16 (0.92-13)	1.17 (0.95-6.73)	1.16 (0.92-13)
MR-proANP day 1 median (range) [pmol/l]	214.0 (2.1-3417.0)	147.2 (2.1-1625.0)	246.6 (7.8-3417.0)

For quantitative variables, median and range (in parenthesis) are given.

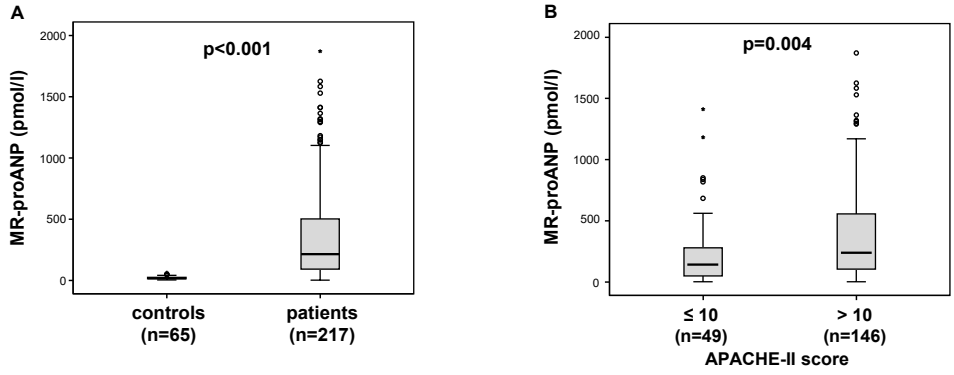


Figure 10.1 MR-proANP levels in critically ill patients. (**A**) MR-proANP plasma concentrations are significantly elevated in critically ill patients compared with healthy controls. (**B**) At ICU admission, MR-proANP levels are significantly elevated in critically ill patients with high initial Acute Physiology and Chronic Health Evaluation (APACHE II) score (>10) in comparison to patients with low APACHE-II scores (≤10). P-values (U-test) are given.

Increased MR-proANP levels have been associated with adverse clinical outcome[43]. In fact, MR-proANP plasma levels correlated positively with established clinical disease severity scores (Table 10.3).

Moreover, critically ill patients with a high (acute physiology and chronic health II (APACHE-II) score above 10 showed significantly higher MR-proANP levels at ICU

admission (median 239.7 pmol/l, range 2.1-1871.0 pmol/l) in comparison to ICU patients admitted with an APACHE-II score of 10 or less (median 143.0 pmol/l, range 2.1-3417.0 pmol/l, p=0.004, Figure 10.1B).

MR-proANP plasma levels are particularly elevated in critically ill patients with sepsis

High MR-proANP plasma levels in critically ill patients had been previously reported to be associated with sepsis[8,11]. Within our cohort of 217 critically ill patients, 144 fulfilled sepsis criteria, while 73 were admitted to the ICU due to other causes of critical illness (Table 10.2). Plasma concentrations of MR-proANP were significantly elevated in patients with sepsis (median 246.6 pmol/l, range 7.8-3417.0 pmol/l) as compared to ICU patients without sepsis (median 147.2 pmol/l, range 2.1-1625.0 pmol/l, p<0.001; Figure 10.2A and Table 10.2).

We analysed the diagnostic value of MR-proANP for sepsis in comparison to classical markers of inflammation and bacterial infection by using ROC curve analyses. Whereas CRP achieved AUC statistics of 0.847 and white blood cell count of 0.585, MR-proANP only reached an AUC of 0.656 (Figure 10.2B). Among the septic or non-septic critically ill patients, there was no association between MR-proANP plasma concentrations and different disease etiologies leading to ICU admission (data not shown).

Table 10.2 Disease etiology of the study population leading to ICU admission.

	Sepsis n=144	**Non-sepsis n=73**
Etiology of sepsis critical illness		
Site of infection n (%)		
Pulmonary	73 (51%)	
Abdominal	26 (18%)	
Urogenital	11 (8%)	
Other	34 (23%)	
Etiology of non-sepsis critical illness n (%)		
Cardio-pulmonary disorder		29 (40%)
Acute pancreatitis		10 (14%)
Acute liver failure		4 (5.5%)
Decompensated liver cirrhosis		9 (12%)
Severe gastrointestinal hemorrhage		4 (5.5%)
Non-sepsis other		17 (23%)

MR-proANP levels in critically ill patients are closely correlated to biomarkers of inflammation, organ dysfunction and clinical scores

Mice lacking a functional NPR 1 gene encoding NPR-A exhibit hypertension and marked cardiac hypertrophy with interstitial fibrosis, in association with enhanced activation of

pro-inflammatory cytokines, probably via nuclear factor kappa mediated signalling pathway[44,45].

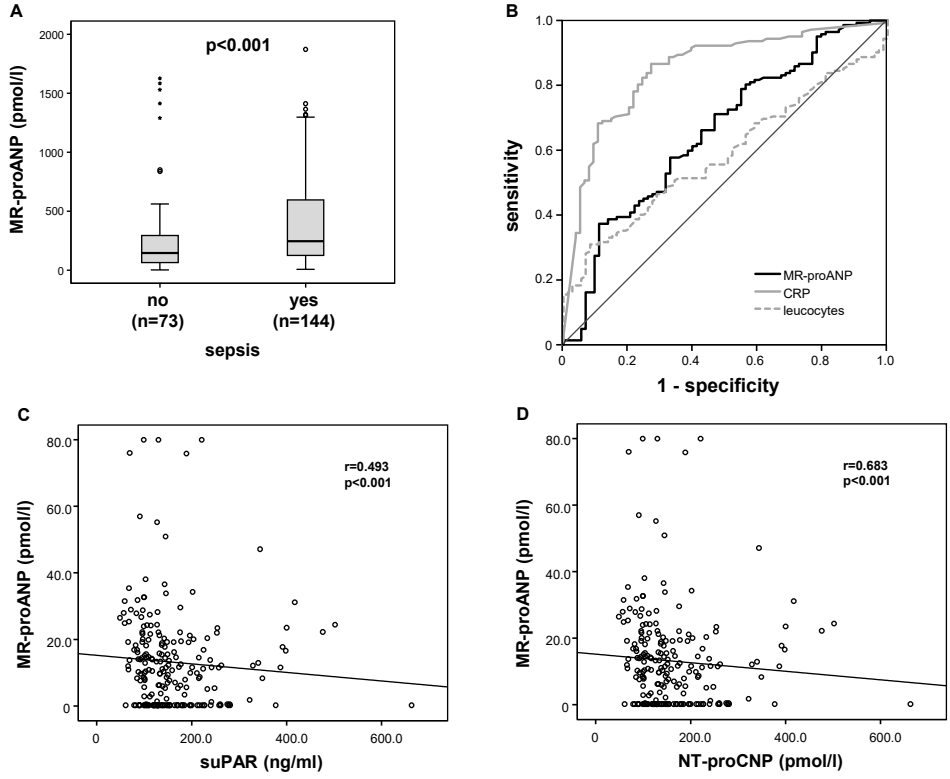


Figure 10.2 MR-proANP levels are elevated in critically ill patients with sepsis and correlate with inflammatory markers. (**A**) ICU patients with sepsis displayed significantly elevated MR-proANP levels compared to patients without sepsis. (**B**) Receiver operating characteristic (ROC) curve analyses comparing the diagnostic power in predicting sepsis of MR-proANP (black line, area under the curve (AUC) 0.656) with classical markers of inflammation and bacterial infection, C-reactive protein (CRP, grey line, AUC 0.847) and white blood cell count (leukocytes, dotted grey line, AUC 0.585). (**C-D**) MR-proANP correlates with experimental markers of inflammation in critical illness like soluble urokinase-type plasminogen activator receptor (suPAR, C) and N-terminal pro C-type natriuretic peptide (NT-proCNP, D). P-values (U-test or Spearman rank correlation) are given.

To determine the factors possibly promoting elevated MR-proANP plasma levels in critically ill patients, correlation analyses with extensive sets of laboratory parameters were performed. At admission to the ICU, plasma MR-proANP concentrations in the total cohort and the subgroups of sepsis and non-sepsis patients were closely correlated with classical markers of inflammation and bacterial infection, such as C-reactive protein (r=0.286, p<0.001), procalcitonin (r=0.378, p<0.001), and

experimental markers of inflammation such as soluble urokinase plasminogen activator receptor (suPAR, r=0.493, p<0.001, Figure 10.2C), and NT-proCNP (r=0.683, p<0.001, Figure 10.2D, Table 10.3).

Table 10.3 Correlations with MR-proANP plasma concentrations at ICU admission.

	ICU patients	
Parameters	**r**	***p***
Disease severity/clinical scoring/therapy		
APACHE II	0.260	*<0.001*
SOFA	0.223	*0.011*
SAPS	0.341	*0.006*
Fluid substitution	-0.233	*0.001*
Markers of inflammation		
White blood cell count	-0.148	*0.029*
C-reactive protein	0.286	*<0.001*
Procalcitonin	0.378	*<0.001*
suPAR	0.493	*<0.001*
NT-proCNP	0.683	*<0.001*
Markers of organ function		
NT-proBNP	0.740	*<0.001*
Urea	0.623	*<0.001*
Creatinine	0.629	*<0.001*
GFR-cystatin C	-0.675	*<0.001*
Cystatin C	0.675	*<0.001*
Lipase	-0.191	*0.012*
Pancreatic amylase	-0.317	*0.006*
Alanine aminotransferase	-0.172	*0.012*
Glutamate dehydrogenase	-0.151	*0.037*
Pseudocholinesterase activity	-0.339	*<0.001*
Albumin	-0.190	*0.045*
Total protein	-0.263	*<0.001*
INR	0.207	*0.003*
aPTT	0.324	*<0.001*
Antithrombin III	-0.216	*0.015*
Adipocytokines/ metabolic markers		
Adiponectin	0.434	*0.001*
Resistin	0.349	*0.008*
RBP4	0.306	*0.012*
HOMA-β	0.332	*0.007*
Parathyroid hormone	0.299	*0.014*
Calcium	-0.288	*<0.001*
Phosphorus	0.241	*0.001*

Spearman rank correlation test, only significant results are shown.

With regard to organ function, we could reveal strong associations with renal and hepatic function for the total study cohort and the subgroups of sepsis and non-sepsis patients.

Specifically, we could demonstrate an inverse association with renal function as displayed by highly significant correlations with the glomerular filtration rate of cystatin C ($r=-0.675$, $p<0.001$), cystatin C ($r=0.675$, $p<0.001$), creatinine ($r=0.629$, $p<0.001$) and urea ($r=0.623$, $p<0.001$) serum concentrations (Table 10.3), indicating renal clearance of MR-proANP[47].

Interestingly, MR-proANP levels inversely correlated with parameters reflecting the liver's biosynthetic and functional capacity, namely albumin ($r=-0.190$, $p=0.045$), pseudocholinesterase activity ($r=-0.339$, $p<0.001$), antithrombin III ($r=-0.216$, $p=0.015$), glutamate dehydrogenase ($r=-0.151$, $p=0.037$) and alanine aminotransferase ($r=-0.172$, $p=0.012$) (Table 10.3). MR-proANP levels also correlated with the amino-terminal brain natriuretic peptide (NT-proBNP) (Table 10.3).

For the total cohort of critically ill patients a strong association of MR-proANP plasma concentrations and established clinical scores like sequential organ failure assessment (SOFA; $r=0.223$, $p=0.011$), simplified acute physiology score 2 (SAPS2; $r=0.341$, $p=0.006$), and acute physiology and chronic health II (APACHE II; $r=0.260$, $p<0.001$) scores could be shown, corroborating that MR-proANP levels are closely linked to disease severity in critical illness (Table 10.3).

Measures of circulatory stabilization such as volume substitution and vasopressor therapy showed a significant inverse correlation of fluid therapy with plasma MR-proANP levels ($r=-0.233$, $p=0.001$), but not with vasopressor administration (Table 10.3).

MR-proANP plasma levels in critically ill patients are not associated with diabetes and obesity

Prior studies have shown an inverse association with natriuretic peptides and metabolic syndrome, fasting glucose, insulin resistance and diabetes development[46,47].

We therefore assessed whether metabolic comorbidities, specifically pre-existing obesity or diabetes, might have an influence on MR-proANP levels also in patients with critical illness. However, neither pre-existing type 2 diabetes (n=65, median 226.6 pmol/l, range 2.1-1871.0 pmol/l, $p=0.196$; Figure 10.3A) nor obesity (n=36, median 248.1 pmol/l, range 17.5-1319.0 pmol/l, $p=0.126$; Figure 10.3B), as defined by a body mass index (BMI) above 30 kg/m², were associated with MR-proANP plasma concentrations. Moreover, by Spearman rank correlation analysis, no correlation between MR-proANP and serum glucose levels, glycosylated hemoglobin A1c (HbA1c) or BMI was present (data not shown).

In addition, MR-proANP did not show any correlations with other key markers of glucose metabolism, such as insulin, C-peptide or the homeostasis model assessment-insulin resistance (HOMA-IR) in ICU patients (data not shown). However, β-cell function (HOMA-β) correlated with MR-proANP (r=3.332, p=0.007, Table 10.3).

Adipose tissue inflammation attributes to dysregulated production and release of inflammatory cytokines and adipocytokines, including interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α) as well as leptin, resistin and adiponectin[48].
We investigated the potential association between MR-proANP and adipocytokine responses in critically ill patients. In agreement with the proposed pro-inflammatory association of MR-proANP, we observed significant correlations between MR-proANP and a broad range of adipocytokines including resistin (r=0.349, p=0.008, Figure 10.3C), adiponectin (r=0.434, p=0.001, Figure 10.3D), and RBP4 (r=0.306, p=0.012, Table 10.3).

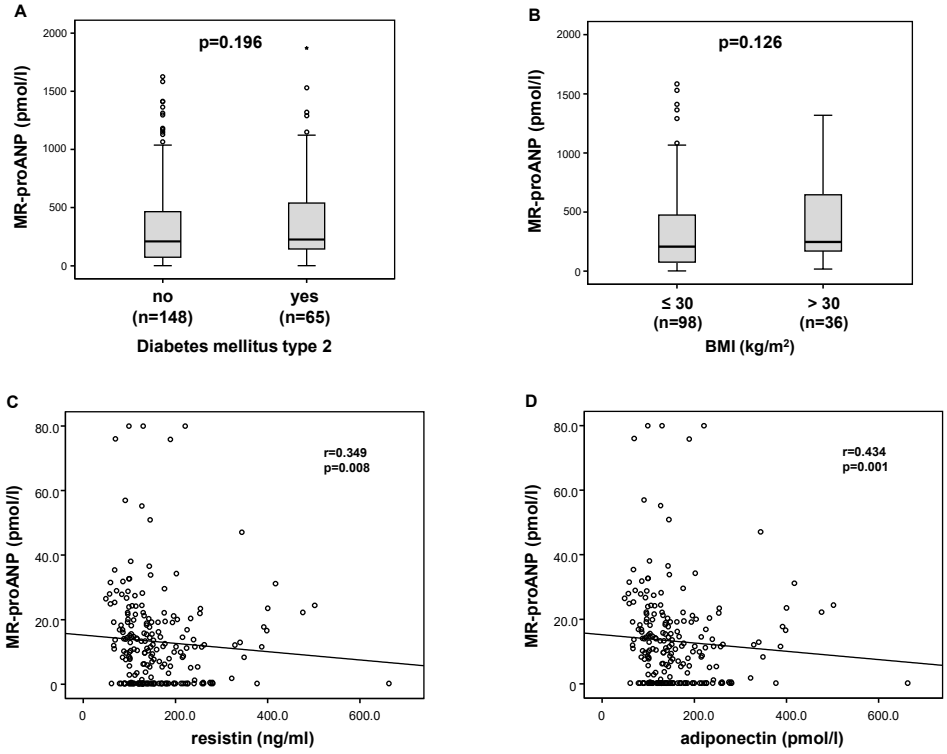


Figure 10.3 MR-proANP levels are not related to metabolic comorbidities. (**A-B**) MR-proANP plasma concentrations in ICU patients are neither associated with pre-existing type 2 diabetes (**A**) nor obesity, as defined by a body-mass index (BMI) above 30 kg/m² (**B**). (**C-D**) We observed significant correlations between MR-proANP and several adipocytokines including resistin (**C**) and adiponectin (**D**). P-values (U-test or Spearman rank correlation) are given.

Elevated MR-proANP plasma levels are associated with mortality in critically ill patients

Circulating natriuretic peptides like NT-proCNP have been previously suggested as biomarkers for disease severity as well as short- and long-term survival in various conditions of critical illness[42].

We assessed long-term survival in 206 out of 217 patients by contacting the patients, their relatives or their general practitioner during the first three years after ICU discharge. MR-proANP levels at ICU admission were significantly elevated in patients that subsequently died (n=86, median 309.0, range 2.1-3417.0) compared with survivors (n=120, median 171.1, range 2.1-1625.0; p<0.001, Figure 10.4A).

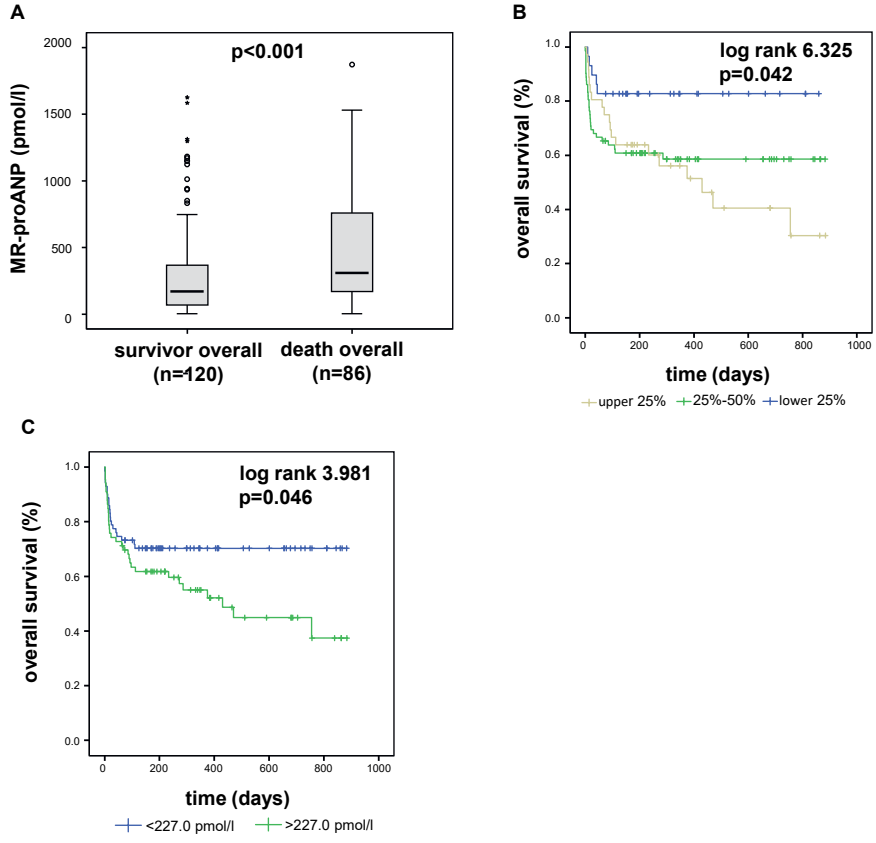


Figure 10.4 MR-proANP is a biomarker for mortality in critically ill patients. (**A**) Patients that died during or after ICU treatment displayed significant higher MR-proANP levels at ICU admission compared to patients that survived in the long-term follow-up. (**B**) High vs. low MR-proANP plasma concentrations discriminated survival of the critically ill patients, as displayed by Kaplan-Meier survival curve analysis for MR-proANP separated into quartiles. (**C**) Elevated MR-proANP plasma concentrations at ICU admission (optimal cut-off: 227.0 pmol/l) predicted the overall mortality in critically ill patients. P-values (U-test or log rank test) are given.

In univariate analysis, including markers of inflammation/infection (CRP (p=0.111), lactate (p=0.198)), hepatic (bilirubin (p=0.161)) and renal (creatinine (p=0.427)) function at admission, MR-proANP showed highest prognostic value (p=0.013) for ICU mortality.

In multivariate Cox regression analyses MR-proANP remained an independent and the only significant prognostic parameter (p=0.012) to predict overall ICU mortality. In this respect, MR-proANP levels showed comparable prognostic accuracy like established multifactorial scores such as APACHE II (AUC = 0.654 for MR-proANP, 0.638 for APACHE II score changes in ROC analyses).

This finding was corroborated by Kaplan-Meier survival curves analyses, demonstrating that patients with MR-proANP plasma levels of the lower quartile (<25%, corresponding to 91.3 pmol/l) had the best survival rates, while patients with admission MR-proANP levels of the upper quartile (>75%, corresponding to 506.6 pmol/l) had the highest long-term mortality (Figure 10.4B).

Using the calculated optimal cut-off for MR-proANP of 227.0 pmol/l, patients with high MR-proANP demonstrated a high mortality rate, as depicted by Kaplan-Meier survival curve analysis (Figure 10.4C).

Discussion

The expression and secretion of the atrial natriuretic polypeptide (ANP) hormone has been mainly studied in the context of cardiac diseases[49]. In particular, increases in ANP or MR-proANP concentrations in blood circulation were often considered to be dependent on the prevalence of cardiac insufficiency and classical cardiac risk factors such as diabetes and renal failure[50]. Furthermore, ANP has been linked to both inflammatory and metabolic responses that typically occur during critical illness[9,28,29].

However, ANP is expressed and secreted in the cells of the heart atria and BNP mainly in the ventricles that is therefore less sensitive to intraventricular pressure increase and hemodynamic stress than BNP. NT-proBNP is currently recognized as the clinical gold standard for the diagnosis of acute destabilized heart failure in patients with dyspnea[51].

In critically ill patients, elevated plasma concentrations of natriuretic peptides are found in severe hemodynamic disturbances such as cardiogenic or septic shock due to impaired ventricular dysfunction and the release of proinflammatory cytokines[5,9]. In accordance to the positive correlation between MR-proANP and NT-pro BNP in our

study, dramatically increased proinflammatory cytokines in critically ill patients may also contribute to ANP and BNP secretion from the heart.

In our study, we demonstrated that MR-proANP is elevated in critically ill patients already at admission to the ICU as compared with healthy controls, in agreement with prior studies[8,11,32].

Moreover, using correlation analyses our study revealed significant associations between MR-proANP and established biomarkers reflecting inflammation, metabolic alterations, and organ dysfunction in medical ICU patients.

Although MR-proANP levels were further elevated in critically ill patients with sepsis, their diagnostic power for sepsis was inferior to routinely used inflammatory markers such as CRP or procalcitonin. In line with our findings, it has been reported that MR-proANP is neither a direct sepsis marker nor a predictor of bacteraemia[32,52,53]. Interestingly, in ventilator-associated pneumonia and lower respiratory tract infections, implementing MR-proANP improved survival prediction of clinical severity scores, especially when used in combination with procalcitonin (PCT)[54,55].

In septic shock patients, MR-proANP was significantly associated with 28-day mortality[56]. Moreover, MR-proANP was associated with cardiorenal dysfunction and an increased risk of terminal kidney disease and mortality[57]. In this context, MR-proANP showed a high accuracy for predicting survival in critical ill patients in our study.

Several studies have shown that obese individuals display lower circulating natriuretic peptide concentrations, indicating that obesity or BMI may be confounding factors for clinical and prognostic utility of MR-proANP[58-60]. In our cohort, we found that MR-proANP is strongly correlated with adipocytokines such as adiponectin, RBP4 and resistin, which are important mediators of insulin resistance and metabolic alterations[36,42]. Interestingly, MR-proANP did only correlate with markers reflecting adipose tissue inflammation, but not with patient's BMI or pre-existing obesity. Critically ill patients show dramatic metabolic and inflammatory dysfunctions, including dysregulated adipocytokines[30,31].

Within this context, ANP-binding to the natriuretic peptide receptor A activates the cyclic guanylyl monophosphate (cGMP) to mediate a variety of systemic effects such as lipolysis and free fatty acid mobilization in human adipocytes[19,23,24], which may provoke adipocytokine secretion from adipose tissue. The effects of ANP on adipose tissue might sustain inflammatory responses, possibly supporting systemic inflammation in critical illness and sepsis.

Our findings demonstrate the potential diagnostic and prognostic value of MR-proANP in critically ill patients with sepsis.and may contribute to implement MR-proANP as a potential novel biomarker in critical disease.

Conclusion

Our study emphasizes the role of circulating MR-proANP as a potential novel biomarker in critically ill patients, in which high MR-proANP plasma concentrations indicate organ dysfunction, sepsis, disease severity and mortality risk.

The association between high MR-proANP and inflammatory as well as adipose tissue-derived endocrine mediators warrants further pathophysiological investigations. Knowledge of these interactions will enhance the understanding of the pathogenic role of natriuretic peptides in critical illness.

References

1. Volpe M. Natriuretic peptides and cardio-renal disease. Int J Cardiol. 2014;176(3):630-639.
2. Woodard GE, Rosado JA. Natriuretic peptides in vascular physiology and pathology. Int Rev Cell Mol Biol. 2008;268:59-93.
3. Bie P. Natriuretic Peptides and Normal Body Fluid Regulation. Compr Physiol. 2018;8(3):1211-1249.
4. Fu S, Ping P, Wang F, Luo L. Synthesis, secretion, function, metabolism and application of natriuretic peptides in heart failure. J Biol Eng. 2018;12:2.
5. Wong PC, Guo J, Zhang A. The renal and cardiovascular effects of natriuretic peptides. Adv Physiol Educ. 2017;41(2):179-185.
6. Edwards BS, Zimmerman RS, Schwab TR, Heublein DM, Burnett JC Jr. Atrial stretch, not pressure, is the principal determinant controlling the acute release of atrial natriuretic factor. Circ Res. 1988;62(2): 191-195.
7. Vesely DL. Atrial natriuretic peptide prohormone gene expression: hormones and diseases that upregulate its expression. IUBMB Life. 2002;53(3):153-159.
8. Lipinska-Gediga M, Mierzchala M, Durek G. Pro-atrial natriuretic peptide (pro-ANP) level in patients with severe sepsis and septic shock: prognostic and diagnostic significance. Infection. 2012;40(3): 303-309.
9. Witthaut R. Science review: natriuretic peptides in critical illness. Crit Care. 2004;8(5):342-349.
10. Morgenthaler NG, Struck J, Thomas B, Bergmann A. Immunoluminometric assay for the midregion of pro-atrial natriuretic peptide in human plasma. Clin Chem. 2004;50(1):234-236.
11. Morgenthaler NG, Struck J, Christ-Crain M, Bergmann A, Müller B. Pro-atrial natriuretic peptide is a prognostic marker in sepsis, similar to the APACHE II score: an observational study. Crit Care. 2005;9(1):R37-45.
12. Lee CS, Tkacs NC. Current concepts of neurohormonal activation in heart failure: mediators and mechanisms. AACN Adv Crit Care. 2008;19(4):364-385.
13. Pandey KN. Emerging Roles of Natriuretic Peptides and their Receptors in Pathophysiology of Hypertension and Cardiovascular Regulation. J Am Soc Hypertens. 2008;2(4):210-226.
14. Theilig F, Wu Q. ANP-induced signaling cascade and its implications in renal pathophysiology. Am J Physiol Renal Physiol. 2015;308(10):F1047-1055.
15. Buckley MG, Marcus NJ, Yacoub MH. Cardiac peptide stability, aprotinin and room temperature: importance for assessing cardiac function in clinical practice. Clin Sci (Lond). 1999;97(6):689-695.
16. Seidler T, Pemberton C, Yandle T, Espiner E, Nicholls G, Richards M. The amino terminal regions of proBNP and proANP oligomerise through leucine zipper-like coiled-coil motifs. Biochem Biophys Res Commun. 1999;255(2):495-501.
17. Baertschi AJ, Monnier D, Schmidt U, Levitan ES, Fakan S, Roatti A. Acid prohormone sequence determines size, shape, and docking of secretory vesicles in atrial myocytes. Circ Res. 2001;89(3) E23-29.
18. Cappellin E, Gatti R, Spinella P, De Palo CB, Woloszczuk W, Maragno I, De Palo EF. Plasma atrial natriuretic peptide (ANP) fragments proANP (1-30) and proANP (31-67) measurements in chronic heart failure: a useful index for heart transplantation? Clin Chim Acta. 2001;310(1):49-52.
19. de Bold AJ, Borenstein HB, Veress AT, Sonnenberg H. A rapid and potent natriuretic response to intravenous injection of atrial myocardial extract in rats. Reprinted from Life Sci. 28:89-94, 1981. J Am Soc Nephrol. 2001;12(2):403-409.
20. Wiedermann CJ, Niedermühlbichler M, Braunsteiner H, Widermann CJ. Priming of polymorphonuclear neutrophils by atrial natriuretic peptide in vitro. J Clin Invest. 1992;89(5):1580-1586.
21. Fujita S, Shimojo N, Terasaki F, Otsuka K, Hosotani N, Kohda Y, Tanaka T, Nishioka T, Yoshida T, Hiroe M, et al. Atrial natriuretic peptide exerts protective action against angiotensin II-induced cardiac remodeling by attenuating inflammation via endothelin-1/endothelin receptor A cascade. Heart Vessels. 2013;28(5):646-657.
22. Vollmar AM. The role of atrial natriuretic peptide in the immune system. Peptides. 2005;26(6):1086-94.
23. Moro C, Lafontan M. Natriuretic peptides and cGMP signaling control of energy homeostasis. Am J Physiol Heart Circ Physiol. 2013;304(3):H358-368.

24. Sengenès C, Berlan M, De Glisezinski I, Lafontan M, Galitzky J. Natriuretic peptides: a new lipolytic pathway in human adipocytes. FASEB J. 2000;14(10):1345-1351.
25. Tsukamoto O, Fujita M, Kato M, Yamazaki S, Asano Y, Ogai A, Okazaki H, Asai M, Nagamachi Y, Maeda N, et al. Natriuretic peptides enhance the production of adiponectin in human adipocytes and in patients with chronic heart failure. J Am Coll Cardiol. 2009;53(22):2070-2077.
26. Birkenfeld AL, Boschmann M, Engeli S, Moro C, Arafat AM, Luft FC, Jordan J. Atrial natriuretic peptide and adiponectin interactions in man. PLoS One. 2012;7(8):e43238.
27. Birkenfeld AL, Boschmann M, Jordan J. Metabolic regulation: effects of natriuretic peptide interactions. Expert Rev Endocrinol Metab. 2007;2(5):607-614.
28. Mazul-Sunko B, Zarkovic N, Vrkic N, Klinger R, Peric M, Bekavac-Beslin M, Novkoski M, Krizmanic A, Gvozdenovic A, Topic E. Pro-atrial natriuretic peptide hormone from right atria is correlated with cardiac depression in septic patients. J Endocrinol Invest. 2001;24(7):RC22-24.
29. Berendes E, Van Aken H, Raufhake C, Schmidt C, Assmann G, Walter M. Differential secretion of atrial and brain natriuretic peptide in critically ill patients. Anesth Analg. 2001;93(3):676-682.
30. Alipoor E, Mohammad Hosseinzadeh F, Hosseinzadeh-Attar MJ. Adipokines in critical illness: A review of the evidence and knowledge gaps. Biomed Pharmacother. 2018;108:1739-1750.
31. Robinson K, Prins J, Venkatesh B. Clinical review: adiponectin biology and its role in inflammation and critical illness. Crit Care. 2011;15(2):221.
32. Gille J, Schmidt J, Kremer T, Sablotzki A. Evaluation of MR-proANP and copeptin for sepsis diagnosis after burn injury. J Crit Care. 2019;52:149-155.
33. Koch A, Weiskirchen R, Kunze J, Dückers H, Bruensing J, Buendgens L, Matthes M, Luedde T, Trautwein C, Tacke F. Elevated asymmetric dimethylarginine levels predict short- and long-term mortality risk in critically ill patients. J Crit Care. 2013;28(6):947-953.
34. Buendgens L, Yagmur E, Bruensing J, Herbers U, Baeck C, Trautwein C, Koch A, Tacke F. Growth Differentiation Factor-15 Is a Predictor of Mortality in Critically Ill Patients with Sepsis. Dis Markers. 2017;5271203.
35. Rhodes A, Evans LE, Alhazzani W, Levy MM, Antonelli M, Ferrer R, Kumar A, Sevransky JE, Sprung CL, Nunnally ME, et al. Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock: 2016. Intensive Care Med. 2017;43(3):304-377.
36. Koch A, Voigt S, Kruschinski C, Sanson E, Dückers H, Horn A, Yagmur E, Zimmermann H, Trautwein C, Tacke F. Circulating soluble urokinase plasminogen activator receptor is stably elevated during the first week of treatment in the intensive care unit and predicts mortality in critically ill patients. Crit Care. 2011;15(1):R63.
37. Koch A, Weiskirchen R, Zimmermann HW, Sanson E, Trautwein C, Tacke F. Relevance of serum leptin and leptin-receptor concentrations in critically ill patients. Mediators Inflamm. 2010. pii: 473540.
38. Koch A, Weiskirchen R, Sanson E, Zimmermann HW, Voigt S, Dückers H, Trautwein C, Tacke F. Circulating retinol binding protein 4 in critically ill patients before specific treatment: prognostic impact and correlation with organ function, metabolism and inflammation. Crit Care. 2010;14(5):R179.
39. Koch A, Sanson E, Voigt S, Helm A, Trautwein C, Tacke F. Serum adiponectin upon admission to the intensive care unit may predict mortality in critically ill patients. J Crit Care. 2011;26(2):166-174.
40. Koch A, Sanson E, Helm A, Voigt S, Trautwein C, Tacke F. Regulation and prognostic relevance of serum ghrelin concentrations in critical illness and sepsis. Crit Care. 2010;14(3):R94.
41. Koch A, Gressner OA, Sanson E, Tacke F, Trautwein C. Serum resistin levels in critically ill patients are associated with inflammation, organ dysfunction and metabolism and may predict survival of non-septic patients. Crit Care. 2009;13(3):R95.
42. Koch A, Voigt S, Sanson E, Dückers H, Horn A, Zimmermann HW, Trautwein C, Tacke F. Prognostic value of circulating amino-terminal pro-C-type natriuretic peptide in critically ill patients. Crit Care. 2011;15(1):R45.
43. Odermatt J, Hersberger L, Bolliger R, Graedel L, Christ-Crain M, Briel M, Bucher HC, Mueller B, Schuetz P. The natriuretic peptide MR-proANP predicts all-cause mortality and adverse outcome in community patients: a 10-year follow-up study. Clin Chem Lab Med. 2017;55(9):1407-1416.
44. Oliver PM, Fox JE, Kim R, Rockman HA, Kim HS, Reddick RL, Pandey KN, Milgram SL, Smithies O, Maeda N. Hypertension, cardiac hypertrophy, and sudden death in mice lacking natriuretic peptide receptor A. Proc Natl Acad Sci U S A. 1997;94(26):14730-14735.

45. Vellaichamy E, Kaur K, Pandey KN . Enhanced activation of pro-inflammatory cytokines in mice lacking natriuretic peptide receptor-A. Peptides. 2007;28(4):893-899.
46. Rubattu S, Sciarretta S, Ciavarella GM, Venturelli V, De Paolis P, Tocci G, De Biase L, Ferrucci A, Volpe M. Reduced levels of N-terminal-proatrial natriuretic peptide in hypertensive patients with metabolic syndrome and their relationship with left ventricular mass. J Hypertens. 2007;25(4):833-839.
47. Salomaa V, Havulinna A, Saarela O, Zeller T, Jousilahti P, Jula A, Muenzel T, Aromaa A, Evans A, Kuulasmaa K, et al. Thirty-one novel biomarkers as predictors for clinically incident diabetes. PLoS One. 2010;5(4):e10100.
48. Tsai S, Clemente-Casares X, Revelo XS, Winer S, Winer DA. Are obesity-related insulin resistance and type 2 diabetes autoimmune diseases? Diabetes. 2015;64(6):1886-1897.
49. de Bold AJ. Cardiac natriuretic peptides gene expression and secretion in inflammation. J Investig Med. 2009;57(1):29-32.
50. Dieplinger B, Mueller T, Kollerits B, Struck J, Ritz E, von Eckardstein A, Haltmayer M, Kronenberg F. Pro-A-type natriuretic peptide and pro-adrenomedullin predict progression of chronic kidney disease: the MMKD Study. Kidney Int. 2009;75(4):408-414.
51. Gegehuber A, Struck J, Poelz W, Pacher R, Morgenthaler NG, Bergmann A, Haltmayer M, Mueller T. Midregional pro-A-type natriuretic peptide measurements for diagnosis of acute destabilized heart failure in short-of-breath patients: Comparison with B-type natriuretic peptide (BNP) and amino-terminal proBNP. Clin Chem. 2006;52(5):827-831.
52. Hicks CW, Engineer RS, Benoit JL, Dasarathy S, Christenson RH, Peacock WF. Procalcitonin as a biomarker for early sepsis in the emergency department. Eur J Emerg Med. 2014;21(2):112-117.
53. Guinard-Barbier S, Grabar S, Chenevier-Gobeaux C, Quinquis L, Schmidt J, Kierzek G, Guérin S, Hausfater P, Bernot B, Brun P, et al. Is mid-regional pro-atrial natriuretic peptide (MRproANP) an accurate marker of bacteremia in pyelonephritis? Biomarkers. 2011;16(4):355-363.
54. Boeck L, Eggimann P, Smyrnios N, Pargger H, Thakkar N, Siegemund M, Marsch S, Rakic J, Tamm M, Stolz D. Midregional pro-atrial natriuretic peptide and procalcitonin improve survival prediction in VAP. Eur Respir J. 2011;37(3):595-603.
55. Vazquez M, Jockers K, Christ-Crain M, Zimmerli W, Müller B, Schuetz P. MR-pro-atrial natriuretic peptide (MR-proANP) predicts short- and long-term outcomes in respiratory tract infections: a prospective validation study. Int J Cardiol. 2012;156(1):16-23.
56. Guignant C, Venet F, Voirin N, Poitevin F, Malcus C, Bohè J, Lepape A, Monneret G. Proatrial natriuretic peptide is a better predictor of 28-day mortality in septic shock patients than proendothelin-1. Clin Chem Lab Med. 2010;48(12):1813-1820.
57. Theilade S, Hansen TW, Goetze JP, Rossing P. Increased plasma concentrations of midregional proatrial natriuretic Peptide is associated with risk of cardiorenal dysfunction in type 1 diabetes. Am J Hypertens. 2015;28(6):772-779.
58. Asferg CL, Nielsen SJ, Andersen UB, Linneberg A, Møller DV, Hedley PL, Christiansen M, Gøtze JP, Jeppesen JL. Metabolic rather than body composition measurements are associated with lower serum natriuretic peptide concentrations in normal weight and obese men. Am J Hypertens. 2014;27(4): 620-627.
59. Asferg CL, Nielsen SJ, Andersen UB, Linneberg A, Goetze JP, Jeppesen JL. Serum proatrial natriuretic peptide concentrations during oral glucose-induced acute hyperinsulinemia in lean and obese men. Peptides. 2019;111:98-102.
60. Asferg CL, Andersen UB, Linneberg A, Goetze JP, Jeppesen JL. Obese Hypertensive Men Have Lower Circulating Proatrial Natriuretic Peptide Concentrations Despite Greater Left Atrial Size. Am J Hypertens. 2019;31(6):645-650.

Chapter 11

Diagnostic and Prognostic Value of Clusterin Plasma Concentrations in Critically Ill Patients

Eray Yagmur
Samira Abu Jhaisha
Nadezhda Sapundzhieva
Jonathan F. Brozat
Philipp Hohlstein
Theresa Wirtz
Ger H. Koek
Ralf Weiskirchen
Christian Trautwein
Frank Tacke
Alexander Koch

Submitted

Abstract

Background
Clusterin is a multifunctional protein that is recognized to mediate cellular stress response associated with organ failure, systemic inflammation and metabolic alteration. The aim of the study was to determine the value of Clusterin as a clinical biomarker in critical illness and/or sepsis.

Methods
We analyzed Clusterin plasma concentrations in 200 critically ill patients (133 with sepsis, 67 without sepsis) on admission to the medical intensive care unit (ICU). The results were compared with 66 healthy controls.

Results
Clusterin plasma concentration was significantly elevated in critically ill patients compared to healthy subjects. Clusterin levels were found significantly higher in non-septic ICU patients.

Clusterin correlated inversely with markers of inflammatory response. Furthermore, Clusterin levels were higher in ICU patients with pre-existing obesity and type 2 diabetes. Clusterin was not associated with disease severity, organ failure or mortality in the ICU.

Conclusion
This study highlights significantly elevated Clusterin levels in critically ill patients, predominantly in non-sepsis, and associates circulating Clusterin to inflammatory and metabolic dysfunctions.

Introduction

The polyfunctional protein Clusterin is permanently expressed and located in a broad range of tissues and body secretions[1]. Its highly diversified functions include chaperone function, regulation of humoral immune, cellular function and energy metabolism. These diverse characteristics demonstrate the complex involvement of Clusterin in health and disease[2-5].

Thus, Clusterin has been described as sensitive to different types of stressors, such as pro-inflammatory, oxidative, or endoplasmic reticulum-associated cell dysfunction. Clusterin modulates the immunological complement system by regulating the formation of the membrane attack complex (MAC) from complement components C5b, C6, and C7. Furthermore, Clusterin modulates or reduces pro-inflammatory processes, including NF-κB-signaling and several cytokines, including transforming growth factor-β (TGF-β), tumor necrosis factor-α (TNF-α), and interleukin-2 (IL-2)[6-11].

Clusterin is considered both a secreted and intracellular protein[12-14]. The secreted Clusterin is a highly glycosylated 75-80 kDa heterodimer consisting of two disulfide-linked chains of 40 kDa each[15]. After cytoplasmatic release, Clusterin can be internalized by cells to participate in intracellular pathways when cell damage or stress requires a protective mechanism to prevent cell death[16,17]. In this context, Clusterin expression is elevated in injured arteries due to atherosclerosis[18].

Clusterin removes cholesterol from macrophage foam cells in atherosclerotic lesions[19]. Together with apolipoprotein E and apolipoprotein A-I, Clusterin forms HDL cholesterol and promotes its hepatic metabolization[1]. Therefore, it is obvious that Clusterin is also expressed in adipose tissue[20].

Thus, on the one hand, Clusterin is positively associated with metabolic parameters such as glycosylated hemoglobin A1c, insulin resistance by HOMA-IR, and fasting C-peptide levels[21]. But, on the other hand, bariatric surgery followed by weight loss also results in decreased Clusterin expression[22].

In consideration of its cellular, immunologic and metabolic effects, Clusterin has associations to various systemic inflammatory, metabolic and neurological disorders and their clinical outcome and phenotype, respectively[23-27].

Due to the frequent presence of both inflammatory and metabolic alterations in critically ill patients, It is reasonable to consider Clusterin as a beneficial diagnostic tool in terms of disease severity, immunologic modulation, metabolic changes, and prediction of mortality in an ICU setting. However, the associations between Clusterin

and these previously mentioned clinical alterations in ICU patients have not been thoroughly investigated so far.

For this reason, we have conducted a thoughtful and detailed investigation on the clinical relevance of Clusterin in a thoroughly documented cohort of ICU patients with and without sepsis.

Materials and methods

Selection and inclusion of patients

For this study, we included patients admitted to the intensive care unit of the University Hospital RWTH Aachen, Germany. Furthermore, these patients were classified into sepsis and non-sepsis following the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3)[28] and treatment was performed in accordance with the current guidelines for the treatment of sepsis (Surviving Sepsis Campaign)[29]. However, patients referred for follow-up after invasive procedures or after elective surgical intervention only were excluded[30].

To assess long-term patient survival, we contacted the patients, their relatives, and/or primary care physicians at 6-month intervals for 2 years after discharge from intensive care[31]. Blood donors were selected as a healthy control group with the following criteria: normal blood count, no biochemical evidence of hepatic or metabolic disease or viral disease, such as viral hepatitis or HIV[31].

The local ethics committee in accordance with the ethical standards of the Declaration of Helsinki (reference number EK 150/06) approved our study.

Laboratory analysis of circulating clusterin in patients' plasma

Measurement of Clusterin concentration was conducted from blood samples obtained prior to therapeutic treatment of patients. According to the manufacturer's instructions, each blood sample was centrifuged immediately after collection and the plasma was stored at -80°C until analysis with a quantitative sandwich enzyme-linked immunosorbent assay (ELISA) (Clusterin (human) Competitive ELISA, #AG-45A-0013EK-KI01, Adipogen AG, Liestal, Switzerland).

Statistical analysis

An explorative descriptive analysis based on a non-parametric distribution was performed for the statistical evaluation of the parameters. All quantitative parameters

were therefore presented as medians including range and showed graphically as box-and-whiskers plots. The association between different variables and Clusterin was examined using the Spearman rank correlation test.

The significance of parameters between two different groups were assessed with the Mann-Whitney U test. Statistically significant was assumed to be any comparison with a p-value less than 0.05. Statistical analyses were carried out using IBM SPSS Statistics (SPSS; Chicago, Illinois).

Results

Clusterin concentrations are increased in critically ill patients but significantly lower in ICU patients with sepsis

In a large a cohort of 200 critically ill patients (median 52.9 µg/ml, range 4.8-144.1 µg/ml; Table 11.1), we showed that Clusterin plasma concentrations were significantly increased upon ICU admission compared with 66 healthy individuals (median 30.1 µg/ml, range 0.1-61.4 ng, p<0.001; Figure 11.1A).

Table 11.1 Basic parameters and characteristics of ICU patients.

Parameter	All patients	Non-sepsis	Sepsis	p-*value**
Number	200	67	133	
Sex (male/female)	122 / 78	44 / 23	78 / 55	0.360
Age median (range) [years]	64 (18-90)	62 (18-85)	65 (20-90)	0.612
APACHE-II score median (range)	18 (2-43)	14 (2-33)	19 (4-43)	0.002
SOFA score median (range)	9 (0-17)	8 (0-17)	9 (2-17)	0.055
SAPS-2 score median (range)	41 (0-73)	41 (13-72)	41.5 (0-73)	0.280
ICU days median (range)	7 (1-137)	6 (1-45)	9 (1-137)	0.013
Death during ICU n(%)	43 (21.5%)	9 (13.4%)	34 (25.6%)	0.067
Death during follow-up (total) n(%)	78 (41.3%)	21 (33.3%)	57 (45.2%)	0.158
Mechanical ventilation n(%)	134 (67.7%)	46 (68.7%)	91 (44.8%)	0.915
Ventilation time median (range) [h]	116 (0-3628)	66 (0-3628)	123.5 (0-2966)	0.350
Vasopressor therapy n(%)	123 (61.5%)	33 (16.3%)	92 (45.3%)	0.011
Pre-existing diabetes n(%)	62 (31.6%)	22 (33.8%)	43 (32.1%)	0.061
BMI median (range) [m^2/kg]	25.8 (15.3-86.5)	25.7 (15.9-40.5)	25.8 (15.3-86.5)	0.621
WBC median (range) [x10^3/µl]	12.7 (0-208)	12.1 (1.8-29.6)	13.6 (0-208)	0.041
CRP median (range) [mg/dl]	96 (0-230)	17 (5-230)	154 (0-230)	0.001
Procalcitonin median (range) [µg/l]	0.6 (0-207.5)	0.2 (0.03-17.4)	1.8 (0-207.5)	0.001
Cystatin C median (range) [mg/dL]	1.6 (0-7.3)	1.17 (0.41-7.3)	2.06 (0-6.33)	0.001
GFR Cystatin C median (range) [ml/min]	35 (0-379)	63 (5-379)	21.5 (0-218)	0.001
INR median (range)	1.16 (0-133)	1.16 (0.95-6.73)	1.16 (0-133)	0.989
Clusterin day 1 median (range) [µg/mL]	52.9 (4.8-144.1)	60.0 (17.7-112.9)	48.9 (4.8-144.1)	0.026

ALT, alanine aminotransferase; APACHE, Acute Physiology And Chronic Health Evaluation; AST, aspartate aminotransferase; BMI, body mass index; CRP, C-reactive protein; ICU, intensive care unit; INR, international normalized ration; WBC, white blood cell. For quantitative variables, median and range (in parenthesis) are given. *p-values for the comparison of sepsis and non-sepsis patients are given (*U*-test for quantitative variables or chi-square test for qualitative parameters).

Nevertheless, we did not observe this increase in Clusterin concentration in all ICU patients. In particular, plasma Clusterin concentrations were significantly lower in patients with sepsis (n=133, median 48.9 µg/ml, range 4.8-144.1 µg/ml) compared with patients without sepsis (n=67, median 60.0 µg/ml, range 17.7-112.9 µg/ml; Figure 11.1B). These observations are consistent with findings from previous reported studies that decreased Clusterin levels are particularly found in patients with a systemic inflammatory response such as sepsis or septic shock[32,33].

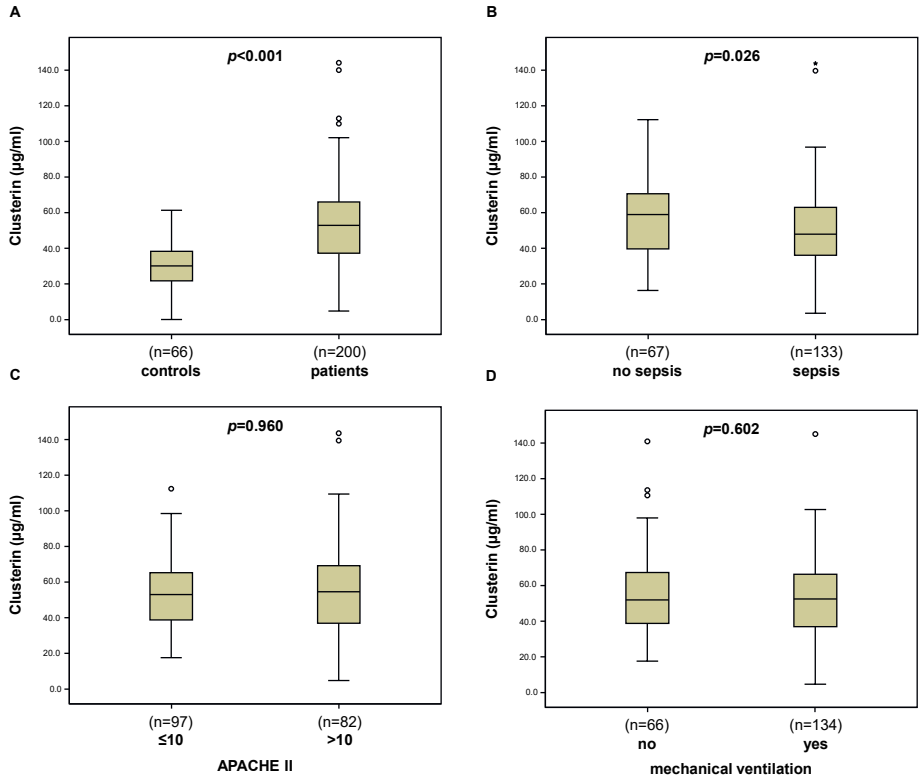


Figure 11.1 Clusterin levels in critically ill patients. (**A**) Clusterin plasma concentrations are significantly elevated in critically ill patients compared with healthy controls. (**B**) Clusterin levels are significantly lower in ICU patients with sepsis compared to ICU patients without sepsis. (**C**) High disease severity, as defined by an APACHE-II score above 10, is not associated with altered plasma Clusterin. (**D**) The need of mechanical ventilation was not associated with Clusterin levels at ICU admission. *p*-values (U-test) are given in the figure.

Clusterin is not associated with different disease etiologies of the study population, disease severity, vasopressor therapy or mechanical ventilation

In our study cohort, typical sepsis-related infection sites were pneumonia, abdomen, and genitourinary tract, while non-sepsis-related causes of critical illness included cardiopulmonary disease, acute pancreatitis, and decompensated liver cirrhosis.(Table 11.2). However, among critically ill patients, there was no consistent correlation between Clusterin plasma concentrations and the various disease etiologies resulting ICU admission (all p>0.05; data not shown).

Table 11.2 Etiology of disease in the study population.

	Sepsis n=133	**Non-sepsis n=67**
Etiology of sepsis critical illness		
Site of infection n (%)		
Pulmonary	68 (51.1%)	
Abdominal	26 (19.1%)	
Urogenital	8 (5.9%)	
Other	31 (22.8%)	
Etiology of non-sepsis critical illness n (%)		
Cardio-pulmonary disorder		28 (41.8%)
Acute pancreatitis		9 (13.4%)
Acute liver failure		3 (4.5%)
Decompensated liver cirrhosis		8 (11.9%)
Severe gastrointestinal hemorrhage		4 (6.0%)
Non-sepsis other		15 (22.4%)

Although Clusterin has associations to various systemic inflammatory, metabolic and neurological disorders (24-27), Clusterin plasma levels are not associated with established clinical disease severity scores such as in critically ill patients with a high Acute Physiology And Chronic Health Evaluation-II (APACHE-II) score above 10 (p=0.960) (Figure 11.1C).

Additionally, vasopressor therapy did not significantly alter plasma Clusterin concentrations in ICU patients (data not shown). Furthermore, there was also no significant difference in plasma Clusterin levels between ventilated or non-ventilated critically ill patients (p=0.602) (Figure 11.1D).

Relationship of Clusterin plasma concentrations at ICU admission with metabolic alterations and inflammatory response

Clusterin has been linked to metabolic[23] and inflammatory diseases[6,8-11] as well as hepatic function[19]. Accordingly, we investigated the impact of metabolic alterations, including pre-existing obesity or diabetes, on Clusterin concentrations in ICU patients.

Interestingly, patients with pre-existing type 2 diabetes had numerically higher Clusterin concentrations (median 59.5 µg/ml in diabetics vs. median 50.8 µg/ml in non-diabetics, p=0.064; Figure 11.2A).

This result is consistent with the positive association between Clusterin and blood glucose (Table 11.3, Figure 11.3A). Patients with obesity characterized by body mass index above 30 kg/m², tended to have higher Clusterin levels (median 58.9 µg/ml vs. median 51.1 µg/ml in non-obese patients, p=0.066) (Figure 11.2B).

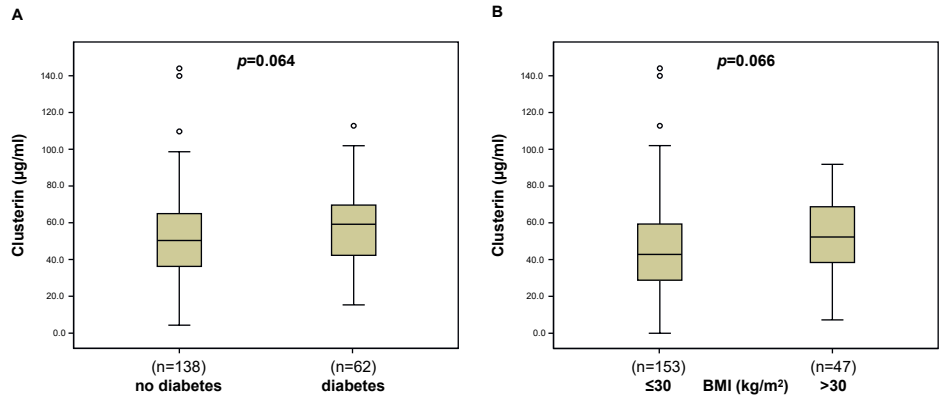


Figure 11.2 Impact of metabolic comorbidities on Clusterin levels. Clusterin plasma concentrations did not differ between ICU patients with or without pre-existing type 2 diabetes (**A**) and obesity, as defined by a body-mass index (BMI) above 30 kg/m² (**B**). *p*-values (U-test) are given in the figure.

Regarding a possible association between Clusterin and inflammation, we showed that Clusterin was inversely associated with interleukin-6 (Figure 11.3B) and procalcitonin (Figure 11.3C). These findings are consistent with the lower Clusterin concentrations in ICU patients with vs. without sepsis (Table 11.3).

In these patients, Receiver Operating Curve (ROC) analysis showed lower diagnostic sensitivity and specificity of Clusterin for the detection of sepsis compared with interleukin-6 and procalcitonin (data not shown).

Interestingly, elevated Clusterin concentrations in the ICU cohort were associated with more prominent hypocoagulability based on global coagulation tests such as prothrombin time, international normalized ratio (INR), partial thromboplastin time (PTT) and fibrinogen, respectively (Table 11.3).

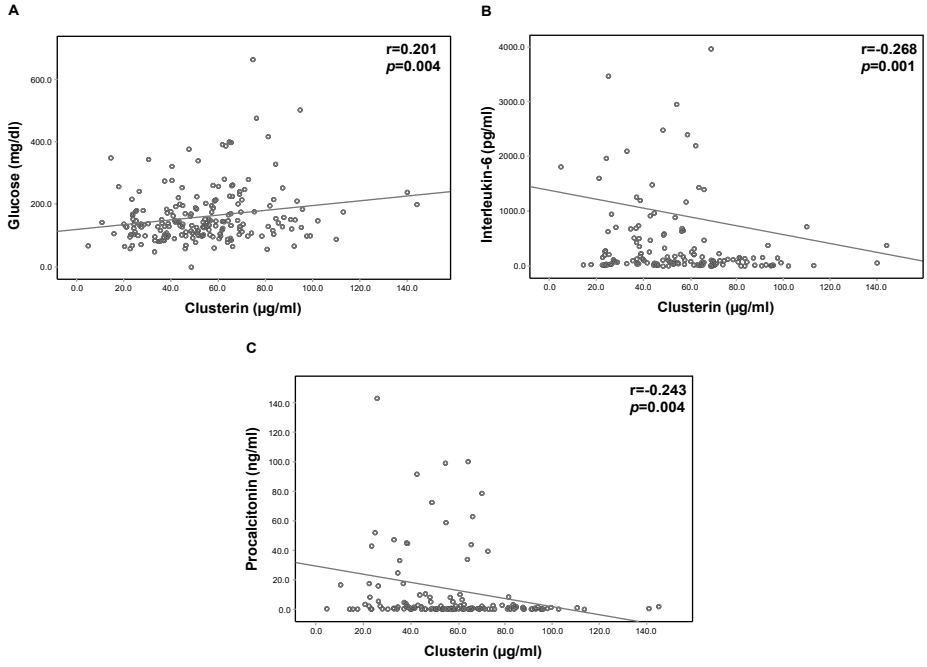


Figure 11.3 Clusterin levels correlate with glucose, as well as inflammatory parameters. Correlation analyses revealed associations between plasma Clusterin and plasma glucose (A), interleukin-6 (B) and procalcitonin (C), respectively. *p*-values (U-test) are given in the figure.

Clusterin plasma concentrations at ICU admission have no association with ICU patient mortality

In ICU patients, there was a tendency for decreased Clusterin levels in patients who died compared with those who have survived (median 48.5 µg/ml vs. median 54.3 µg/ml in ICU survivors) (Figure 11.4A), while there was no significant difference for overall mortality either (Figure 11.4B). Even so, in critically ill patients who died subsequently during ICU treatment (n=43), we did not find significantly altered Clusterin levels. These findings suggest that Clusterin is not a biomarker of prognosis in critical illness.

Table 11.3 Correlations of laboratory parameters with Clusterin plasma concentrations (Spearman rank correlation test, only significant results are shown).

	ICU patients	
Parameters	**r**	***p***
Markers of inflammatory response		
IL-6	-0.268	0.001
PCT	-0.243	0.004
Markers of coagulation		
Prothrombin time	0.183	0.011
INR	-0.163	0.023
PTT	-0.243	0.001
Fibrinogen	-0.241	0.007
AT III	0.242	0.007
Markers of organ function		
Bilirubin conjugated	-0.315	0.001
PCHE	0.19	0.011
Albumin	0.296	0.002
Protein	0.218	0.005
Lipase	-0.203	0.011
Marker of diabetes		
Glucose	0.201	0.004
Markers of lipid metabolism		
Triglycerides	0.237	0.003
Total cholesterol	0.288	<0.001
LDL cholesterol	0.384	0.002
HDL cholesterol	0.292	0.02
Adipocytokines / metabolic markers		
RBP4	0.336	0.007
Myostatin	0.261	0.037
Sclerostin	0.287	<0.001

AT III, antithrombin III; HDL cholesterol, high density lipoprotein cholesterol; IL-6 interleukin-6; INR international normalized ratio; LDL cholesterol, high density lipoprotein cholesterol; PCHE, pseudocholinesterase; PCT, procalcitonin; RBP4, retinol binding protein 4.

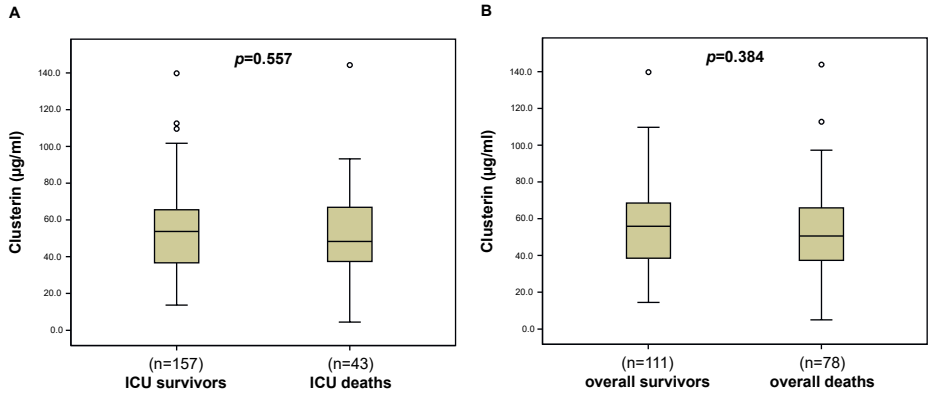


Figure 11.4 Clusterin is not a prognostic biomarker for mortality in critically ill patients**.** Patients that died during the course of ICU treatment (**A**) and/or during follow-up (**B**) are characterized by tendency towards lower plasma Clusterin concentrations already at ICU admission. *p*-values are given in the figure. ICU, intensive care unit.

Discussion

Multiple pathways are dysregulated in critical illness, as homeostatic pathways switch towards stress responses and inflammation upon life-threatening conditions. Based on the central role of Clusterin regarding to regulation of humoral immune, cellular function and energy metabolism[2-5], we hypothesized that Clusterin reveals a beneficial diagnostic performance in terms of disease severity, immunologic modulation, metabolic changes, and prediction of mortality in an ICU setting.

From a metabolic function view, Clusterin is found in high-density and low-density lipoproteins HDL and LDL, indicating an association between Clusterin and insulin effect. Clusterin in HDL is related to insulin sensitivity, whereas Clusterin in LDL is closely correlated with insulin resistance[34].

This finding is consistent with the lack of association of Clusterin in HDL with insulin resistance described by Hoofnagle and colleagues[34]. Correspondingly, previous clinical data implicated that circulating Clusterin correlates closely with insulin resistance. Furthermore, Clusterin decreases in line with improving insulin sensitivity in type 2 diabetes[35]. Notably, evidence from experimental Clusterin knockout animal models suggests liver is the predominant site of Clusterin plasma concentration[36]. The liver is interconnected with several metabolic tissues, such as adipose tissue. Therefore, Clusterin is involved in cellular metabolic activities[36].

In this study, we demonstrated a trend to higher Clusterin concentrations in diabetic ICU patients and a strong association between serum glucose and Clusterin. Based these findings, we hypothesize that Clusterin is released by the liver to improve insulin-dependent glucose uptake by muscles due to impaired metabolic function in critically ill patients[36]. Clusterin of hepatic origin is transported to skeletal muscle, where it binds to its cell surface receptor LRP2 (Low Density Lipoprotein Receptor-related Protein 2; Megalin) to increase insulin action and to regulate glucose hemostasis, insulin signaling, and energy metabolism[37-39].

Although Hoofnagle and colleagues (34) found a negative relationship between the Clusterin concentration in HDL and body mass index, we found a trend towards elevated Clusterin levels in obese critically ill ICU patients as well as a strong correlation between Clusterin levels and triglycerides[34]. As triglycerides are key components of the metabolic syndrome, elevated Clusterin may indicate improved lipid transport and metabolism to preserve a stable metabolic hemostasis and/or favorable cardioprotection[34].

Among obese patients, however, plasma levels of Clusterin were found to be increased as well as directly related to obesity-related metabolic disease complications, among which were insulin resistance, dyslipidemia, hepatic steatosis[40]. These results propose that peripheral-derived Clusterin may play a novel role in the pathophysiology of critically ill patients, particularly in the context of coexisting metabolic impairment. In obesity characterized by body mass index above 30 kg/m², Clusterin concentrations are increased and associated inflammatory markers, and insulin resistance[41].

Notably, Garcia-Obregon and colleagues found lowered Clusterin concentrations in patients with sepsis[32]. Furthermore, DeCoux et al. reported that decreased Clusterin is associated with mortality in critically ill septic patients[33]. In line with these previous findings, ICU patients with sepsis showed significantly lower Clusterin levels than non-sepsis patients.

Nevertheless, for the first time, we were able to demonstrate that ICU patients in general showed a significant increase of Clusterin. This finding corresponds with the inverse correlation of Clusterin with inflammatory markers such as interleukin-6, procalcitonin, or retinol binding protein-4. Elevated Clusterin and the inverse association with pro-inflammatory biomarkers may be the result of a Clusterin-derived modulatory process to reduce hyperinflammation as a defensive mechanism to prevent cytokine storm and cell death[7-11,16,17].

Conclusion

In conclusion, our study with a thoroughly documented cohort of ICU patients demonstrates elevated Clusterin plasma concentrations as compared to healthy subjects. Moreover, we confirm decreased Clusterin plasma concentrations in ICU patients with sepsis versus non-septic critically ill patients, confirming the extracellular characteristics of secreted Clusterin as a molecular response to cell dysfunction requiring a defensive measure to block cell death.

Clusterin is not an indicator of disease severity, organ failure or mortality, but associated with metabolic and inflammatory alterations. This obvious discordance between the key pathogenic function of Clusterin in cellular stress and its weak capacity as a diagnostic clinical biomarker for predicting outcome in critical illness appears to be related to its complex and distinct molecular functions such as secreted cytoprotective and intracellular apoptotic biological role.

Prospective investigations with particular reference to the simultaneous measurement and assessment of the ratio of secreted and intracellular Clusterin in critically ill patients are recommended.

References

1. Rizzi F, Coletta M, Bettuzzi S. Clusterin (CLU): from one gene and two transcripts to many proteins. Adv Cancer Res. 2009;104:9-23.
2. Jenne DE, Tschopp J. Molecular structure and functional characterization of a human complement cytolysis inhibitor found in blood and seminal plasma: identity to sulfated glycoprotein 2, a constituent of rat testis fluid. Proc Natl Acad Sci USA. 1989;86:7123-7127.
3. Humphreys DT, Carver JA, Easterbrook-Smith SB, Wilson MR. Clusterin has chaperone-like activity similar to that of small heat shock proteins. J Biol Chem. 1999:274;6875-6881.
4. Wang H, Eckel RH. What are lipoproteins doing in the brain. Trends Endocrinol Metab. 2014;25:8-14.
5. Kim N, Yoo JC, Han JY, et al. Human nuclear clusterin mediates apoptosis by interacting with Bcl-XL through C-terminal coiled coil domain. J Cell Physiol. 2012;227:1157-1167.
6. Murphy BF, Saunders JR, Bryan MKO, Kirszbaum L, Walker ID, Apice AJF. SP-40,40 is an inhibitor of C5b-6-initiated haemolysis. Int Immunol. 1989;1:551-554.
7. Liu G, Zhang H, Hao F, et al. Clusterin reduces cold ischemia-reperfusion injury in heart transplantation through regulation of NF-kB signaling and Bax/Bcl-xL expression. Cell Physiol Biochem. 2018;45:1003-1012.
8. Santilli G, Aronow BJ, Sala A. Essential requirement of apolipoprotein J (clusterin) signaling for IκB expression and regulation of NF-κB activity. J Biol Chem. 2003;278:38214-38219.
9. Morgan TE, Laping NJ, Rozovsky I, et al. Clusterin expression by astrocytes is influenced by transforming growth factor β1 and heterotypic cell interactions. J Neuroimmunol. 1995;58:101-110.
10. Shim YJ, Kang BH, Choi BK, Park IS, Min BH. Clusterin induces the secretion of TNF-α and the chemotactic migration of macrophages. Biochem Biophys Res Commun. 2012;422:200-205.
11. Sonn CH, Yu YB, Hong YJ, et al. Clusterin synergizes with IL-2 for the expansion and IFN-γ production of natural killer cells. J Leukoc Biol. 2010;88:955-963.
12. Kirszbaum L, Bozas SE, Walker ID. SP-40,40, a protein involved in the control of the complement pathway, possesses a unique array of disulphide bridges. FEBS Lett. 1992;297:70-76.
13. Yang CR, Leskov K, Hosley-Eberlein K, et al. Nuclear clusterin/XIP8, an x-ray-induced Ku70-binding protein that signals cell death. Proc. Natl. Acad. Sci. U.S.A. 2000;97:5907-5912.
14. Rohne P, Prochnow H, Koch-Brandt C. The CLU-files: disentanglement of a mystery. Biomol Concepts. 2016;7:1-15.
15. Choi-Miura NH, Oda T. Relationship between multifunctional protein “clusterin” and Alzheimer disease. Neurobiol Aging. 1996;17:717-722.
16. Kang SW, Shin YJ, Shim YJ, Jeong SY, Park IS, Min BH. Clusterin interacts with SCLIP (SCG10-like protein) and promotes neurite outgrowth of PC12 cells. Exp Cell Res. 2005;309:305-315.
17. Morgan TE, Laping NJ, Rozovsky I, et al. Clusterin expression by astrocytes is influenced by transforming growth factor β1 and heterotypic cell interactions. J. Neuroimmunol. 1995;58:101- 110.
18. Ishikawa Y, Akasaka Y, Ishii T, et al. Distribution and synthesis of apolipoprotein J in the atherosclerotic aorta. Arterioscler Thromb Vasc Biol.1998;18:665-672.
19. Gelissen IC, Hochgrebe T, Wilson MR, et al. Apolipoprotein J (clusterin) induces cholesterol export from macrophage-foam cells: a potential anti-atherogenic function? Biochem J. 1998;331:231-237.
20. Bradley D, Blaszczak A, Yin Z, et al. Clusterin impairs hepatic insulin sensitivity and adipocyte clusterin associates with cardiometabolic risk. Diabetes Care 2019;42:466-475.
21. Ha J, Moon MK, Kim H, et al. Plasma clusterin as a potential link between diabetes and Alzheimer’s disease. J Clin Endocrinol Metab. 2020;105:dgaa378.
22. Ghanim H, Monte SV, Sia CL, et al. Reduction in inflammation and the expression of amyloid precursor protein and other proteins related to Alzheimer’s disease following gastric bypass surgery. J Clin Endocrinol Metab. 2012;97:E1197-E1201.
23. Bradley D. Clusterin as a potential biomarker of obesity-related Alzheimer`s disease risk. Biomarkers Insight. 2020;15:1-7.
24. Miwa Y, Takiuchi S, Kamide K, et al. Insertion/deletion polymorphism in clusterin gene influences serum lipid levels and carotid intima-media thickness in hypertensive Japanese females. Biochem Biophys Res Commun. 2005;331:1587-1593.

25. Gil SY, Youn BS, Byun K, et al. Clusterin and LRP2 are critical components of the hypothalamic feeding regulatory pathway. Nat Commun. 2013;4:1862.
26. Kujiraoka T, Hattori, H, Miwa Y, et al. Serum apolipoprotein j in health, coronary heart disease and type 2 diabetes mellitus. J Atheroscler Thromb. 2006;13:314-322.
27. Thambisetty M, Simmons A, Velayudhan L, et al. Association of plasma clusterin concentration with severity, pathology, and progression in Alzheimer disease. Arch Gen Psychiatry. 2010;67:739-748.
28. Buendgens L, Yagmur E, Bruensing J, et al. Growth Differentiation Factor-15 Is a Predictor of Mortality in Critically Ill Patients with Sepsis. Dis Markers 2017;5271203.
29. Rhodes A, Evans LE, Alhazzani W, et al. Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock: 2016. Intensive Care Med. 2017;43:304-377.
30. Koch A, Weiskirchen R, Kunze J, et al. Elevated asymmetric dimethylarginine levels predict short- and long-term mortality risk in critically ill patients. J Crit Care. 2013;28:947-953.
31. Koch A, Voigt S, Kruschinski C, et al. Circulating soluble urokinase plasminogen activator receptor is stably elevated during the first week of treatment in the intensive care unit and predicts mortality in critically ill patients. Crit Care. 2011;15:R63.
32. Garcia-Obregon S, Azkargorta M, Seijas I, et al. Identification of a panel of serum protein markers in early stage of sepsis and its validation in a cohort of patients. J Microbiol Immunol Infect. 2018;51: 465-472.
33. DeCoux A, Tian Y, DeLeon-Pennell K, et al. Plasma Glycoproteomics Reveals Sepsis Outcomes Linked to Distinct Proteins in Common Pathways. Crit Care Med. 2015;43:2049-2058.
34. Hoofnagle AN, Wu M, Gosmanova AK, et al. Low clusterin levels in high-density lipoprotein associate with insulin resistance, obesity, and dyslipoproteinemia. Arterioscler Thromb Vasc Biol. 2010;30: 2528-2534.
35. Seo JA, Kang MC, Ciaraldi TP, et al. Circulating ApoJ is closely associated with insulin resistance in human subjects. Metabolism. 2018;78:155-166.
36. Seo JA, Kang MC, Yang WM, et al. Apolipoprotein J is a hepatokine regulating muscle glucose metabolism and insulin sensitivity. Nat Commun. 2020;11:2024.
37. Stefan N, Haring HU. The role of hepatokines in metabolism. Nat. Rev. Endocrinol. 2013;9:144-152.
38. Elmquist JK, Coppari R, Balthasar N, Ichinose M, Lowell BB. Identifying hypothalamic pathways controlling food intake, body weight, and glucose homeostasis. J Comp Neurol. 2005;493:63-71.
39. Coppari R, Ichinose M, Lee CE, et al. The hypothalamic arcuate nucleus: a key site for mediating leptin's effects on glucose homeostasis and locomotor activity. Cell Metab. 2005;1:63-72.
40. Bradley D, Blaszczak A, Yin Z, et al. Clusterin impairs hepatic insulin sensitivity and adipocyte clusterin associates with cardiometabolic risk. Diabetes Care. 2019;42:466-475.
41. Won JC, Park CY, Oh SW, Lee ES, Youn BS, Kim MS. Plasma clusterin (ApoJ) levels are associated with adiposity and systemic inflammation. PLoS One. 2014;9:e103351.

Part IV

General Discussion and Summary

Chapter 12

General Discussion

General discussion

The term “biomarker” comprises simple physiologic measurements such as pulse and blood pressure up to multifaceted molecular patterns. In all fields of medicine, the research on biomarkers have been increased during the last 40 years. In this context, research into novel biomarkers in critically ill patients occupies a special position, since critical illnesses are very heterogeneous, usually affect multiple organ systems, and rarely correspond to clearly defined disease patterns. Hereby the development of novel biomarkers in the field of critical care has been delayed as for instance in oncology, where biomarkers play a major clinical role in diagnostication, prognostication and assessment of therapeutic response.

Generally, biomarkers can be categorized according to their clinical applications as markers of risk, diagnosis, monitoring, prognosis, prediction, therapeutic response and safety as proposed by the US Food and Drug Administration (FDA) and the National Institutes of Health (NIH)[134].

In this PhD thesis, novel biomarkers are investigated in a large, prospectively collected cohort of critically ill patients with focus on diagnostic and prognostic applicability from a clinical perspective. This thesis comprises three parts: a) Biomarkers of Systemic inflammation in Critical Disease, b) Role of Specific Adipokines in Critical Ill Patients, c) Biomarkers reflecting Biological Stress in Critical Illness.

PART 1: Biomarkers of systemic inflammation in critical disease

Major findings

In **part 1** we studied novel **Biomarkers of Systemic inflammation in Critical Disease** in a large cohort of medical intensive care patients.

We found that serum **calprotectin** concentrations are significantly increased in critically ill patients with sepsis and associated with renal and respiratory dysfunction. High calprotectin concentrations at ICU admission predict long-term mortality risk. This was the first study to demonstrate a prognostic value of rising calprotectin concentrations during ICU treatment.

We further demonstrated that circulating levels of the **apoptosis-related keratin fragment M30** are significantly elevated in critically ill patients as compared with healthy controls, independent of the presence of sepsis. M30 levels are correlated with

clinical scoring systems for disease severity as well as biomarkers indicating organ dysfunction and inflammation.

The remarkably high levels in patients with cirrhosis and the association with liver function tests indicate that hepatocyte apoptosis might contribute substantially to high circulating M30 in critically ill patients. M30 levels above 250.8 U/L at admission to the ICU indicate an unfavourable short-term prognosis.

We demonstrated significantly elevated **HMGB1** plasma concentrations in critically ill patients, corroborating the extracellular properties of HMGB1 as an alarmin signal. However, our study did not reveal a significant association between HMGB1 levels at ICU admission and clinical outcomes in critically ill patients.

This overt discrepancy of HMGB1's important pathogenic role in experimental sepsis and its poor performance as a clinical biomarker for prediction of outcome in critical illness might be related to its function during late stages of critical disease, which cannot be accurately captured at admission to the ICU.

Discussion

Calprotectin

The patients who are admitted to our ICU display a broad spectrum of specific diseases and comorbidities. Interestingly, we observed significantly decreased calprotectin levels in patients with coronary artery disease (CAD), although we expected in increased serum concentration, as CAD is related to chronic inflammation and patients with acute myocardial infarction have been reported with high calprotectin levels[135]. We explained this by a potential selection bias, as CAD patients are underrepresented in our cohort of ICU patients.

In line, patients with liver cirrhosis displayed significantly decreased calprotectin serum concentrations as compared to ICU patients with other disease etiologies, which might be also explainable by selection bias, as only a small number of patients in our cohort had previous diagnosis of end stage liver disease.

Additionally, we found significantly lower calprotectin serum concentrations in patients with chronic obstructive pulmonary disease (COPD) as compared to non-COPD patients. Nevertheless, our data might also hint at other triggers of calprotectin release in critical disease as the specific underlying disease, which led to ICU admission.

Calprotectin expression is closely linked to systemic inflammation as calprotectin derives from neutrophils and macrophages. According to this, we observed a close correlation of calprotectin with CRP, but not with leucocyte count or PCT.

In line with previous studies, we describe calprotectin as an early biomarker of bacterial infections in critically ill patients[136]. Furthermore, we performed a multitude of correlation analyses with biomarkers reflecting organ dysfunction. In patients with increased calprotectin serum concentrations a positive correlation with higher creatinine levels and intensity of RRT was observed.

This completes data from previous clinical studies in critically ill surgical patients in which calprotectin was linked to acute kidney injury[137]. The aspect that calprotectin might serve as potential biomarker of sepsis-related organ failure is further supported by our observation that calprotectin is strongly correlated with parameters of mechanical ventilation such as fraction of inspired oxygen (FiO_2), maximum or peak airway pressure (P_{max}) and positive end-expiratory pressure (PEEP), reflecting the extent of respiratory failure.

Most recently, calprotectin has been suggested as a diagnostic and prognostic biomarker in COVID-19 infection, where high calprotectin serum concentrations have been demonstrated to discriminate severe from non-severe clinical courses of COVID-19 infections[25].

Given the likely potential of calprotectin as a prognostic biomarker in critical disease, we investigated for the first time the prognostic value of calprotectin serum concentrations in long-term and overall survival of critically ill patients with and without sepsis. High calprotectin concentrations (>2.001 µg/mL) obtained at ICU admission have been demonstrated as a powerful predictor of overall survival in septic patients. However, inclusion of calprotectin at day 1 as a continuous parameter to avoid effect overestimation, Cox regression analysis only revealed a trend towards an impaired overall survival.

Interestingly, both, septic and non-septic ICU patients with increasing calprotectin levels between day 1 and day 7 displayed improved overall survival. Potentially, calprotectin initially mirrors severe systemic inflammation and in latter stages of critical disease regulates inflammatory responses, thereby preventing hyperinflammation[138].

M30

In apoptosis, activated caspases 3,7, and 9 mediate the cleavage of cytokeratin 18, which results in a neoepitope formation that can be detected by the M30 antibody.

M30 reflects the extent of apoptosis and has been suggested as a biomarker in the setting of acute and chronic liver diseases[31,32].

We found significantly increased serum levels of M30 in a heterogeneous cohort of critically ill patients, which were closely associated with disease severity, as expressed by highly significant correlations with clinical scores such as APACHE II, SAPS2 and SOFA.

This finding indicates that apoptosis is a common feature of critical illness and directly influences prognosis. M30 levels were closely correlated to inflammatory biomarkers such as PCT, IL-6 and TNF-α, but was not associated with the presence of sepsis. These findings contrast previous studies from ICU patients, which have linked M30 to the presence of sepsis.

Our study might indicate, that M30 as a biomarker of apoptosis rather reflects disease severity than systemic inflammation itself in critically ill patients. In line, independent from infectious or non-infectious cause of critical illness, M30 serum concentration correlated with biomarkers of organ dysfunction, e.g. liver and kidney. We found highest levels of M30 in patients with severe acute and chronic hepatic dysfunction. These data suggest that liver and kidney are major cellular sources of M30.

Mouse models demonstrated that a substantial quantity of apoptosis in critical illness also proceeds in the gut[128]. Translocation of bacteria, alteration of intestinal immunity and changes in the gut microbiota have been identified as novel targets to improve management of critical ill patients[129].

Our data highlight, that the prognosis of critically ill patients is not only determined by hypoxia- or hypoperfusion triggered necrosis, but also by activation of apoptotic pathways.

We identified M30 as an early predictor of mortality in ICU patients, independent of the presence of sepsis. M30 levels >250.8 U/L at admission to the ICU indicated an unfavourable short-term prognosis.

HMGB1

HMGB1 is regarded as a blueprint of danger-associated molecular patterns (DAMP). DAMPs are released by stressed, hypoxic, injured or dying cells in order to activate the immune system, promote inflammation and to initiate tissue repair. Severe systemic inflammation, infection, tissue hypoperfusion and cell death are key characteristics of critical illness in which DAMPs are highly pathogenetically involved[139]. Data from animal models suggested that HMGB1 is involved in the pathogenesis of sepsis, although it is

not upregulated in the early phase of sepsis, but is a characteristic finding in the late phase with association to lethality[140,141]. Neutralization of HMGB1 with specific antibodies even prevented rodents from sepsis-related death[142].

Given its pathogenetic role and the promising animal data, HMGB1 has been suggested as a potential clinical biomarker in critically ill patients[41,42]. However, the association between HMGB1 and clinical outcome is controversial.

Therefore, we investigated HMGB1 in a large heterogenous cohort of medical ICU patients with and without sepsis. We demonstrated elevated HMGB1 serum concentrations at admission to the ICU as compared with healthy controls in line with previous studies. Surprisingly, HMGB1 levels did either not differ between patients with or without sepsis, nor we could demonstrate any correlation with biomarkers of organ dysfunction and inflammation, clinical scores of disease severity or clinical outcome.

This is line with previous data from a prospective Danish study in patients with suspected community-acquired infections and sepsis, in which no difference in HMGB1 levels in infected and non-infected could be observed[43]. On the other hand, an observational study from Finland reported elevated HMGB1 concentrations in septic patients which did not predict hospital mortality[44]. Another study from Sweden demonstrated sustained high HMGB1 levels in critical ill patients with and without sepsis for one week after ICU admission[41]. The supports the assumption, that HMGB1 is a downstream and late mediator of systemic inflammation in critically ill patients.

Our study clearly demonstrated, that HMGB1 serum concentrations, obtained upon admission to the ICU are of limited value as a biomarker for critically ill patients. Nevertheless, the lack of clinical benefit of HMGB1 in the early phase of critical disease, does not preclude its potential in latter phases of the clinical course. Therefore, future studies should focus on longitudinal measurements of HMGB1 levels in the ICU setting.

PART 2: Role of specific adipokines in critical ill patients

Major findings

In **part 2** we elucidated the **Role of Specific Adipokines in Critical Ill Patients.** We demonstrated in our study comprising 229 critically ill medical patients that circulating levels of the adipokine **visfatin** were significantly elevated at admission to the ICU, as compared with healthy controls.

Visfatin serum concentrations were strongly associated with disease severity, organ failure and sepsis, but not with obesity or type 2 diabetes. High visfatin levels at ICU admission indicated an increased mortality, both at the ICU and during long-term follow-up.

In our large, prospectively enrolled study, **CTRP1** levels were significantly elevated in critically ill patients and were associated with inflammation and sepsis as well as diabetes and metabolic disturbances.

Our comprehensive analysis of **CTRP3** plasma concentrations in a large, prospectively enrolled cohort of critically ill medical patients support that CTRP3 is an interesting biomarker in this setting. Low CTRP3 values may have diagnostic implications by pointing towards inflammatory and infectious diseases as well as prognostic implications by indicating a high risk of mortality.

Serum **perilipin 2 (PLIN2)** may be a useful marker of MOD both at ICU admission and after 48 h with potential for clinical risk stratification. Although these independent and predictive associations are intriguing, the pathomechanisms leading to the observed changes remain to be elucidated.

Discussion

Visfatin

In addition to their important functions in metabolism, adipokines have been increasingly recognized as central regulators of immune response. In our previous work we have described specific adipokines (e.g., resistin and adiponectin) as strongly associated with inflammation and mortality in critically ill patients[143,144].

As the adipokine vistatin is mainly derived from leucocytes and physiologically involved in activation and attraction of inflammatory cells, it seems to be a promising biomarker in the ICU setting.

We therefore investigated the potential of visfatin as a clincal biomarker in 229 critically ill patients. In fact, visfatin levels are significantly elevated in critically ill patients, associated with sepsis and disease severity, correlated to organ dysfunction and we identified visfatin as a reliable predictor of mortality. Its potential as a biomarker predicting short- and long-term mortality is most likely based on the strong correlation of visfatin to inflammatory mediators, such as CRP, PCT and suPAR) and cytokines (IL-6, IL-6, TNF), biomarkers reflecting organ dysfunction, and as well prognostic clinical scores like APACHE-II, SAPS2, and SOFA score.

Furthermore, visfatin has been proposed as directedly involved in the pathogenesis of critical illness and systemic inflammation owing to its (patho)physiological functions (e.g., chemoattraction and promoting release of cytokines and oxidative stress factors) and by this directly contributing to tissue damage and organ failure[60,63,145]. Supporting this hypothesis, it has been demonstrated in mouse models of ventilator-associated lung injury, that experimental inhibition of visfatin results in reduced neutrophil infiltration, organ dysfunction and mortality[60].

Owing to the physiological properties of visfatin as an adipokine, we correlated visfatin to metabolic biomarkers such as BMI, preexisting diabetes mellitus and cholesterol, but did not find relevant associations. This supports the hypothesis, that in critical illness, visfatin levels are primarily associated with the extend of inflammation and not obesity itself.

CTRP1 and CTRP3

Members of the adipokine family of C1q/TNF-like protein (CTRP) are physiologically strongly involved in systemic energy homeostasis and insulin sensitivity and play pathophysiologically a critical role in inducing systemic inflammation and insulin resistance.

Systemic inflammation and metabolic alterations are key characteristics of critical disease, we there for investigated the role of CTRP3 and CTRP3 in a large cohort of critical ill patients. CTRP1 levels were significantly elevated in critically ill patients with highest in sepsis. CTRP1 was related to preexisting diabetes mellitus and long-term blood glucose control, reflected by HbA1c.

This is in line with data from non-ICU patients with diabetes and obesity[64,146]. In animal experiments it has been shown, that CTRP1 directly lowers blood glucose levels without altering insulin or adiponectin levels and that high CTRP1 concentrations protect from insulin-resistance and obesity[147].

To elucidate the pathophysiological role of CTRP1 in critical disease we performed extensive correlation analyses with metabolic biomarkers and (adipo)cytokines. Remarkably, we could not detect correlations of CTRP1 and insulin, leptin, leptin receptor, ghrelin, adiponectin, resistin or retinol-binding protein 4 (RBP4). But we found highly significant correlations between CTRP1 and biomarkers of systemic inflammation such as CRP, PCT, IL-6 and suPAR, as wells as biomarkers of renal function.

Taken this together, we postulated that the involvement of CTRP1 in processes of systemic inflammation during critical disease overlays its physiological metabolic functions.

In contrast to CTRP1, we found significantly reduced CTRP3 levels as compared with healthy controls, and CTRP3 was particularly low in patients with sepsis. CTRP3 plasma concentrations were inversely correlated with inflammatory cytokines and classical sepsis markers. We could not detect a correlation with CTRP3 and metabolic comorbidities. CTRP3 levels below 620,6 ng/mL demonstrated a high mortality rate at the ICU.

CTRP3 has been associated with beneficial metabolic and anti-inflammatory functions in animal models. CTRP3 protected against sepsis-related heart failure[73]. CTRP3 specifically blocks the binding to its receptor TLR4 and by this inhibits proinflammatory responses. Reversely, LPS inhibits differentiation of adipose tissue, induces insulin resistance, and prevents expression of CTRP3[74,148].

In our study, circulating CTRP3 inversely correlated to with inflammatory biomarkers and cytokines, supporting a counter-regulatory mechanism of CTRP3 in critically ill patients.

However, we cannot conclude, whether low CTRP3 levels are a physiological response of critical disease or inadequately low CTRP3 is rather a driver of critical disease. The close correlation of low CTRP3 with mortalities seems to support the hypothesis, that low CTRP3 is more like a promotor of disease progression.

In summary, the members of the CTRP family seem to be promising novel biomarkers, or even potential therapeutic targets in critical disease.

However, their distinct involvement in a plethora of physiological and pathophysiological pathways makes it challenging to differentiate its clinical relevance from cofounding factors in observational studies. Future research should aim at investigating the exact pathogenic functions of members of the CTRP family for a more detailed knowledge of their different roles in systemic inflammation and metabolism.

Perilipin 2 (PLIN2)

Perilipin 2 (PLIN2), also referred to as adipose differentiation-related protein (ADRP) or adipophilin is a lipid droplet protein with numerous systemic, metabolic and age-related functions.

Lipid droplets are ubiquitous in nature and have long been regarded as functionally inert fat drops. Lately, lipid droplets have been identified as multi-functional organelles exerting key functions in cellular metabolism and homeostasis[77].

To our knowledge, our study is the first to analyse the usefulness of serum PLIN2 in a large cohort of well-characterized critically ill patients admitted to a medical ICU. PLIN2 is elevated in critically ill patients as compared with healthy controls. Our most important finding is, that PLIN2 obtained at ICU admission can reliably predict occurrences MOD (defined as a SOFA score >9 points) 48h after ICU admission.

To evaluate PLIN2 as a biomarker in the ICU setting, we performed extensive correlation analyses with established biomarkers of systemic inflammation, metabolism, organ dysfunction, comorbidities (e.g., cardiovascular, hepatic, pancreatic, malignant disease) and patients' characteristics such as age, sex, BMI. In summary, our analyses indicate that PLIN2 is independent of the investigated biomarkers and conditions and does not reflect metabolic dysregulation in our cohort of critically ill patients.

For a subset of patients (n=36) in our cohort, CT-scan body composition data, reflecting sarcopenia (reflecting quantity of muscle) and sarcopenia (reflecting quality of muscle), were available. Although PLIN2 is strongly physiologically involved in regulation of lipid metabolism, lipid storage and lipolysis, we could not detect relevant correlations between CT-scan body composition markers and PLIN2 serum concentrations.

Our analyses with regard to ICU- and long-term mortality, did not reveal significant differences of PLIN2 levels between survivors and non-survivors. We identified PLIN2 as a useful biomarker for prediction of MOD 48h hours after admission to the ICU. Despite extensive analyses, we could not reveal clinically relevant correlations between PLIN2 serum concentrations and numerous established and novel biomarkers as well as a large dataset of patients' characteristics and treatment measures.

Although, from a physiological perspective, PLIN2 seems to be a promising biomarker in the ICU setting, the pathophysiological mechanisms of PLIN2 in critical illness have next to be elucidated.

PART 3: Biomarkers reflecting biological stress in critical illness

Major findings

In part 3 we investigated **Biomarkers reflecting Biological Stress in Critical Illness**. **Copeptin** plasma levels were significantly elevated in critically ill patients (n=218) at ICU admission, as compared with 66 healthy controls. Neither sepsis as the cause of critical illness nor pre-existing metabolic disorders (type 2 diabetes, obesity) were found to influence copeptin levels.

On the contrary, plasma copeptin was closely associated with disease severity (e.g., APACHE-II, SAPS2 score) and correlated with biomarkers of inflammation, renal failure, metabolism, vascular tone and tissue perfusion. Elevated copeptin levels at ICU admission predicted short-term and long-term mortality.

Mid-regional pro atrial natriuretic peptide (MR-proANP) plasma levels were significantly elevated in critically ill patients, when compared to healthy controls. Notably, MR-proANP levels were significantly higher in ICU patients with sepsis. MR-proANP levels were not associated with metabolic comorbidities like diabetes or obesity. In critically ill patients, MR-proANP plasma concentrations correlated with inflammatory cytokines, markers of organ dysfunction and several adipocytokines, such as resistin, retinol-binding protein 4 (RBP4) and adiponectin.

Importantly, high MR-proANP plasma levels were associated with mortality, as MR-proANP levels above 227.0 pmol/l indicated a particularly increased mortality risk in ICU patients.

Clusterin plasma concentration was significantly elevated in critically ill patients compared to healthy subjects. Clusterin levels were found significantly higher in non-septic ICU patients. Clusterin correlated inversely with markers of inflammatory response. Furthermore, Clusterin levels were higher in ICU patients with pre-existing obesity and type 2 diabetes. Clusterin was not associated with disease severity, organ failure or mortality in the ICU.

Discussion

Copeptin

The severity of critical illness is determined by the extent of biological stress, the degree of systemic inflammation and the resultant hemodynamic changes, organ failures and finally, death.

The activation of neuroendocrine pathways, especially the hypothalamic-pituitary-adrenal axis and vasopressin release is one of the characteristic responses to biological stress in critical disease. Circulating mediators of such core pathways could be used as clinical biomarkers in intensive care medicine. Copeptin is a stable protein, consistently detectable in the circulation and reliably reflects biologically functional vasopressin in both healthy and acutely ill patients[89,149].

We determined the value of copeptin as a biomarker in a large cohort of critically ill patients. In line with previous studies, we demonstrated increased copeptin plasma concentrations in critically ill patients as compared with healthy controls[89,93-95]. However, copeptin were not affected by sepsis or metabolic comorbidities such as diabetes mellitus, metabolic syndrome or obesity, which was reported in the setting of chronic non-intensive care patients[90,91].

Copeptin serum concentrations in our study were closely associated with disease severity, as expressed by strong correlations to the APACHE-II and SAPS2 scores and biomarkers of renal and hepatic dysfunction, altered metabolism and tissue perfusion. Consequentially, high copeptin plasma concentrations at ICU admission were a reliable predictor of short-term as well as long-term mortality in our cohort.

In contrast to our results, a large observational study from China in the emergency care setting, suggested copeptin as an early indicator of sepsis. In line with our study, the authors also reported an association of copeptin levels with disease severity and short-term outcome[150].

Our findings from the highly complex intensive care setting imply, that not the presence of sepsis alone, but the severity of disease, as sum of systemic inflammation, hemodynamic deterioration and organ failure might be the main driver for elevated copeptin plasma concentrations. This might be supported by the results of two smaller observational studies, in which a stepwise increase in circulating copeptin levels from sepsis to severe sepsis to septic shock was reported, which in a particular way reflects the extent of cardiovascular instability[94,151].

Moreover, we observed in our study a strong correlation of copeptin with biomarkers of endothelial dysfunction and systemic regulators of vascular tone such as CT-pro-ET1, ADMA, SDMA or NT-proCNP, as wells as clinical endpoint parameters as lactate or acidosis. This further supports, that copeptin contributes to hemodynamic alterations resulting in tissue hypoperfusion.

Nevertheless, from our correlative analysis, it is impossible to dissect whether high copeptin serum concentrations in critical illness is cause or consequence of biological

stress, vascular dysregulation and tissue hypoperfusion. Previously, other studies in acute illnesses reported an association of high copeptin with increased short-term mortality[89,94,95,150].

Beyond that, our study demonstrated for the first time, that copeptin plasma concentrations at the admission to the ICU can even predict long-term and overall mortality in critically ill patients.

Mid-regional pro atrial natriuretic peptide (MR-proANP)

The natriuretic peptides (ANP, atrial natriuretic peptide, BNP, brain natriuretic peptide and CNP, C-type natriuretic peptide) are members of a family of structurally related, but functionally different hormones.

Physiological functions of natriuretic peptides comprise diuretic, natriuretic and vasoactive actions, which directly affect maintenance of cardiovascular and fluid homeostasis, as wells as blood pressure regulation[96,97]. The assessment of mid-regional proANP (MR-proANP) is the current biochemical detection method of choice for ANP and reliably mirrors ANP serum concentrations[100,101].

Beyond its known use as a biomarker for cardiac insufficiency, ANP has been also linked to inflammatory and metabolic alternations that are characteristic for critical illness[152,153]. Increased concentrations of natriuretic peptides have been reported in patients with severe cardiogenic shock and concomitant release of proinflammatory cytokines[152,154].

In line with this, we found increased MR-proANP levels at admission to the ICU as compared with healthy controls. Using extensive correlation analyses we found highly significant associations of MR-proANP and bioamarkers reflecting inflammation (e.g., CRP, PCT, suPAR, leucocytes), metabolic alterations (adipokines, HOMA-ß), and organ dysfunction (liver kidney, pancreas, coagulation). In our cohort, we found that MR-proANP is strongly correlated with adipocytokines such as adiponectin, RBP4 and resistin, which are important mediators of insulin resistance and metabolic alterations. Interestingly, MR-proANP did only correlate with markers reflecting adipose tissue inflammation, but not with patient's BMI or pre-existing obesity.

We also found increased MR-proANP levels in patients with sepsis, but receiver operating characteristic curve (ROC)-analyses revealed, that MR-proANP has inferior diagnostic power for sepsis as compared with CRP or PCT. This has been previously reported in smaller studies of patients with burn injuries[155] and patients in the emergency department[156], but now for a large cohort of medical ICU patients. MR-proANP has also been associated with 28-day mortality in patients with septic shock[157].

Interestingly, when combined with PCT, the assessment of MR-proANP has been shown to improve the accuracy of survival-prediction in patients with ventilator-associated pneumonia[158].

In our study, we addressed the impact of MR-proANP obtained at admission to the ICU with regard to short- and long-term survival. We assessed long-term survival in 206 out of 217 patients by contacting the patients, their relatives or their general practitioner during the first three years after ICU discharge. MR-proANP levels at ICU admission were significantly elevated in patients that subsequently died. Elevated MR-proANP plasma concentrations at ICU admission (optimal cut-off: 227.0 pmol/l) predicted the overall mortality (short-term and long-term) in critically ill patients

Clusterin

Clusterin is a multifunctional protein, which is expressed in a broad range of tissues and present in almost all body fluids[107]. It is known for mediating cellular stress response on the basis of organ failure, systemic inflammation and metabolic disturbances.

Given its central role in immunity, cellular function and energy metabolism we investigated the clinical usefulness of clusterin as biomarker in critically ill patients with regard to metabolic and immunologic alterations, organ failure, disease severity, as well as mortality. In a cohort of 200 critically ill patients we found increased plasma concentrations of clusterin as compared to healthy controls and significantly lower values in patients with sepsis.

These observations are in line with findings from previous studies that displayed decreased clusterin levels particularly in patients with systemic inflammatory response such as sepsis or septic shock[159,160]. However, among critically ill patients, there was no consistent correlation between clusterin plasma concentrations and the various disease etiologies leading to ICU admission (e.g., typical sepsis-related infection sites were pneumonia, abdomen, and genitourinary tract, while non-sepsis-related causes of critical illness included cardiopulmonary disease, acute pancreatitis, and decompensated liver cirrhosis).

Clusterin was not associated with disease severity or extent of therapeutic measurements such as vasopressor therapy or mechanical ventilation. Regarding a possible association between clusterin and inflammation, we showed that clusterin was inversely associated with interleukin-6 and procalcitonin. These findings are consistent with the lower clusterin concentrations in ICU patients with sepsis.

Clusterin has been previously linked to metabolic conditions and diseases, discussing its value as a biomarker for Alzheimer's disease in obesity-related metabolic disease[161].

Clusterin is found in high-density- (HDL) and low-density- (LDL) lipoproteins. Interestingly, in HDL clusterin has been linked to insulin-sensitivity, whereas in LDL clusterin is associated with insulin-resistance[162].

Circulating clusterin has been shown to also be closely related to the extent of insulin resistance regardless of obesity, and a decrease of clusterin levels in line with therapeutically improved insulin sensitivity in type 2 diabetes mellitus has been reported[163]. In our study, we found a trend to higher clusterin levels in patients with preexisting diabetes mellitus and a strong association with blood glucose concentrations at admission to the ICU prior to therapeutic intervention. Moreover, we found a trend to higher clusterin levels in patients with obesity (BMI >30 kg/m²) and remarkably increased triglycerides, LDL and HDL.

In summary, Clusterin is not an indicator of disease severity, organ failure or mortality, but associated with metabolic and inflammatory alterations. The discordance between the key pathogenic function of clusterin in cellular stress and its weak capacity as a clinical biomarker appears to be related to its complex, distinct not fully understood molecular functions.

References are given in the References section of the Addendum

Chapter 13

Summary

Summary

Biomarkers are widely used in critical care medicine to make diagnosis, prognosticate and monitor treatment. For clinical applicability, biomarkers must have appropriate test characteristics to distinguish between different states of disease.

The prerequisite for this is that in statistical analysis the area under the receiver operating characteristic curve (AUROC) should approach 1 and has to be greater than 0.5. In addition, biomarkers should be easily to obtain, rapidly measurable, and generalizable for instant interpretation in the clinical context and prompt decision making to favorably influence the course of the disease[164].

The present thesis summarizes publications of prospective, non-interventional studies on the identification of novel biomarkers in critical care medicine (Table 13.1). The aim of our studies was to evaluate the significance, regulation, diagnostic and prognostic value of novel biomarkers in serum or plasma of critically ill patients who were admitted to the medical ICU at the University Hospital Aachen, Germany.

Thematically, this thesis is divided into three parts. The first part deals with "Biomarkers of Systemic Inflammation in Critical Disease", in the second part the "Role of Specific Adipokines in Critical Ill Patients" is examined and in the third part "Biomarkers of Biological Stress in Critical Illness" are discussed.

We evaluated **calprotectin**, **caspase-cleaved keratin 18 fragments (M30)** and **high-mobility group box 1 (HMGB1)** as potential novel **Biomarkers of Systemic Inflammation in Critical Disease** in **part 1** of this thesis.

In **chapter 2**, we analysed serum **calprotectin** concentrations critically ill patients with and without sepsis, compared to 24 healthy controls and correlated with clinical parameters, therapeutic interventions, and survival. We found significantly increased serum calprotectin concentrations in ICU patients as well as in septic patients compared to respective controls. In patients with comorbidities i.e., coronary artery disease we detected lower calprotectin concentrations. Calprotectin concentrations strongly correlated with CRP and were closely associated to parameters reflecting the extent of mechanical ventilation (i.e., inspiratory oxygen fraction, FiO_2; oxygen-enriched air has a higher FiO_2 than 0.21; up to 1.00 which means 100% oxygen.).

High baseline calprotectin concentrations were identified as predictive for impaired overall survival in septic patients, whereas increasing calprotectin concentrations during the first week of ICU treatment were associated with a favourable long-term outcome.

Table 13.1 Tabular summary of the most important study results (cumulative impact factor [IF]: 39,7).

Biomarker	ICU patients	Sepsis patients	Relevant associations	Prognostic significance (mortality)	Reference
Biomarkers of Systemic Inflammation in Critical Disease					
Calprotectin	increased	increased	systemic inflammation, parameters of mechanical ventilation	yes, overall survival, low values favourable, increase within the first week is associated with favourable outcome	*Wirtz TH et al. Diagnostics 2020, 10, 990 (IF: 4.13)*
M30	increased	unchanged	disease severity (APACHE-II, SAPS2, SOFA, suPAR), systemic inflammation, liver dysfunction (esp. cirrhosis), renal dysfunction,	yes, ICU survival, (trend to overall survival) low values favourable	*Koch et al. Disease Markers 2018, ID: 8583121 (IF: 3.43)*
HMGB1	increased	unchanged	trend to lower HBGB1 levels in pre-existing obesity, type 2 DM and end-stage renal disease	no	*Yagmur E et al.J Clin Lab Anal 2018; e22584 (IF: 2.91)*
Specific Adipokines in Critical Ill Patients					
Visfatin	increased	increased	disease severity (APACHE-II, SAPS2, SOFA, suPAR), systemic inflammation, liver dysfunction, renal dysfunction, adipokines (leptin, adiponectin, resistin)	yes, ICU and long-term survival, low values favourable	*Koch et al. Disease Markers 2018, ID: 7315356 (IF: 3.43)*
CTRP1	increased	increased	systemic inflammation, cholestasis, renal dysfunction, preexisting type 2 DM, HbA1c	no	*Yagmur E et al. J Clin Med 2019, 8, 661 (IF: 5.1)*
CTRP3	increased	increased	disease severity (APACHE-II, SAPS2, invers), systemic inflammation (invers), resistin (invers), lipid metabolism	yes, ICU and long-term survival, high values favourable	*Yagmur E et al. Diagnostics 2019, 9,63 (IF: 4.13)*
PLIN2	increased	slighty increased	disease severity (APACHE-II, SAPS2), predictor of MOF, severe respiratory failure (PaO_2/FiO_2), length of ICU stay	no, (subgroup analysis: ICU survival in patients >65 years, high values favourable)	*Kurt B et al. Biomedicines 2021, 9, 1210 (IF: 5.22)*
Biomarkers of Biological Stress in Critical Illness					
Copeptin	increased	unchanged	disease severity (APACHE-II, SAPS2), systemic inflammation, renal dysfunction, vascular tonus, tissue perfusion	yes, ICU and long-term survival, low values favourable	*Koch et al. J Clin Lab Anal 2018; e22614 (IF: 2.91)*
MR-proANP	increased	increased	disease severity (APACHE-II, SAPS2, SOFA), systemic inflammation, renal dysfunction, hepatic dysfunction, adipokines (adiponectin, resistin, RBP4)	yes, ICU and long-term survival, low values favourable	*Yagmur E et al. J Transl Med 2019, 17:415 (IF: 8.44)*
Clusterin	increased	decreased	preexisting type 2 DM, obesity, blood glucose, markers of inflammatory response (IL-6, PCT; inverse)	no	*submitted*

As a circulating biomarker of apoptosis, we analysed circulating **M30** levels in critically ill patients (with and without sepsis) at admission to the medical intensive care unit, in comparison to healthy controls in **chapter 3**.

Our study demonstrated that circulating levels of the apoptosis-related keratin fragment M30 are significantly elevated in critically ill patients as compared with healthy controls, independent of the presence of sepsis. Circulating M30 was closely associated with disease severity, as displayed by a close correlation with prognostic scoring systems such as APACHE-II and SOFA score, but did not differ between patients with sepsis and ICU patients without sepsis. We found M30 serum levels correlated with biomarkers of inflammation, cell injury, renal failure and liver failure in critically ill patients. Hepatocyte apoptosis might contribute substantially to high circulating M30 in critically ill patients, as we observed remarkably high levels in patients with chronic liver diseases. High M30 levels (>250.8 U/L) at admission to the ICU indicated an unfavourable short-term outcome.

In **chapter 4** we assessed the potential of **high-mobility group box 1 (HMGB1)** as a clinical biomarker. We found significantly elevated HMGB1 levels in critically ill patients as compared with healthy controls and elevated HMGB1 plasma levels were independent from the presence of sepsis. HMGB1 was not associated with disease severity, organ failure or mortality in the ICU. We observed a trend towards lower HMGB1 levels in ICU patients with pre-existing obesity, type 2 diabetes and end-stage renal disease patients on chronic haemodialysis.

Surprising to us, the study showed no significant associations between HMGB1 levels at ICU admission and clinical outcomes in critically ill patients. Possibly this is due to the fact that HMGB1 exerts its pathogenic role in the latter phases of sepsis and we obtained blood samples immediately at admission to the ICU.

Future studies might assess the potential value of HMGB1 by measuring its plasma concentrations at later time points during the course of critical illness.

In **part 2** we investigated the **Role of Specific Adipokines in Critically Ill Patients**.

Adipokines may represent an important causal link between hyperglycemia, insulin resistance and excessive systemic inflammatory reaction in sepsis and critical illness.

We therefore investigated the potential of **visfatin**, **CTRP1**, **CTRP3** and **perilipin 2 (PLIN2)** as biomarkers in critically ill patients.

We analysed serum levels of **visfatin** in critically ill medical patients upon admission to the intensive care unit in **chapter 5**. Visfatin levels were found to be significantly elevated in medical ICU patients, especially in patients with sepsis as compared with healthy controls.

We demonstrated a strong association of visfatin serum concentrations with disease severity and organ failure. But interestingly, no difference in visfatin concentrations could be observed between patients with or without obesity or type 2 diabetes. This supports the hypothesis, that circulating visfatin levels in critical illness are primarily attributable to the extent of inflammation and not obesity itself.

Visfatin levels correlated with biomarkers of renal failure, liver dysfunction and other adipokines (e.g., resistin, leptin, adiponectin) in critically ill patients.

In our study, high visfatin levels at ICU admission were an excellent predictor of the overall mortality during a two-years follow-up period.

As members of the adipokine family of C1q/TNF-like proteins (CTRP) have been suggested as important regulators of metabolism and meditators in the interaction between insulin resistance, adiposity and inflammation, we aimed at exploring the potential of **CTRP1** and **CTRP3** as biomarkers in critically ill patients in **chapter 6 and 7**.

We found significantly increased CTRP1 plasma concentrations in critically ill patients at admission to the medical intensive care unit (ICU) in comparison to healthy controls. In patients with sepsis CTRP1 levels were significantly higher as compared to patients without sepsis.

Although, circulating CTRP1 has been previously suggested as a biomarker in the non-ICU setting, we could not detect an association between disease severity or mortality in our cohort. We reported a close association of elevated CTRP1 and preexisting diabetes as well as to long-term blood glucose control reflected by HbA1c. CTRP1 correlated also with markers of inflammatory response, renal function, liver damage and cholestasis. Conclusively, we found that CTRP1 is integrated in the complex network of adipokines in the pathogenesis of critical illness, sepsis and organ failure, hinting at a potential clinical usability.

However, we could not demonstrate a clinical use of CTRP1 as a biomarker in our cohort critically ill patients. Therefore, mechanistic studies are warranted to elucidate the pathogenic role of CTRP1 in in metabolic and inflammatory pathways during critical illness.

We also investigated **CTRP3** in critically ill patients upon admission to the ICU (**chapter 7**). In critically ill patients CTRP3 plasma levels were significantly decreased as compared to healthy controls and low CTRP3 levels were highly associated with the presence of sepsis. No association of CTRP3 levels with obesity or diabetes could be demonstrated.

CTRP3 plasma concentrations were inversely correlated with inflammatory cytokines and classical sepsis markers, supporting the anti-inflammatory properties of CTRP3. CTRP3 levels below 620.6 ng/mL predictied overall mortality in critically ill patients.

Perilipin 2 (PLIN2), a member of lipid droplet proteins, is substantially involved in lipid metabolism and was recently linked to conditions of chronic inflammation such cardivascular diseases. This prompted us to measure serum PLIN2 serum concentrations in critically ill patients upon admission to the ICU in comparison to healthy controls (**chapter 8**).

Compared to controls, serum PLIN2 concentrations were elevated in critically ill patients at ICU admission. PLIN2 independently indicated multiple organ dysfunction (MOD) instantely at ICU admission, and was also able to independently predict occurance of MOD 48h after ICU admission. Moreover, serum PLIN2 levels were associated with severe respiratory failure, potentially reflecting a moribund state. Serum PLIN2 may be a useful biomarker for prediction of MOD in the ICU setting.

Part 3 of this thesis deals with the topic of **Biomarkers of Biological Stress in Critical Illness**. Biological stress in critical illness is is the body's method of reacting to a severe insults such as infections, shock, trauma, and metabolic altertations. We have investigated the role of **copeptin**, **mid-regional pro atrial natriuretic peptide (MR-proANP)** and **clusterin** as clincal biomarkers of biological stress in critically ill patients in part 3 of this thesis.

Biological stress activates hypothalamic-pituitary-adrenal axis as well as vasopressin release. **Copeptin** mirrors biologically functional endogenous vaspression and by this the level of biological stress in critically ill patients.

We analyzed plasma copeptin levels in a prospective, single-center, observational study comprising critically ill patients at admission to the medical ICU (**chapter 9**). At ICU admission, copeptin plasma levels were significantly increased in critically ill patients as compared with healthy controls. Neither sepsis as the cause of critical illness nor pre-existing metabolic disorders (type 2 diabetes, obesity) were found to influence copeptin levels.

We found a close correlation of plasma copeptin with disease severity (e.g., APACHE-II score) and biomarkers of inflammation, renal failure, metabolism, vascular tonus and tissue perfusion. Elevated copeptin levels at ICU admission predicted short-term and long-term mortality. Mortality was assessed during a two-year observational follow-up period.

Atrial natruretic peptide (ANP) exerts diuretic, natriuretic and vasoactive actions. Atrial wall stress is the main driver for ANP secretion. In critical disease, ANP appears to take on regulatory functions in systemic inflammation, besides well known effects on vascular and fluid homeostasis.

We investigated **mid-regional pro atrial natriuretic peptide (MR-proANP)** plasma concentrations in critically ill patients with and without sepsis upon admission to the medical ICU (**chapter 10**). MR-proANP plasma levels were significantly elevated in critically ill patients, with highest levels in patients with sepsis, when compared to healthy controls. We observed a close correlation of MR-proANP plasma concentrations with inflammatory cytokines, markers of organ dysfunction and several adipocytokines, such as resistin, retinol-binding protein 4 (RBP4) and adiponectin. High MR-proANP levels above 227.0 pmol/l predicted a significantly increased mortality risk.

We clearly demonstrated, that MR-proANP indicates organ dysfunction, sepsis and mortality risk, thus emphasizing the role of circulating MR-proANP as a diagnostic and prognostic biomarker in critically ill patients.

Clusterin has been suggested as a mediator of cellular stress response inducted by organ failure, systemic inflammation and severe disturbances in metabolism. To determine the value of clusterin as a biomarker in critical conditions, we analysed clusterin plasma concentrations in intensive care patients (**chapter 11**).

Clusterin plasma concentrations were significantly increased in critically ill patients compared to healthy subjects. In patients with sepsis significantly lower were observed. In line, clusterin correlated inversely with markers of inflammatory response, such as CRP and PCT. Furthermore, Clusterin levels were higher in ICU patients with pre-existing obesity and/or type 2 diabetes. This fits previous findings that clusterin directly correlates to insulin resistance and clusterin levels decrease with improving insulin sensitivity in type 2 diabetes.

Clusterin was not associated with disease severity, organ failure or mortality in the ICU. Although clusterin exerts key pathogenic functions in cellular stress pathways we could not demonstrate a significant clinical applicability of clusterin as a biomarker in critical disease.

The knowledge gained from the presented results should contribute to a better understanding of the regulation and pathophysiological role of the investigated biomarkers in critical illness and sepsis. We intended to present them either as novel diagnostic and prognostic biomarkers and potentially open perspectives for new therapeutic approaches in intensive care medicine or to depict their limited clinical utility in this complex clinical setting.

Addendum

Nederlandse samenvatting

Biomarkers worden in de kritieke zorg geneeskunde op grote schaal gebruikt in de diagnostiek, prognostiek en om de behandeling te volgen. Voor klinische toepasbaarheid moeten biomarkers geschikte testkarakteristieken hebben om onderscheid te kunnen maken tussen verschillende ziektetoestanden.

Voorwaarde hiervoor is dat bij statistische analyse de area under the receiver operating characteristic curve (AUROC) de één benadert en groter moet zijn dan 0,5. Bovendien moeten biomarkers gemakkelijk te verkrijgen, snel meetbaar en generaliseerbaar zijn voor onmiddellijke interpretatie in de klinische context en snelle besluitvorming om het ziekteverloop gunstig te beïnvloeden[163].

Dit proefschrift geeft een overzicht van prospectieve, niet-interventionele studies betreffende de identificatie van nieuwe biomarkers in de kritieke zorggeneeskunde (tabel 3). Het doel van onze studies was het evalueren van de betekenis, regulatie, diagnostische en prognostische waarde van nieuwe biomarkers in serum of plasma van kritisch zieke patiënten die werden opgenomen op de medische ICU van het Universitair Ziekenhuis Aken, Duitsland.

Het proefschrift is in drie delen onderverdeeld. Het eerste deel behandelt biomarkers van systemische ontsteking in kritieke ziekte, in het tweede deel wordt de rol van specifieke adipokines in kritisch zieke patiënten onderzocht en in het derde deel worden biomarkers van biologische stress in kritieke ziekte besproken.

In deel 1 van dit proefschrift hebben wij calprotectine, caspase-cleaved keratine 18 fragmenten (M30) en high-mobility group box 1 (HMGB1) geëvalueerd als potentiële nieuwe biomarkers van systemische ontsteking bij kritieke ziekte.

In hoofdstuk 2 analyseerden wij serum calprotectine concentraties van kritisch zieke patiënten met en zonder sepsis, vergeleken met 24 gezonde controles en gecorreleerd met klinische parameters, therapeutische interventies en overleving. Wij vonden significant verhoogde serum calprotectine concentraties bij zowel IC-patiënten als bij septische patiënten in vergelijking met gezonde controles. Bij patiënten met comorbiditeiten, zoals coronaire hartziekte, vonden wij lagere calprotectine concentraties. Calprotectine concentraties correleerden sterk met CRP en waren nauw verbonden met parameters die de mate van mechanische ventilatie weergeven (d.w.z., inspiratoire zuurstoffractie, FiO2; met zuurstof verrijkte lucht heeft een hogere FiO2 dan 0,21; tot 1,00 wat 100% zuurstof betekent).

Hoge baseline calprotectine concentraties bleken voorspellend voor een verminderde algehele overleving bij septische patiënten, terwijl stijgende calprotectine concentraties tijdens de eerste week van de IC-behandeling werden geassocieerd met een gunstige uitkomst op lange termijn.

Als circulerende biomarker van apoptose analyseerden wij in hoofdstuk 3 de circulerende M30 concentraties bij kritisch zieke patiënten (met en zonder sepsis) bij opname op de medische intensive care, in vergelijking met gezonde controles.

Onze studie toonde aan dat circulerende concentraties van het aan apoptose gerelateerde keratinefragment M30 significant verhoogd zijn bij ernstig zieke patiënten in vergelijking met gezonde controles, onafhankelijk van de aanwezigheid van sepsis. Circulerende M30 was geassocieerd met de ernst van de ziekte, zoals bleek uit een sterke correlatie met prognostische scoringssystemen zoals APACHE-II en SOFA-score, maar verschilde niet tussen patiënten met sepsis en IC-patiënten zonder sepsis. Wij vonden een correlatie tussen M30-serumniveaus en biomarkers van ontsteking, celschade, nierfalen en leverfalen bij kritisch zieke patiënten. Apoptose van hepatocyten zou aanzienlijk kunnen bijdragen aan hoge circulerende M30 bij ernstig zieke patiënten, aangezien wij opmerkelijk hoge niveaus vonden bij patiënten met chronische lever ziekten. Hoge M30 niveaus (>250,8 U/L) bij opname op de ICU duidden op een ongunstige prognose op korte termijn.

In hoofdstuk 4 onderzochten wij de waarde van high-mobility group box 1 (HMGB1) als klinische biomarker. Wij vonden significant verhoogde HMGB1-spiegels bij ernstig zieke patiënten in vergelijking met gezonde controles. De verhoogde HMGB1-plasmaspiegels waren onafhankelijk van de aanwezigheid van sepsis. HMGB1 was niet geassocieerd met de ernst van de ziekte, orgaanfalen of sterfte op de ICU. Wij constateerden een trend naar lagere HMGB1-spiegels bij IC-patiënten met reeds bestaande obesitas, diabetes type 2 en patiënten met eindstadium nierziekte met chronische hemodialyse.

Verrassend voor ons was dat de studie geen significante associaties liet zien tussen HMGB1 niveaus bij ICU opname en klinische uitkomsten bij kritisch zieke patiënten. Mogelijk is dit te wijten aan het feit dat HMGB1 zijn pathogene rol uitoefent in de laatste fasen van sepsis en wij onmiddellijk bij opname op de IC bloedmonsters afnamen.

Toekomstige studies zouden de potentiële waarde van HMGB1 kunnen beoordelen door de plasmaconcentraties op verschillende tijdstippen tijdens het verloop van de kritieke ziekte te meten.

In deel 2 onderzochten wij de rol van specifieke adipokines bij kritisch zieke patiënten. Adipokines kunnen een belangrijk oorzakelijk verband vormen tussen hyperglycemie, insuline resistentie en excessieve systemische ontstekingsreactie bij sepsis en kritieke ziekte. We onderzochten visfatine, CTRP1, CTRP3 en perilipine 2 (PLIN2) als biomarkers bij kritiek zieke patiënten.

In hoofdstuk 5 analyseerden wij de serum concentraties van visfatine bij kritiek zieke patiënten tijdens opname op de intensive care afdeling. Visfatine spiegels bleken significant verhoogd bij IC-patiënten, vooral degene met sepsis in vergelijking met gezonde controles.

Wij toonden een sterke associatie aan van de visfatine serum concentraties met de ernst van de ziekte en orgaan falen. Opvallend was dat er geen verschil in visfatine concentraties waargenomen werd tussen patiënten met of zonder obesitas of type 2 diabetes. Dit ondersteunt de hypothese, dat de serum visfatine concentraties bij kritieke ziekte vooral toe te schrijven zijn aan de mate van ontsteking en niet aan de obesitas zelf.

Visfatine spiegels correleerden met biomarkers van nierfalen, leverdisfunctie en andere adipokines (bv. resistine, leptine, adiponectine) in kritisch zieke patiënten.

In onze studie waren hoge visfatine spiegels bij opname op de IC een uitstekende voorspeller van de totale mortaliteit gedurende een follow-up periode van twee jaar.

Aangezien leden van de adipokine familie van C1q/TNF-achtige eiwitten (CTRP) zijn voorgesteld als belangrijke regulatoren van het metabolisme en mediatoren in de interactie tussen insulineresistentie, adipositas en ontsteking, wilden wij in hoofdstuk 6 en 7 het potentieel van CTRP1 en CTRP3 als biomarkers bij kritiek zieke patiënten onderzoeken.

Wij vonden significant verhoogde CTRP1 plasmaconcentraties bij ernstig zieke patiënten bij opname op de medische intensive care unit (ICU) in vergelijking met gezonde controles. Bij patiënten met sepsis waren de CTRP1-spiegels significant hoger dan bij patiënten zonder sepsis.

Hoewel circulerend CTRP1 eerder is voorgesteld als biomarker in de niet-ICU-setting, konden wij in ons cohort geen verband ontdekken tussen ziekte-ernst of mortaliteit. Wij rapporteerden een nauw verband tussen een verhoogd CTRP1 en reeds bestaande diabetes en met de bloedglucose controle op lange termijn, weergegeven door HbA1c. CTRP1 correleerde ook met markers van ontstekingsreactie, nierfunctie, leverschade en cholestase. Wij concludeerden dat CTRP1 geïntegreerd is in het complexe netwerk van

adipokines in de pathogenese van kritieke ziekte, sepsis en orgaan falen, wat wijst op een mogelijke klinische bruikbaarheid.

Wij konden echter geen klinisch gebruik van CTRP1 als biomarker aantonen in ons cohort kritisch zieke patiënten. Daarom zijn mechanistische studies nodig om de pathogene rol van CTRP1 in metabole en inflammatoire pathways tijdens kritieke ziekte te kunnen verklaren.

Wij onderzochten ook CTRP3 bij kritiek zieke patiënten bij opname op de ICU (hoofdstuk 7). Bij ernstig zieke patiënten waren de CTRP3-plasmaspiegels significant verlaagd in vergelijking met gezonde controles, en lage CTRP3-spiegels waren sterk geassocieerd met de aanwezigheid van sepsis. Er kon geen verband worden aangetoond tussen CTRP3-spiegels en obesitas of diabetes.

CTRP3-plasmaconcentraties waren omgekeerd gecorreleerd met ontstekingscytokinen en klassieke sepsismarkers, wat de ontstekingsremmende eigenschappen van CTRP3 ondersteunt. CTRP3-niveaus onder 620,6 ng/mL voorspelden mortaliteit bij kritisch zieke patiënten.

Perilipine 2 (PLIN2), een lid van lipide druppeleiwitten, is in belangrijke mate betrokken bij het vetmetabolisme en werd onlangs in verband gebracht met chronische ontstekings aandoeningen zoals cardiovasculaire ziekten. Dit was voor ons aanleiding om de serum PLIN2-concentraties te meten bij ernstig zieke patiënten bij opname op de IC in vergelijking met gezonde controles (hoofdstuk 8).

Vergeleken met controles waren de serum PLIN2-concentraties bij kritisch zieke patiënten bij opname op de IC verhoogd. PLIN2 was een onafhankelijk voorspeller voor multipel orgaan falen (MOD) direct bij IC-opname en tevens voorspeller voor MOD 48 uur na IC-opname. Bovendien waren serum PLIN2-waarden geassocieerd met ernstig respiratoir falen, wat mogelijk een weerspiegeling is van fatale afloop. Dus serum PLIN2 kan een nuttige biomarker zijn voor het voorspellen van MOD in de IC-setting.

Deel 3 van dit proefschrift behandelt het onderwerp biologische stress biomarkers bij kritieke ziekte. Biologische stress bij kritieke ziekte is de manier waarop het lichaam reageert op ernstige bedreigingen zoals infecties, shock, trauma en metabole veranderingen. Wij hebben de rol van copeptine, mid-regional pro atrial natriuretic peptide (MR-proANP) en clusterin onderzocht als klinische biomarkers van biologische stress bij kritisch zieke patiënten.

Biologische stress activeert de hypothalamus-hypofyse-bijnieras en de afgifte van vasopressine. Copeptine weerspiegelt de biologisch functionele endogene vasopressie en daarmee het niveau van biologische stress bij kritiek zieke patiënten.

Wij analyseerden plasma copeptine concentraties in een prospectieve, single-center, observationele studie met kritisch zieke patiënten bij opname op de ICU (hoofdstuk 9). Bij opname op de IC waren de copeptine-plasmaspiegels significant verhoogd bij ernstig zieke patiënten in vergelijking met gezonde controles. Noch sepsis als oorzaak van de kritieke ziekte, noch reeds bestaande metabole stoornissen (type 2 diabetes, obesitas) bleken de copeptinespiegel te beïnvloeden.

Wij vonden een correlatie tussen copeptine in plasma en de ernst van de ziekte (bv. APACHE-II score) en biomarkers van ontsteking, nierfalen, metabolisme, vaattonus en weefselperfusie. Verhoogde copeptine spiegels bij opname op de IC voorspelden sterfte op korte en lange termijn. De mortaliteit werd beoordeeld tijdens een observatieperiode van twee jaar.

Atrial natruretic peptide (ANP) heeft een diuretische, natriuretische en vasoactieve werking. Atriale wandstress is de belangrijkste oorzaak van ANP secretie. Bij kritieke ziekte blijkt ANP naast de bekende effecten op de vasculaire en vocht homeostase ook regulerende functies te vervullen bij systemische ontsteking.

Wij onderzochten mid-regional pro atrial natriuretic peptide (MR-proANP) plasmaconcentraties in kritisch zieke patiënten met en zonder sepsis bij opname op de medische IC (hoofdstuk 10). MR-proANP plasmaspiegels waren significant verhoogd bij ernstig zieke patiënten, met de hoogste niveaus bij patiënten met sepsis, in vergelijking met gezonde controles. Wij vonden een nauwe correlatie tussen MR-proANP plasmaconcentraties en ontstekingscytokines, markers van orgaanschade en verschillende adipocytokines, zoals resistine, retinol-bindend proteïne 4 (RBP4) en adiponectine. Hoge MR-proANP concentratie boven 227,0 pmol/l voorspelden een significant verhoogd sterfterisico.

Wij toonden duidelijk aan dat MR-proANP wijst op orgaandisfunctie, sepsis en sterfterisico, en benadrukken daarmee de rol van circulerend MR-proANP als diagnostische en prognostische biomarker bij kritisch zieke patiënten.

Clusterine is voorgesteld als een mediator van cellulaire stressrespons geïnduceerd door orgaanfalen, systemische ontsteking en ernstige verstoringen van het metabolisme. Om de waarde van clusterine als biomarker in kritieke omstandigheden te bepalen, analyseerden wij clusterine plasmaconcentraties bij intensive care patiënten (hoofdstuk 11).

Clusterine-plasmaconcentraties waren significant hoger bij kritisch zieke patiënten dan bij gezonde proefpersonen. Bij patiënten met sepsis werden significant lagere concentraties waargenomen. Clusterine correleerde omgekeerd met markers van ontstekingsreactie, zoals CRP en PCT. Bovendien waren de Clusterine niveaus hoger bij IC patiënten met reeds bestaande obesitas en/of type 2 diabetes. Dit sluit aan bij eerdere bevindingen dat clusterine direct correleert met insulineresistentie en dat clusterine niveaus dalen met het verbeteren van insuline gevoeligheid bij type 2 diabetes.

Clusterine was niet geassocieerd met de ernst van de ziekte, orgaanfalen of sterfte op de ICU. Hoewel clusterine belangrijke pathogene functies uitoefent in cellulaire stress pathways konden we geen significante klinische toepasbaarheid van clusterine als biomarker in kritieke ziekte aantonen.

De kennis die is verkregen uit de gepresenteerde resultaten moet bijdragen tot een beter begrip van de regulering en de pathofysiologische rol van de onderzochte biomarkers bij kritieke ziekte en sepsis. Het was onze bedoeling om ze ofwel als nieuwe diagnostische en prognostische biomarkers te presenteren en mogelijk perspectieven te openen voor nieuwe therapeutische benaderingen in de intensive care geneeskunde, ofwel hun beperkte klinische nut in deze complexe klinische setting weer te geven.

Addendum

Deutsche Zusammenfassung

Biomarker werden in der Intensivmedizin in großem Umfang zur Diagnose, Prognose und Überwachung der Behandlung eingesetzt. Für die klinische Anwendbarkeit müssen Biomarker geeignete Testeigenschaften aufweisen, um zwischen verschiedenen Krankheitszuständen zu unterscheiden zu können.

Voraussetzung dafür ist, dass in der statistischen Analyse die Fläche unter der „receiver operating characteristic curve“ (AUROC) nahe bei 1 liegt und größer als 0,5 ist. Darüber hinaus sollten Biomarker leicht zu gewinnen, schnell messbar und allgemein anwendbar sein, damit sie im klinischen Kontext sofort interpretiert werden können, eine schnelle Entscheidungsfindung ermöglichen und um damit den Krankheitsverlauf günstig zu beeinflussen[163].

Die vorliegende Arbeit fasst Veröffentlichungen von prospektiven, nicht-interventionellen Studien zur Identifizierung neuer Biomarker in der Intensivmedizin zusammen (Tabelle 13.1). Das Ziel unserer Studien war es, die klinische Relevanz, die Messwerte in unterschiedlichen klinischen Situationen, sowie den diagnostischen und prognostischen Wert neuartiger Biomarker im Serum oder Plasma kritisch kranker Patienten, die auf der medizinischen Intensivstation des Universitätsklinikums Aachen aufgenommen wurden, zu untersuchen.

Thematisch gliedert sich diese Arbeit in drei Teile. Der erste Teil befasst sich mit "Biomarkern der systemischen Entzündungsreaktion bei kritischer Erkrankung", im zweiten Teil wird die "Rolle spezifischer Adipokine bei kritisch kranken Patienten" untersucht und im dritten Teil werden "Biomarker der biologischen Stress-Reaktion bei kritischer Erkrankung" diskutiert.

In Teil 1 dieser Arbeit haben wir **Calprotectin**, **Caspase-gespaltene Keratin 18 Fragmente (M30)** und **High-Mobility Group Box 1 (HMGB1)** als potenzielle neue Biomarker für die **systemische Entzündungsreaktion bei kritischen Erkrankungen** untersucht.

In **Kapitel 2** analysierten wir die Serum-**Calprotectin**-Konzentrationen kritisch kranker Patienten mit und ohne Sepsis im Vergleich zu 24 gesunden Kontrollpersonen und setzten sie in Beziehung zu klinischen Parametern, therapeutischen Interventionen und Überleben. Wir fanden signifikant erhöhte Serum-Calprotectin-Konzentrationen bei Patienten auf der Intensivstation sowie bei septischen Patienten im Vergleich zu den jeweiligen Kontrollen. Bei Patienten mit Begleiterkrankungen, z. B. einer koronaren Herzkrankheit, wurden niedrigere Calprotectin-Konzentrationen festgestellt. Die Calprotectin-Konzentrationen korrelierten stark mit dem CRP und standen in engem

Zusammenhang mit Parametern, die das Ausmaß der mechanischen Beatmung widerspiegeln (beispielsweise der inspiratorischen Sauerstofffraktion, FiO2).

Hohe Ausgangs-Calprotectin-Konzentrationen wurden als prädiktiv für ein schlechteres Gesamtüberleben bei septischen Patienten identifiziert, wohingegen steigende Calprotectin-Konzentrationen während der ersten Woche der Intensivbehandlung mit einem günstigen Langzeitergebnis verbunden waren.

Als Biomarker für Apoptose analysierten wir in **Kapitel 3** die **M30**-Serumkonzentrationen bei kritisch kranken Patienten (mit und ohne Sepsis) bei der Aufnahme auf die medizinische Intensivstation im Vergleich zu gesunden Kontrollpersonen.

Unsere Studie zeigte, dass die Serumspiegel der mit Apoptose assoziierten Keratin-Fragmente, die mittels spezifischem Antikörper M30 gemessen wurden, bei schwerkranken Patienten im Vergleich zu gesunden Kontrollpersonen signifikant erhöht sind, und zwar unabhängig vom Vorliegen einer Sepsis. Das zirkulierende M30 stand in engem Zusammenhang mit dem Schweregrad der Erkrankung, was sich in einer engen Korrelation mit prognostischen Scoring-Systemen wie APACHE-II und SOFA-Score zeigte, es unterschied sich jedoch nicht zwischen Patienten mit Sepsis und Intensivpatienten ohne Sepsis. Wir fanden heraus, dass die M30-Serumspiegel mit Biomarkern für Entzündungen, Zellschäden, Nieren- und Leberversagen bei kritisch kranken Patienten korrelierten. Die Apoptose der Hepatozyten könnte wesentlich zu den hohen zirkulierenden M30-Werten bei schwerkranken Patienten beitragen, da wir bei Patienten mit chronischen Lebererkrankungen bemerkenswert hohe Werte beobachteten. Hohe M30-Werte (>250,8 U/L) bei der Aufnahme in die Intensivstation deuten auf ein schlechtes ICU-Überleben hin.

In **Kapitel 4** bewerteten wir das Potenzial der **High-Mobility Group Box 1 (HMGB1)** als klinischen Biomarker. Wir fanden signifikant erhöhte HMGB1-Spiegel bei kritisch kranken Patienten im Vergleich zu gesunden Kontrollpersonen, und erhöhte HMGB1-Plasmaspiegel waren unabhängig vom Vorliegen einer Sepsis. HMGB1 stand nicht im Zusammenhang mit dem Schweregrad der Erkrankung, dem Organversagen oder der Sterblichkeit auf der Intensivstation. Wir beobachteten einen Trend zu niedrigeren HMGB1-Spiegeln bei Intensivpatienten mit vorbestehender Adipositas, Typ-2-Diabetes und Patienten mit terminaler dialysepflichtiger Nierenerkrankung.

Überraschenderweise zeigte unsere Studie keinen signifikanten Zusammenhang zwischen den HMGB1-Werten bei der Aufnahme in die Intensivstation und den klinischen Ergebnissen bei schwerkranken Patienten. Möglicherweise ist dies auf die Tatsache zurückzuführen, dass HMGB1 seine pathogene Rolle in den späteren Phasen

der Sepsis ausübt und wir die Blutproben unmittelbar bei der Aufnahme in die Intensivstation entnommen haben.

Künftige Studien sollten die potenzielle Wertigkeit von HMGB1 durch Messung seiner Plasmakonzentrationen zu späteren Zeitpunkten im Verlauf der kritischen Erkrankung bewerten.

In **Teil 2** untersuchten wir die **Rolle spezifischer Adipokine bei kritisch kranken Patienten**. Adipokine stellen eine wichtige kausale Verbindung zwischen Hyperglykämie, Insulinresistenz und übermäßigen systemischen Entzündungsreaktionen bei Sepsis und kritischen Erkrankungen dar.

Wir untersuchten daher das Potenzial von **Visfatin**, **CTRP1**, **CTRP3** und **Perilipin 2 (PLIN2)** als Biomarker bei kritisch kranken Patienten.

In **Kapitel 5** analysierten wir die Serumspiegel von **Visfatin** bei kritisch kranken Patienten bei der Aufnahme auf die Intensivstation. Es zeigte sich, dass die Visfatin-Werte bei Patienten auf der Intensivstation, insbesondere bei Patienten mit Sepsis, im Vergleich zu gesunden Kontrollpersonen deutlich erhöht waren.

Wir konnten einen starken Zusammenhang zwischen den Visfatin-Serumkonzentrationen und dem Schweregrad der Erkrankung sowie vorliegenden Organversagen nachweisen. Interessanterweise konnte jedoch kein Unterschied in den Visfatin-Konzentrationen zwischen Patienten mit oder ohne Adipositas oder Typ-2-Diabetes festgestellt werden. Dies stützt die Hypothese, dass die zirkulierenden Visfatin-Konzentrationen bei kritischen Erkrankungen in erster Linie auf das Ausmaß der Entzündung und nicht auf die Adipositas selbst zurückzuführen sind.

Die Visfatin-Spiegel korrelierten mit Biomarkern für Nierenversagen, Leberdysfunktion und anderen Adipokinen (z. B. Resistin, Leptin, Adiponektin) bei kritisch kranken Patienten.

In unserer Studie waren hohe Visfatin-Werte bei der Aufnahme in die Intensivstation ein hervorragender Prädiktor für die Gesamtmortalität während einer zweijährigen Nachbeobachtungszeit.

Da Mitglieder der Adipokin-Familie der C1q/TNF-ähnlichen Proteine (CTRP) als wichtige Regulatoren des Stoffwechsels und Vermittler in der Interaktion zwischen Insulinresistenz, Adipositas und Entzündung beschrieben worden, haben wir das Potenzial von **CTRP1** und **CTRP3** als Biomarker bei kritisch kranken Patienten in **Kapitel 6 und 7** untersucht.

Wir fanden signifikant erhöhte CTRP1-Plasmakonzentrationen bei kritisch kranken Patienten bei der Aufnahme auf die internistische Intensivstation (ICU) im Vergleich zu gesunden Kontrollen. Bei Patienten mit Sepsis waren die CTRP1-Konzentrationen im Vergleich zu Patienten ohne Sepsis signifikant höher.

Obwohl zirkulierendes CTRP1 bereits früher als Biomarker für Patienten außerhalb der Intensivstation vorgeschlagen wurde, konnten wir in unserer Kohorte keinen Zusammenhang mit dem Schweregrad der Erkrankung oder der Sterblichkeit feststellen. Wir konnten einen engen Zusammenhang zwischen erhöhtem CTRP1 und bereits bestehendem Diabetes sowie der langfristigen Blutzuckereinstellung, die sich im HbA1c-Wert widerspiegelt nachweisen. CTRP1 korrelierte auch mit Markern für die systemische Entzündungsreaktion, Nierenfunktion, Leberschaden und Cholestase. Zusammenfassend stellten wir fest, dass CTRP1 in das komplexe Netzwerk von Adipokinen in der Pathogenese von kritischen Erkrankungen, Sepsis und Organversagen eingebunden ist, was auf eine mögliche klinische Verwendbarkeit als Biomarker hindeutet.

Wir konnten jedoch keinen klinischen Nutzen von CTRP1 als Biomarker in unserer Kohorte schwerkranker Patienten nachweisen. Daher sind mechanistische Studien erforderlich, um die pathogene Rolle von CTRP1 in Stoffwechsel- und Entzündungsprozessen bei kritischen Erkrankungen zu klären.

Wir untersuchten auch **CTRP3** bei kritisch kranken Patienten bei der Aufnahme auf die Intensivstation (**Kapitel 7**). Bei kritisch kranken Patienten waren die CTRP3-Plasmaspiegel im Vergleich zu gesunden Kontrollpersonen signifikant erniedrigt, und niedrige CTRP3-Spiegel waren in hohem Maße mit dem Vorliegen einer Sepsis verbunden. Es konnte kein Zusammenhang zwischen den CTRP3-Spiegeln und Adipositas oder Diabetes nachgewiesen werden.

Die CTRP3-Plasmakonzentrationen korrelierten invers mit inflammatorischen Zytokinen und klassischen Sepsismarkern, was die anti-inflammatosichen Eigenschaften von CTRP3 untermauert. CTRP3-Konzentrationen unter 620,6 ng/ml waren Prädiktoren der Gesamtmortalität bei schwerkranken Patienten.

Perilipin 2 (PLIN2), ein Mitglied der Lipid-Droplet-Proteine, ist maßgeblich am Fettstoffwechsel beteiligt und wurde kürzlich mit chronischen Entzündungen wie Herz-Kreislauf-Erkrankungen in Verbindung gebracht. Dies veranlasste uns, die Serumkonzentration von PLIN2 bei schwerkranken Patienten bei der Aufnahme auf die Intensivstation im Vergleich zu gesunden Kontrollpersonen zu messen (**Kapitel 8**).

Im Vergleich zu den Kontrollen waren die Serum-PLIN2-Konzentrationen bei kritisch kranken Patienten bei der Aufnahme auf die Intensivstation erhöht. PLIN2 war ein unabhängiger Indikator für ein Multiorganversagen bei der Aufnahme auf die Intensivstation und konnte auch unabhängig das Auftreten eines Multiorganversagens 48 Stunden nach der Aufnahme in die Intensivstation vorhersagen. Darüber hinaus waren die PLIN2-Serumspiegel mit schwerem respiratorischen Organversagen assoziiert. Insgesamt könnte Serum-PLIN2 könnte ein nützlicher Biomarker für die Vorhersage von Multiorganversagen auf der Intensivstation sein.

Teil 3 dieser Arbeit befasst sich mit dem Thema **Biomarker für die biologischen Stress-Reaktion bei kritischen Erkrankungen**. Biologischer Stress bei kritischen Erkrankungen ist die Art und Weise, wie der Körper auf schwere Insulte wie Infektionen, Schock, Trauma und Stoffwechselveränderungen reagiert. In Teil 3 dieser Arbeit haben wir die Rolle von **Copeptin**, **mid-regional pro atrial natriuretic peptide (MR-proANP)** und **Clusterin** als klinische Biomarker für biologischen Stress bei kritisch kranken Patienten untersucht.

Biologischer Stress aktiviert die Hypothalamus-Hypophysen-Nebennieren-Achse sowie die Vasopressin-Ausschüttung. **Copeptin** spiegelt die biologisch funktionelle endogene Vasopressinausschüttung und damit den Grad des biologischen Stresses bei schwerkranken Patienten wider.

Wir analysierten die Copeptin-Spiegel im Plasma in einer prospektiven, monozentrischen Beobachtungsstudie, die kritisch kranke Patienten bei der Aufnahme auf die medizinische Intensivstation einschloss (**Kapitel 9**). Bei der Aufnahme auf die Intensivstation waren die Copeptin-Plasmaspiegel bei kritisch kranken Patienten im Vergleich zu gesunden Kontrollpersonen signifikant erhöht. Weder die Sepsis als Ursache der kritischen Erkrankung noch vorbestehende Stoffwechselstörungen (Typ-2-Diabetes, Adipositas) hatten einen Einfluss auf die Copeptin-Spiegel.

Wir fanden eine enge Korrelation von Plasma-Copeptin mit dem Schweregrad der Erkrankung (z. B. APACHE-II-Score) und Biomarkern für Entzündung, Nierenversagen, Stoffwechsel, Gefäßtonus und Gewebeperfusion. Erhöhte Copeptin-Werte bei der Aufnahme in die Intensivstation sagten die Kurz- und Langzeitmortalität voraus. Die Sterblichkeit wurde während einer zweijährigen Nachbeobachtungszeit bewertet.

Atriales natruretisches Peptid (ANP) hat diuretische, natriuretische und vasoaktive Wirkungen. Atrialer Wandstress ist die Hauptursache für die ANP-Sekretion. Bei kritischen Erkrankungen scheint ANP neben den bekannten Auswirkungen auf die Gefäß- und Flüssigkeitshomöostase auch regulatorische Funktionen bei systemischen Entzündungen zu übernehmen.

Wir untersuchten die Plasmakonzentrationen von **mid-regional pro atrial natriuretic peptide (MR-proANP)** bei kritisch kranken Patienten mit und ohne Sepsis bei der Aufnahme auf die internistische Intensivstation (**Kapitel 10**). Die MR-proANP-Plasmaspiegel waren bei kritisch kranken Patienten signifikant erhöht, wobei die höchsten Werte bei Patienten mit Sepsis im Vergleich zu gesunden Kontrollpersonen nachgewiesen worden. Wir beobachteten eine enge Korrelation der MR-proANP-Plasmakonzentrationen mit inflammatorischen Zytokinen, Markern für Organdysfunktion und verschiedenen Adipozytokinen wie Resistin, Retinol-bindendes Protein 4 (RBP4) und Adiponektin. Hohe MR-proANP-Werte über 227,0 pmol/l sagten ein signifikant erhöhtes Sterberisiko voraus.

Wir konnten eindeutig nachweisen, dass MR-proANP mit Organdysfunktion, Sepsis und Sterblichkeitsrisiko assoziiert ist, was die Rolle von zirkulierendem MR-proANP als diagnostischem und prognostischem Biomarker bei schwerkranken Patienten unterstreicht.

Clusterin wurde als Mediator der zellulären Stressreaktion beschrieben, die durch Organversagen, systemische Entzündungen und schwere Stoffwechselstörungen ausgelöst wird. Um die Wertigkeit von Clusterin als Biomarker bei kritisch kranken Patienten zu bestimmen, haben wir die Clusterin-Plasmakonzentrationen bei Intensivpatienten analysiert (**Kapitel 11**).

Die Clusterin-Plasmakonzentrationen waren bei kritisch kranken Patienten im Vergleich zu gesunden Probanden deutlich erhöht. Bei Patienten mit Sepsis wurden signifikant niedrigere Werte beobachtet. Clusterin korrelierte invers mit Markern der systemischen Entzündungsreaktion wie CRP und PCT. Darüber hinaus waren die Clusterin-Werte bei Intensivpatienten mit vorbestehender Fettleibigkeit und/oder Typ-2-Diabetes höher. Dies passt zu Erkenntnissen aus vorherigen Studien, wonach Clusterin direkt mit der Insulinresistenz korreliert und die Clusterin-Spiegel mit zunehmender Insulinsensitivität bei Typ-2-Diabetes abnehmen.

Wir konnten Clusterin nicht mit dem Schweregrad der Erkrankung, Organversagen oder Sterblichkeit auf der Intensivstation assoziieren. Obwohl Clusterin wichtige pathogene Funktionen der zellulären Stressreaktion ausübt, konnten wir keine klinische Anwendbarkeit von Clusterin als Biomarker bei kritischen Erkrankungen nachweisen.

Die aus den vorgestellten Ergebnissen gewonnenen Erkenntnisse sollen zu einem besseren Verständnis der Regulation und der pathophysiologischen Rolle der untersuchten Biomarker bei kritischen Erkrankungen und Sepsis beitragen. Ziel unserer Studien war es, sie einerseits als potentielle neuartige diagnostische und prognostische Biomarker zu präsentieren und möglicherweise Perspektiven für neue therapeutische

Ansätze in der Intensivmedizin zu eröffnen oder anderseits ihren begrenzten klinischen Nutzen in diesem komplexen klinischen Umfeld darzustellen.

Addendum

Impact paragraph

Diagnostic and prognostic implications

To date, it has not been possible to identify a biomarker that can reliably distinguish infection or sepsis from sterile, non-infectious inflammation. Currently, most frequently used biomarkers for this purpose are procalcitonin (PCT) and C-reactive protein (CRP).

PCT has been proven as an useful biomarker for early diagnosis of sepsis in critically ill patients[13]. More important than a single absolute value is the kinetics of PCT. When procalcitonin decreased by at least 80%, the negative predictive value for ICU/in-hospital mortality was reported with 90%. Stagnation of PCT levels or even increase was associated with an unfavorable prognosis, with respective positive predictive values for mortality of approximately 50%[165]. Moreover, PCT is of great importance with regard to guide the duration of an anti-infective therapy in critically ill patients[8,166].

CRP is an acute phase protein, that is secreted by hepatocytes in response to inflammation, infection and tissue damages. The accuracy of determination of CRP levels for the diagnosis of bacterial infection have been shown inferior to PCT, most likely due to a delayed rise (6 to 10 hours after infection) and a prolonged half-life (up to 48h) in comparison to PCT (increase 2h after infection, half-life 4 to 6h)[167].

In the intensive care setting, biomarkers are not only used to confirm the diagnosis of the underlying disease, for instance sepsis, but also to differentiate from other critical clinical conditions, monitor the effectiveness of therapeutic interventions and to predict prognosis. In a recently published systematic review, prognostic associations of routine blood measurements in the intensive care unit have been examined. A total of 128 studies in adult critical care investigating associations between parameters measured routinely in whole blood, plasma or serum, and outcome parameters such as length of stay or mortality have been identified[168].

Interestingly, for the majority of examined biomarkers the certainty of evidence for associations with outcome was low or moderate. Only increased red cell distribution width, low platelet count, increased neutrophil-to-lymphocyte ratio and decreased serum albumin have been demonstrated to be consistently associated with mortality, whereas data on CRP were inconsistent.

The studies we performed and the results that are presented in this thesis aimed at improving the understanding of the regulation and pathophysiological role of the investigated biomarkers in critical illness and sepsis and to demonstrate their potential as novel diagnostic and prognostic biomarkers.

We identified calprotectin as predictive for poor 180- and 365-days outcome in septic patients, with increasing calprotectin during the course of critical illness indicates an improved overall survival (**chapter 2**). Circulating M30 was closely associated with disease severity and mortality, supporting the utility of circulating levels of the apoptosis-related keratin fragment M30 as a prognostic biomarker at ICU admission (**chapter 3**). Visfatin was strongly associated with disease severity and organ failure and we demonstrated the validity and performance of visfatin as a biomarker for the prediction of ICU or overall survival in critically ill patients (**chapter 5**). Low CTRP3 plasma concentrations at ICU admission predicted the overall mortality in critically ill patients (**chapter 7**). Elevated copeptin levels at ICU admission predicted short-term and long-term mortality (**chapter 9**). High MR-proANP plasma concentrations indicated organ dysfunction, sepsis, disease severity and mortality risk in ICU patients (**chapter 10**).

An ideal biomarker has a high sensitivity and specificity and is suitable for clinical application in terms of diagnosis, staging, prognosis, and treatment of disease. Currently, there are just a few routinely clinically used biomarkers which meet these criteria in the setting of intensive care medicine. Nevertheless, laboratory values are widely used in daily clinical practice on the ICU and generate high healthcare costs.

A profound understanding of causative biological mechanisms and ongoing (biomarker) research in critical disease will a) allow to identify novel molecules as biomarkers, b) define biomarker thresholds and c) specify the adequate timepoint of assessment for implementation in clinical practice and by this improving patient care[169]. Humbly, we hope to have contributed a modest part to the large and steadily growing field of clinical biomarker research with our work.

Socio-economic implications

The identification, validation and integration of novel biomarkers into clinical routine is most likely associated with an increase in health care expenditure. Therefore, biomarker development is not only a matter of the clinical benefit of a specific tool, but also of monetary benefits.

Yet, cost-effectiveness and cost-utility studies are uncommon in the economic assessment of new clinical laboratory tests. However, it must first be noted, that a new biomarker will never make economic sense without clinical benefit[170]. Cost-utility studies can evaluate the ratio between the cost of a clinical test and the resulting benefit, displayed as the numbers of clinical events (e.g., early diagnosis of sepsis, prevention of cardiovascular events) or the amount of money gained per quality adjusted life year (QALY). According to the definition of the British National Institute for Health and Care Excellence (NICE), QALY is “a measure of the state of health of a person

or group in which the benefits, in terms of length of life, are adjusted to reflect the quality of life. One quality-adjusted life year (QALY) is equal to 1 year of life in perfect health"[171].

Generally, a threshold for financial expenditure of 50.000 US Dollar (USD) per QALY gained is considered as cost-effective[170].

As an example, in the US, early treatment of chronic left-ventricular heart failure with ACE inhibitors has been demonstrated as cost-effective with approximately 5600 USD per QALY. Screening (all patients >55 years) for asymptomatic left ventricular dysfunction by echocardiography has been proven to be not cost effective. Importantly, screening with BNP testing and performing echocardiography if the BNP is abnormal has been found to be is cost-effective in all populations over 55 years at USD 19.000 per QALY compared to no screening[172].

However, cost-effectiveness studies not common for clinical laboratory tests and very rarely in the critically ill patients. A recent meta-analysis revealed just a few publications on health economic evaluations in critically ill patients, comprising, with regard to laboratory biomarkers, PCT-guided antibiotic therapy and lactate testing[173].

With respect to the high economic and social burden of critical diseases, high quality economic studies in cooperation of scientists, economists and clinicians are urged, to improve understanding of cost-effectiveness in the complex setting of intensive care medicine[174].

References are given in the References section of the Addendum

Addendum

Abbreviations

ACE	angiotensin converting enzyme
ACTH	adrenocorticotropic hormone
ADH	anti-diuretic hormone
ADMA	asymmetric dimethylarginine
ADRP	adipose differentiation-related protein
ALF	acute liver failure
AMPK	AMP-activated protein kinase
ANP	atrial natriuretic peptide
APACHE-II	acute physiology and chronic health evaluation score II
AUROC	area under the receiver operating characteristic curve
AVP	arginine vasopressin
BMI	body mass index
BNP	brain natriuretic peptide
CAD	coronary artery disease
CCL	chemoattractant protein C-C motif chemokine ligand
cGMP	intracellular cyclic guanosine monophosphate
CNP	C-type natriuretic peptide
COPD	chronic obstructive pulmonary disease
COVID-19	coronavirus disease 2019
CRH	corticotropin-releasing hormone
CRP	C-reactive protein
CT	computertomography
CT-proET1	C-terminal proendothelin-1
CTRP	C1q tumor necrosis factor-like protein
CXCR	C-X-C chemokine receptor
DAMP	danger-associated molecular patterns
DM	diabetes mellitus
DNA	deoxyribonucleic acid
e.g.	exempli gratia (for example)
eNampt	extracellular molecule of nicotinamide phosphoribosyltransferase
esp.	especially
FDA	US Food and Drug Administration

FiO_2	inspiratory oxygen fraction
GFR	glomerular filtration rate
GPCR	G-protein-coupled receptor
HbA1c	glycated hemoglobin
HDL	high density lipoprotein
HMG	high-mobility group
HMGB1	high-mobility group box 1
HOMA	homeostasis model assessment
HSP	heat shock protein
i.e.	id est (that is)
ICAM	intracellular adhesion molecule
ICU	intensive care unit
IL	interleukin
iNampt	intracellular molecule of nicotinamide phosphoribosyltransferase
K18	keratin18
kDa	kilodalton
LDL	low density lipoprotein
LPS	lipopolysaccharide
MCP	monocyte chemoattractant protein
MELD	model of end stage liver disease
MOD	multi-organ dysfunction
MOF	multi-organ failure
MR-proANP	mid-regional pro atrial natriuretic peptide
NAD+	nicotinamide adenine dinucleotide
NAFLD	non-alcoholic fatty liver disease
NF-κB	nuclear factor 'kappa-light-chain-enhancer' of activated B-cells
NICE	(British) National Institute for Health and Care Excellence
NIH	National Institutes of Health
NLR	nucleotide-binding oligomerization domain-like receptors (NOD)-like receptor
NMN	nicotinamide mononucleotide
NT-proANP	N-terminal proatrial natriuretic peptide
NT-proCNP	N-terminal pro-C-type natriuretic peptide
PAMP	pathogen-associated molecular patterns
PaO_2	partial pressure of arterial oxygen

PBEF	pre-B-cell colony-enhancing factor
PCT	procalcitonin
PEEP	positive end-expiratory pressure
PLIN	perilipin
P_{max}	maximum airway pressure
PRR	pattern recognition receptors
QALY	quality adjusted life year
RAGE	receptor for advanced glycation end products
RBP4	retinol-binding protein 4
RLR	retinoic acid-inducible gene I (RIGI)-like receptor
ROC	receiver operating characteristic curve
RRT	renal replacement therapy
SAPS2	simplified acute physiology score 2
SARS-CoV-2	severe acute respiratory syndrome coronavirus type 2
SDMA	symmetric dimethylarginine
SOFA	sequential organ failure assessment score
STEMI	ST-elevation myocardial infarction
suPAR	soluble urokinase-type plasminogen activator receptor
TIP47	tail-interacting protein of 47kDa
TLR	toll-like receptor
TNF	tumor necrosis factor
TREM	triggering receptor expressed on myeloid cells
US	United States (of America)
USD	US Dollar
WHO	World Health Organization

Addendum

References

1. Safety WHOIPoC. Biomarkers in risk assessment : validity and validation. World Health Organization. 2001.
2. Biomarkers Definitions Working G. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther. 2001;69:89-95.
3. Vanden Berghe T, Hoste E. Paving the way for precision medicine v2.0 in intensive care by profiling necroinflammation in biofluids. Cell Death Differ. 2019;26:83-98.
4. Singer M, Deutschman CS, Seymour CW, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA. 2016;315:801-810.
5. Angus DC, van der Poll T. Severe sepsis and septic shock. N Engl J Med. 2013;369:2063.
6. Levy MM, Artigas A, Phillips GS, et al. Outcomes of the Surviving Sepsis Campaign in intensive care units in the USA and Europe: a prospective cohort study. Lancet Infect Dis. 2012;12:919-924.
7. Iwashyna TJ, Ely EW, Smith DM, et al. Long-term cognitive impairment and functional disability among survivors of severe sepsis. JAMA. 2010;304:1787-1794.
8. Evans L, Rhodes A, Alhazzani W, et al. Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock 2021. Crit Care Med. 2021;49:e1063-e1143.
9. van Engelen TSR, Wiersinga WJ, Scicluna BP, et al. Biomarkers in Sepsis. Crit Care Clin. 2018;34:139-152.
10. Aronson JK. Biomarkers and surrogate endpoints. Br J Clin Pharmacol. 2005;59:491-494.
11. Pepys MB, Hirschfield GM. C-reactive protein: a critical update. J Clin Invest. 2003;111:1805-1812.
12. Silvestre JP, Coelho LM, Povoa PM. Impact of fulminant hepatic failure in C-reactive protein? J Crit Care. 2010;25:657 e657-612.
13. Wacker C, Prkno A, Brunkhorst FM, et al. Procalcitonin as a diagnostic marker for sepsis: a systematic review and meta-analysis. Lancet Infect Dis. 2013;13:426-435.
14. Kyriazopoulou E, Giamarellos-Bourboulis EJ. Antimicrobial Stewardship Using Biomarkers: Accumulating Evidence for the Critically Ill. Antibiotics (Basel). 2022;11:367.
15. Selberg O, Hecker H, Martin M, et al. Discrimination of sepsis and systemic inflammatory response syndrome by determination of circulating plasma concentrations of procalcitonin, protein complement 3a, and interleukin-6. Crit Care Med. 2000;28:2793-2798.
16. Jawa RS, Anillo S, Huntoon K, et al. Analytic review: Interleukin-6 in surgery, trauma, and critical care: part I: basic science. J Intensive Care Med. 2011;26:3-12.
17. Striz I, Trebichavsky I. Calprotectin - a pleiotropic molecule in acute and chronic inflammation. Physiol Res. 2004; 53: 245-253.
18. Steinbakk M, Naess-Andresen CF, Lingaas E, et al. Antimicrobial actions of calcium binding leucocyte L1 protein, calprotectin. Lancet. 1990;336:763-765.
19. Ma L, Sun P, Zhang JC, et al. Proinflammatory effects of S100A8/A9 via TLR4 and RAGE signaling pathways in BV-2 microglial cells. Int J Mol Med. 2017;40:31-38.
20. Jukic A, Bakiri L, Wagner EF, et al. Calprotectin: from biomarker to biological function. Gut. 2021;70: 1978-1988.
21. Sipponen T, Savilahti E, Kolho KL, et al. Crohn's disease activity assessed by fecal calprotectin and lactoferrin: correlation with Crohn's disease activity index and endoscopic findings. Inflamm Bowel Dis. 2008;14:40-46.
22. De Jong HK, Achouiti A, Koh GC, et al. Expression and function of S100A8/A9 (calprotectin) in human typhoid fever and the murine Salmonella model. PLoS Negl Trop Dis. 2015;9:e0003663.
23. Raju MS, V J, Kamaraju RS, et al. Continuous evaluation of changes in the serum proteome from early to late stages of sepsis caused by Klebsiella pneumoniae. Mol Med Rep. 2016;13:4835-4844.
24. Larsson A, Tyden J, Johansson J, et al. Calprotectin is superior to procalcitonin as a sepsis marker and predictor of 30-day mortality in intensive care patients. Scand J Clin Lab Invest. 2020;80:156-161.
25. Udeh R, Advani S, de Guadiana Romualdo LG, et al. Calprotectin, an Emerging Biomarker of Interest in COVID-19: A Systematic Review and Meta-Analysis. J Clin Med. 2021;10:775.
26. Wan Y, Li J, Shen L, et al. Enteric involvement in hospitalised patients with COVID-19 outside Wuhan. Lancet Gastroenterol Hepatol. 2020;5:534-535.

27. Cinel I, Dellinger RP. Advances in pathogenesis and management of sepsis. Curr Opin Infect Dis. 2007; 20:345-352.
28. Chen GY, Nunez G. Sterile inflammation: sensing and reacting to damage. Nat Rev Immunol. 2010;10: 826-837.
29. Barichello T, Generoso JS, Singer M, et al. Biomarkers for sepsis: more than just fever and leukocytosis-a narrative review. Crit Care. 2022;26:14.
30. Nedeva C. Inflammation and Cell Death of the Innate and Adaptive Immune System during Sepsis. Biomolecules 2021;11:1011.
31. Ku NO, Strnad P, Bantel H, et al. Keratins: Biomarkers and modulators of apoptotic and necrotic cell death in the liver. Hepatology. 2016;64:966-976.
32. Mazzolini G, Sowa JP, Canbay A. Cell death mechanisms in human chronic liver diseases: a far cry from clinical applicability. Clin Sci. 2016;130:2121-2138.
33. Ma KC, Schenck EJ, Pabon MA, et al. The Role of Danger Signals in the Pathogenesis and Perpetuation of Critical Illness. Am J Respir Crit Care Med. 2018;197:300-309.
34. Klune JR, Dhupar R, Cardinal J, et al. HMGB1: endogenous danger signaling. Mol Med. 2008;14:476-484.
35. Kang R, Chen R, Zhang Q et al. HMGB1 in health and disease. Mol Aspects Med. 2014;40:1-116.
36. Chiba S, Baghdadi M, Akiba H, et al. Tumor-infiltrating DCs suppress nucleic acid-mediated innate immune responses through interactions between the receptor TIM-3 and the alarmin HMGB1. Nat Immunol. 2012;13:832-842.
37. Chen GY, Tang J, Zheng P, et al. CD24 and Siglec-10 selectively repress tissue damage-induced immune responses. Science 2009;323:1722-1725.
38. Xue J, Suarez JS, Minaai M, et al. HMGB1 as a therapeutic target in disease. J Cell Physiol. 2021;236: 3406-3419.
39. Ulloa L, Ochani M, Yang H, et al. Ethyl pyruvate prevents lethality in mice with established lethal sepsis and systemic inflammation. Proc Natl Acad Sci USA.2002;99:12351-12356.
40. Wang H, Liao H, Ochani M, et al. Cholinergic agonists inhibit HMGB1 release and improve survival in experimental sepsis. Nat Med. 2004;10:1216-1221.
41. Sunden-Cullberg J, Norrby-Teglund A, Rouhiainen A, et al. Persistent elevation of high mobility group box-1 protein (HMGB1) in patients with severe sepsis and septic shock. Crit Care Med. 2005;33: 564-573.
42. Angus DC, Yang L, Kong L, et al. Circulating high-mobility group box 1 (HMGB1) concentrations are elevated in both uncomplicated pneumonia and pneumonia with severe sepsis. Crit Care Med. 2007; 35:1061-1067.
43. Gaini S, Pedersen SS, Koldkjaer OG, et al. High mobility group box-1 protein in patients with suspected community-acquired infections and sepsis: a prospective study. Crit Care. 2007;11:R32.
44. Karlsson S, Pettila V, Tenhunen J, et al. HMGB1 as a predictor of organ dysfunction and outcome in patients with severe sepsis. Intensive Care Med. 2008;34:1046-1053.
45. Gibot S, Massin F, Cravoisy A, et al. High-mobility group box 1 protein plasma concentrations during septic shock. Intensive Care Med. 2007;33:1347-1353.
46. Chiappalupi S, Salvadori L, Donato R, et al. Hyperactivated RAGE in Comorbidities as a Risk Factor for Severe COVID-19-The Role of RAGE-RAS Crosstalk. Biomolecules 2021;11:876.
47. Passos FRS, Heimfarth L, Monteiro BS, et al. Oxidative stress and inflammatory markers in patients with COVID-19: Potential role of RAGE, HMGB1, GFAP and COX-2 in disease severity. Int Immunopharmacol. 2022;104:108502.
48. Yamada H, Okamoto M, Nagasaki Y, et al. Analysis of Early Biomarkers Associated with the Development of Critical Respiratory Failure in Coronavirus Disease 2019 (COVID-19). Diagnostics (Basel). 2022;12: 339.
49. Kovalaske MA, Gandhi GY. Glycemic control in the medical intensive care unit. J Diabetes Sci Technol. 2009;3:1330-1341.
50. Finfer S, Heritier S, Committee NSM, et al. The NICE-SUGAR (Normoglycaemia in Intensive Care Evaluation and Survival Using Glucose Algorithm Regulation) Study: statistical analysis plan. Crit Care Resusc. 2009;11:46-57.
51. Karetnikova V, Gruzdeva O, Uchasova E, et al. Glucose levels as a prognostic marker in patients with ST-segment elevation myocardial infarction: a case-control study. BMC Endocr Disord. 2016;16:31.

52. Lee TF, Burt MG, Heilbronn LK, et al. Relative hyperglycemia is associated with complications following an acute myocardial infarction: a post-hoc analysis of HI-5 data. Cardiovasc Diabetol. 2017;16:157.
53. Planer D, Witzenbichler B, Guagliumi G, et al. Impact of hyperglycemia in patients with ST-segment elevation myocardial infarction undergoing percutaneous coronary intervention: the HORIZONS-AMI trial. Int J Cardiol. 2013;167:2572-2579.
54. Ng KW, Allen ML, Desai A, et al. Cardioprotective effects of insulin: how intensive insulin therapy may benefit cardiac surgery patients. Circulation. 2012;125:721-728.
55. Scheen M, Giraud R, Bendjelid K. Stress hyperglycemia, cardiac glucotoxicity, and critically ill patient outcomes current clinical and pathophysiological evidence. Physiol Rep. 2021;9:e14713.
56. Ouchi N, Parker JL, Lugus JJ, et al. Adipokines in inflammation and metabolic disease. Nat Rev Immunol. 2011;11:85-97.
57. Vachharajani V, Vital S. Obesity and sepsis. J Intensive Care Med. 2006;21:287-295.
58. Ouchi N, Kihara S, Funahashi T, et al. Obesity, adiponectin and vascular inflammatory disease. Curr Opin Lipidol. 2003;14:561-566.
59. Jia SH, Li Y, Parodo J, et al. Pre-B cell colony-enhancing factor inhibits neutrophil apoptosis in experimental inflammation and clinical sepsis. J Clin Invest. 2004;113:1318-1327.
60. Hong SB, Huang Y, Moreno-Vinasco L, et al. Essential role of pre-B-cell colony enhancing factor in ventilator-induced lung injury. Am J Respir Crit Care Med. 2008;178:605-617.
61. Moschen AR, Kaser A, Enrich B, et al. Visfatin, an adipocytokine with proinflammatory and immunomodulating properties. J Immunol. 2007;178:1748-1758.
62. Lee K, Huh JW, Lim CM, et al. Clinical role of serum pre-B cell colony-enhancing factor in ventilated patients with sepsis and acute respiratory distress syndrome. Scand J Infect Dis. 2013;45:760-765.
63. Hajri T, Gharib M, Kaul S, et al. Association between adipokines and critical illness outcomes. J Trauma Acute Care Surg. 2017;83:507-519.
64. Peterson JM, Aja S, Wei Z, et al. CTRP1 protein enhances fatty acid oxidation via AMP-activated protein kinase (AMPK) activation and acetyl-CoA carboxylase (ACC) inhibition. J Biol Chem. 2012;287: 1576-1587.
65. Wong GW, Krawczyk SA, Kitidis-Mitrokostas C, et al. Molecular, biochemical and functional characterizations of C1q/TNF family members: adipose-tissue-selective expression patterns, regulation by PPAR-gamma agonist, cysteine-mediated oligomerizations, combinatorial associations and metabolic functions. Biochem J. 2008;416:161-177.
66. Kim D, Park SY. C1q and TNF related protein 1 regulates expression of inflammatory genes in vascular smooth muscle cells. Genes Genomics. 2019;41:397-406.
67. Rhodes A, Evans LE, Alhazzani W, et al. Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock: 2016. Intensive Care Med. 2017;43:304-377.
68. Seldin MM, Tan SY, Wong GW. Metabolic function of the CTRP family of hormones. Rev Endocr Metab Disord. 2014;15:111-123.
69. Chen L, Wu F, Yuan S, et al. Identification and characteristic of three members of the C1q/TNF-related proteins (CTRPs) superfamily in Eudontomyzon morii. Fish Shellfish Immunol. 2016;59:233-240.
70. Yi W, Sun Y, Yuan Y, et al. C1q/tumor necrosis factor-related protein-3, a newly identified adipokine, is a novel antiapoptotic, proangiogenic, and cardioprotective molecule in the ischemic mouse heart. Circulation. 2012;125:3159-3169.
71. Peterson JM, Wei Z, Wong GW. C1q/TNF-related protein-3 (CTRP3), a novel adipokine that regulates hepatic glucose output. J Biol Chem. 2010;285:39691-39701.
72. Yoo HJ, Hwang SY, Hong HC, et al. Implication of progranulin and C1q/TNF-related protein-3 (CTRP3) on inflammation and atherosclerosis in subjects with or without metabolic syndrome. PLoS One. 2013;8: e55744.
73. Wei WY, Ma ZG, Zhang N, et al. Overexpression of CTRP3 protects against sepsis-induced myocardial dysfunction in mice. Mol Cell Endocrinol. 2018;476:27-36.
74. Kopp A, Bala M, Buechler C, et al. C1q/TNF-related protein-3 represents a novel and endogenous lipopolysaccharide antagonist of the adipose tissue. Endocrinology. 2010;151:5267-5278.
75. Schmid A, Kopp A, Hanses F, et al. C1q/TNF-related protein-3 (CTRP-3) attenuates lipopolysaccharide (LPS)-induced systemic inflammation and adipose tissue Erk-1/-2 phosphorylation in mice in vivo. Biochem Biophys Res Commun. 2014;452:8-13.

76. Schmid A, Vlacil AK, Schuett J, et al. Anti-Inflammatory Effects of C1q/Tumor Necrosis Factor-Related Protein 3 (CTRP3) in Endothelial Cells. Cells. 2021;10:2146.
77. Beller M, Herker E, Fullekrug J. Grease on-Perspectives in lipid droplet biology. Semin Cell Dev Biol. 2020;108:94-101.
78. Sztalryd C, Brasaemle DL. The perilipin family of lipid droplet proteins: Gatekeepers of intracellular lipolysis. Biochim Biophys Acta Mol Cell Biol Lipids. 2017;1862:1221-1232.
79. Bosma M, Hesselink MK, Sparks LM, et al. Perilipin 2 improves insulin sensitivity in skeletal muscle despite elevated intramuscular lipid levels. Diabetes. 2012;61:2679-2690.
80. Liu F, Wang C, Zhang L, et al. Metformin prevents hepatic steatosis by regulating the expression of adipose differentiation-related protein. Int J Mol Med. 2014;33:51-58.
81. Xu B, Zhao H, Wang S, et al. Increased ADRP expression in human atherosclerotic lesions correlates with plaque instability. Int J Clin Exp Med. 2015;8:5414-5421.
82. Matsubara J, Honda K, Ono M, et al. Identification of adipophilin as a potential plasma biomarker for colorectal cancer using label-free quantitative mass spectrometry and protein microarray. Cancer Epidemiol Biomarkers Prev. 2011;20:2195-2203.
83. Zhang XD, Li W, Zhang N, et al. Identification of adipophilin as a potential diagnostic tumor marker for lung adenocarcinoma. Int J Clin Exp Med. 2014;7:1190-1196.
84. Conte M, Santoro A, Collura S, et al. Circulating perilipin 2 levels are associated with fat mass, inflammatory and metabolic markers and are higher in women than men. Aging (Albany NY). 2021;13: 7931-7942.
85. Conte M, Vasuri F, Bertaggia E, et al. Differential expression of perilipin 2 and 5 in human skeletal muscle during aging and their association with atrophy-related genes. Biogerontology. 2015;16: 329-340.
86. Conte M, Vasuri F, Trisolino G, et al. Increased Plin2 Expression in Human Skeletal Muscle Is Associated with Sarcopenia and Muscle Weakness. PLoS One. 2013;8:e73709.
87. Schetz M, De Jong A, Deane AM, et al. Obesity in the critically ill: a narrative review. Intensive Care Med. 2019;45:757-769.
88. Bolignano D, Cabassi A, Fiaccadori E, et al. Copeptin (CTproAVP), a new tool for understanding the role of vasopressin in pathophysiology. Clin Chem Lab Med. 2014;52:1447-1456.
89. Jochberger S, Morgenthaler NG, Mayr VD, et al. Copeptin and arginine vasopressin concentrations in critically ill patients. J Clin Endocrinol Metab. 2006;91:4381-4386.
90. Morgenthaler NG. Copeptin: a biomarker of cardiovascular and renal function. Congest Heart Fail. 2010;16 Suppl 1:S37-44.
91. Saleem U, Khaleghi M, Morgenthaler NG, et al. Plasma carboxy-terminal provasopressin (copeptin): a novel marker of insulin resistance and metabolic syndrome. J Clin Endocrinol Metab. 2009;94: 2558-2564.
92. Smaradottir MI, Ritsinger V, Gyberg V, et al. Copeptin in patients with acute myocardial infarction and newly detected glucose abnormalities - A marker of increased stress susceptibility? A report from the Glucose in Acute Myocardial Infarction cohort. Diab Vasc Dis Res. 2017;14:69-76.
93. Jochberger S, Luckner G, Mayr VD, et al. Course of vasopressin and copeptin plasma concentrations in a patient with severe septic shock. Anaesth Intensive Care. 2006;34:498-500.
94. Seligman R, Papassotiriou J, Morgenthaler NG, et al. Copeptin, a novel prognostic biomarker in ventilator-associated pneumonia. Crit Care. 2008;12:R11.
95. Krychtiuk KA, Honeder MC, Lenz M, et al. Copeptin Predicts Mortality in Critically Ill Patients. PLoS One. 2017;12:e0170436.
96. Bie P. Natriuretic Peptides and Normal Body Fluid Regulation. Compr Physiol. 2018;8:1211-1249.
97. Fu S, Ping P, Wang F et al. Synthesis, secretion, function, metabolism and application of natriuretic peptides in heart failure. J Biol Eng. 2018;12:2.
98. Volpe M, Carnovali M, Mastromarino V. The natriuretic peptides system in the pathophysiology of heart failure: from molecular basis to treatment. Clin Sci. 2016;130:57-77.
99. Vesely DL. Atrial natriuretic peptide prohormone gene expression: hormones and diseases that upregulate its expression. IUBMB Life. 2002;53:153-159.
100. Morgenthaler NG, Struck J, Thomas B, et al. Immunoluminometric assay for the midregion of pro-atrial natriuretic peptide in human plasma. Clin Chem. 2004;50:234-236.

101. Seidler T, Pemberton C, Yandle T, et al. The amino terminal regions of proBNP and proANP oligomerise through leucine zipper-like coiled-coil motifs. Biochem Biophys Res Commun. 1999;255:495-501.
102. Li N, Song Z, Wang J, et al. Prognostic value of natriuretic peptides in severe trauma patients with multiple organ dysfunction syndrome. Exp Ther Med. 2015;10:792-796.
103. Vollmar AM. The role of atrial natriuretic peptide in the immune system. Peptides. 2005;26:1086-1094.
104. Moro C, Lafontan M. Natriuretic peptides and cGMP signaling control of energy homeostasis. Am J Physiol Heart Circ Physiol. 2013;304:H358-368.
105. Lipinska-Gediga M, Mierzchala M, Durek G. Pro-atrial natriuretic peptide (pro-ANP) level in patients with severe sepsis and septic shock: prognostic and diagnostic significance. Infection. 2012;40:303-309.
106. Rodriguez-Rivera C, Garcia MM, Molina-Alvarez M, et al. Clusterin: Always protecting. Synthesis, function and potential issues. Biomed Pharmacother. 2021;134:111174.
107. Rizzi F, Coletta M, Bettuzzi S. Chapter 2: Clusterin (CLU): From one gene and two transcripts to many proteins. Adv Cancer Res. 2009;104:9-23.
108. Bass-Stringer S, Ooi JYY, McMullen JR. Clusterin is regulated by IGF1-PI3K signaling in the heart: implications for biomarker and drug target discovery, and cardiotoxicity. Arch Toxicol. 2020;94: 1763-1768.
109. Strnad P, Tacke F, Koch A, et al. Liver - guardian, modifier and target of sepsis. Nat Rev Gastroenterol Hepatol. 2017;14:55-66.
110. Chousterman BG, Swirski FK, Weber GF. Cytokine storm and sepsis disease pathogenesis. Semin Immunopathol. 2017;39:517-528.
111. Aziz M, Jacob A, Wang P. Revisiting caspases in sepsis. Cell Death Dis 2014;5:e1526.
112. Wesche-Soldato DE, Swan RZ, Chung CS, et al. The apoptotic pathway as a therapeutic target in sepsis. Curr Drug Targets. 2007;8:493-500.
113. Huttunen R, Aittoniemi J. New concepts in the pathogenesis, diagnosis and treatment of bacteremia and sepsis. J Infect. 2011;63:407-419.
114. Harjai M, Bogra J, Kohli M, et al. Is suppression of apoptosis a new therapeutic target in sepsis? Anaesth Intensive Care 2013;41:175-183.
115. Roth GA, Krenn C, Brunner M, et al. Elevated serum levels of epithelial cell apoptosis-specific cytokeratin 18 neoepitope m30 in critically ill patients. Shock. 2004;22:218-220.
116. Hofer S, Brenner T, Bopp C, et al. Cell death serum biomarkers are early predictors for survival in severe septic patients with hepatic dysfunction. Crit Care. 2009;13:R93.
117. Lorente L, Martin MM, Gonzalez-Rivero AF, et al. Serum levels of caspase-cleaved cytokeratin-18 and mortality are associated in severe septic patients: pilot study. PLoS One. 2014;9:e109618.
118. Moore DJ, Greystoke A, Butt F, et al. A pilot study assessing the prognostic value of CK18 and nDNA biomarkers in severe sepsis patients. Clin Drug Invest. 2012;32:179-187.
119. Koch A, Weiskirchen R, Kunze J, et al. Elevated asymmetric dimethylarginine levels predict short- and long-term mortality risk in critically ill patients. J Crit Care. 2013;28:947-953.
120. Koch A, Voigt S, Kruschinski C, et al. Circulating soluble urokinase plasminogen activator receptor is stably elevated during the first week of treatment in the intensive care unit and predicts mortality in critically ill patients. Crit Care. 2011;15:R63.
121. Buendgens L, Yagmur E, Bruensing J, et al. C-terminal proendothelin-1 (CT-proET-1) is associated with organ failure and predicts mortality in critically ill patients. J Intensive Care. 2017;5:25.
122. Backes Y, van der Sluijs KF, Mackie DP, et al. Usefulness of suPAR as a biological marker in patients with systemic inflammation or infection: a systematic review. Intensive Care Med. 2012;38:1418-1428.
123. Adebayo D, Morabito V, Andreola F, et al. Mechanism of cell death in acute-on-chronic liver failure: a clinico-pathologic-biomarker study. Liver Int. 2015;35:2564-2574.
124. Chu PG, Weiss LM. Keratin expression in human tissues and neoplasms. Histopathology. 2002;40: 403-439.
125. Roth GA, Lebherz-Eichinger D, Ankersmit HJ, et al. Increased total cytokeratin-18 serum and urine levels in chronic kidney disease. Clin Chim Acta. 2011;412:713-717.
126. Hotchkiss RS, Schmieg RE, Jr., Swanson PE, et al. Rapid onset of intestinal epithelial and lymphocyte apoptotic cell death in patients with trauma and shock. Crit Care Med. 2000;28:3207-3217.
127. Hiramatsu M, Hotchkiss RS, Karl IE, et al. Cecal ligation and puncture (CLP) induces apoptosis in thymus, spleen, lung, and gut by an endotoxin and TNF-independent pathway. Shock. 1997;7:247-253.

128. Clark JA, Coopersmith CM. Intestinal crosstalk: a new paradigm for understanding the gut as the "motor" of critical illness. Shock. 2007;28:384-393.
129. Haak BW, Wiersinga WJ. The role of the gut microbiota in sepsis. Lancet Gastroenterol Hepatol. 2017; 2:135-143.
130. Bechmann LP, Jochum C, Kocabayoglu P, et al. Cytokeratin 18-based modification of the MELD score improves prediction of spontaneous survival after acute liver injury. J Hepatol 2010;53:639-647.
131. Hotchkiss RS, Chang KC, Swanson PE, et al. Caspase inhibitors improve survival in sepsis: a critical role of the lymphocyte. Nat Immunol. 2000;1:496-501.
132. Wesche-Soldato DE, Chung CS, Lomas-Neira J, et al. In vivo delivery of caspase-8 or Fas siRNA improves the survival of septic mice. Blood. 2005;106:2295-2301.
133. Hotchkiss RS, Nicholson DW. Apoptosis and caspases regulate death and inflammation in sepsis. Nat Rev Immunol. 2006;6:813-822.
134. FDA-NIH Biomarker Working Group. BEST (Biomarkers, EndpointS, and other Tools) Resource [Internet]. Silver Spring (MD): Food and Drug Administration (US); 2016-. Glossary. 2016 Jan 28 [Updated 2021 Nov 29]. Co-published by National Institutes of Health (US), Bethesda (MD).
135. Pedersen L, Nybo M, Poulsen MK, et al. Plasma calprotectin and its association with cardiovascular disease manifestations, obesity and the metabolic syndrome in type 2 diabetes mellitus patients. BMC Cardiovasc DCisord. 2014;14:196.
136. Jonsson N, Nilsen T, Gille-Johnson P, et al. Calprotectin as an early biomarker of bacterial infections in critically ill patients: an exploratory cohort assessment. Crit Care Resusc. 2017;19:205-213.
137. Lee CW, Kou HW, Chou HS, et al. A combination of SOFA score and biomarkers gives a better prediction of septic AKI and in-hospital mortality in critically ill surgical patients: a pilot study. World J Emerg Surg. 2018;13:41.
138. Dreschers S, Ohl K, Lehrke M, et al. Impaired cellular energy metabolism in cord blood macrophages contributes to abortive response toward inflammatory threats. Nat Commun. 2019;10:1685.
139. Hotchkiss RS, Moldawer LL, Opal SM et al. Sepsis and septic shock. Nat Rev Dis Primers. 2016;2:16045.
140. Wang H, Ward MF, Sama AE. Targeting HMGB1 in the treatment of sepsis. Expert Opin Ther Targets. 2014;18:257-268.
141. Wang H, Bloom O, Zhang M, et al. HMG-1 as a late mediator of endotoxin lethality in mice. Science 1999;285:248-251.
142. Qin S, Wang H, Yuan R, et al. Role of HMGB1 in apoptosis-mediated sepsis lethality. J Exp Med. 2006; 203:1637-1642.
143. Koch A, Gressner OA, Sanson E, et al. Serum resistin levels in critically ill patients are associated with inflammation, organ dysfunction and metabolism and may predict survival of non-septic patients. Crit Care. 2009;13:R95.
144. Koch A, Sanson E, Voigt S, et al. Serum adiponectin upon admission to the intensive care unit may predict mortality in critically ill patients. J Crit Care. 2011;26:166-174.
145. Luk T, Malam Z, Marshall JC. Pre-B cell colony-enhancing factor (PBEF)/visfatin: a novel mediator of innate immunity. J Leukoc Biol. 2008;83:804-816.
146. Chalupova L, Zakovska A, Adamcova K. Development of a novel enzyme-linked immunosorbent assay (ELISA) for measurement of serum CTRP1: a pilot study: measurement of serum CTRP1 in healthy donors and patients with metabolic syndrome. Clin Biochem. 2013;46:73-78.
147. Shabani P, Emamgholipour S, Doosti M. CTRP1 in Liver Disease. Adv Clin Chem. 2017;79:1-23.
148. Schmid A, Kopp A, Hanses F et al. The novel adipokine C1q/TNF-related protein-3 is expressed in human adipocytes and regulated by metabolic and infection-related parameters. Exp Clin Endocrinol Diabetes. 2012;120:611-617.
149. Szinnai G, Morgenthaler NG, Berneis K, et al. Changes in plasma copeptin, the c-terminal portion of arginine vasopressin during water deprivation and excess in healthy subjects. J Clin Endocrinol Metab. 2007;92:3973-3978.
150. Zhang Q, Dong G, Zhao X, et al. Prognostic significance of hypothalamic-pituitary-adrenal axis hormones in early sepsis: a study performed in the emergency department. Intensive Care Med. 2014;40: 1499-1508.
151. Laribi S, Lienart D, Castanares-Zapatero D, et al. CT-proAVP IS NOT A GOOD PREDICTOR OF VASOPRESSOR NEED IN SEPTIC SHOCK. Shock 2015; 44: 330-335. DOI: 10.1097/SHK.0000000000000436

152. Witthaut R. Science review: natriuretic peptides in critical illness. Crit Care. 2004;8:342-349.
153. Berendes E, Van Aken H, Raufhake C, et al. Differential secretion of atrial and brain natriuretic peptide in critically ill patients. Anesth Analg. 2001;93:676-682.
154. Wong PC, Guo J, Zhang A. The renal and cardiovascular effects of natriuretic peptides. Adv Physiol Educ. 2017;41:179-185.
155. Gille J, Schmidt J, Kremer T, et al. Evaluation of MR-proANP and copeptin for sepsis diagnosis after burn injury. J Crit Care. 2019;52:149-155.
156. Hicks CW, Engineer RS, Benoit JL, et al. Procalcitonin as a biomarker for early sepsis in the emergency department. Eur J Emerg Med. 2014;21:112-117.
157. Guignant C, Venet F, Voirin N, et al. Proatrial natriuretic peptide is a better predictor of 28-day mortality in septic shock patients than proendothelin-1. Clin Chem Lab Med. 2010;48:1813-1820.
158. Boeck L, Eggimann P, Smyrnios N, et al. Midregional pro-atrial natriuretic peptide and procalcitonin improve survival prediction in VAP. Eur Respir J. 2011;37:595-603.
159. Garcia-Obregon S, Azkargorta M, Seijas I, et al. Identification of a panel of serum protein markers in early stage of sepsis and its validation in a cohort of patients. J Microbiol Immunol Infect. 2018;51: 465-472.
160. DeCoux A, Tian Y, DeLeon-Pennell KY, et al. Plasma Glycoproteomics Reveals Sepsis Outcomes Linked to Distinct Proteins in Common Pathways. Crit Care Med. 2015;43:2049-2058.
161. Bradley D. Clusterin as a Potential Biomarker of Obesity-Related Alzheimer's Disease Risk. Biomark Insights. 2020;15:1177271920964108.
162. Hoofnagle AN, Wu M, Gosmanova AK, et al. Low clusterin levels in high-density lipoprotein associate with insulin resistance, obesity, and dyslipoproteinemia. Arterioscler Thromb Vasc Biol. 2010;30: 2528-2534.
163. Seo JA, Kang MC, Ciaraldi TP, et al. Circulating ApoJ is closely associated with insulin resistance in human subjects. Metabolism. 2018;78:155-166.
164. Conway SR, Wong HR. Biomarker Panels in Critical Care. Crit Care Clin. 2020;36:89-104.
165. Schuetz P, Maurer P, Punjabi V, et al. Procalcitonin decrease over 72 hours in US critical care units predicts fatal outcome in sepsis patients. Crit Care. 2013;17:R115.
166. Bouadma L, Luyt CE, Tubach F, et al. Use of procalcitonin to reduce patients' exposure to antibiotics in intensive care units (PRORATA trial): a multicentre randomised controlled trial. Lancet. 2010;375: 463-474.
167. Simon L, Gauvin F, Amre DK, et al. Serum procalcitonin and C-reactive protein levels as markers of bacterial infection: a systematic review and meta-analysis. Clin Infect Dis. 2004;39:206-217.
168. Wan YI, Brayne AB, Haines RW, et al. Prognostic association of routinely measured biomarkers in patients admitted to critical care: a systematic review. Biomarkers. 2021;26:1-12.
169. Leisman DE, Harhay MO, Lederer DJ, et al. Development and Reporting of Prediction Models: Guidance for Authors From Editors of Respiratory, Sleep, and Critical Care Journals. Crit Care Med. 2020;48: 623-633.
170. Scott MG. When do new biomarkers make economic sense? Scand J Clin Lab Invest Suppl. 2010;242: 90-95.
171. (NICE) NIfHaCE. NICE Glossary. In: 2016:
172. Heidenreich PA, Gubens MA, Fonarow GC, et al. Cost-effectiveness of screening with B-type natriuretic peptide to identify patients with reduced left ventricular ejection fraction. J Am Coll Cardiol. 2004;43: 1019-1026.
173. Higgins AM, Brooker JE, Mackie M, et al. Health economic evaluations of sepsis interventions in critically ill adult patients: a systematic review. J Intensive Care. 2020;8:5.
174. Kluytmans A, Deinum J, Jenniskens K, et al. Clinical biomarker innovation: when is it worthwhile? Clin Chem Lab Med. 2019;57:1712-1720.

Addendum

List of publications

Full research papers of this thesis (* = 1st Author or last Author):

Koch A*, Yagmur E, Linka J, Schumacher F, Bruensing J, Buendgens L, Herbers U, Koek GH, Weiskirchen R, Trautwein C, Tacke F. High Circulating Caspase-Cleaved Keratin 18 Fragments (M30) Indicate Short-Term Mortality in Critically Ill Patients (2018). Dis Markers. 2018:8583121. http://dx.doi.org/10.1155/2018/8583121. Published.

Koch A*, Weiskirchen Ralf, Krusch A, Bruensing J, Buendgens L, Herbers U, Yagmur E, Koek GH, Trautwein C, Tacke F. Visfatin Serum Levels Predict Mortality in Critically Ill Patients (2018). Dis Markers. 2018:7315356. http://dx.doi.org/10.1155/2018/7315356. Published.

Koch A*, Yagmur E, Hoss A, Buendgens B, Herbers U, Weiskirchen R, Koek GH, Trautwein C, Tacke F. Clinical relevance of copeptin plasma levels as a biomarker of disease severity and mortality in critically ill patients (2018). J Clin Lab Anal. 2018:32(9):e22614. http://dx.doi.org/10.1002/jcla.22614. Published.

Yagmur E, Buendgens L, Herbers U, Beeretz A, Weiskirchen R, Koek GH, Trautwein C, Tacke F, **Koch A***. High mobility group box 1 as a biomarker in critically ill patients (2018). J Clin Lab Anal. 2018:2(8):e22584. http://dx.doi.org/10.1002/jcla.22584. Published.

Yagmur E, Buergerhausen D, Koek GH, Weiskirchen R, Trautwein C, **Koch A***, Tacke F*. Elevated CTRP1 Plasma Concentration Is Associated with Sepsis and Pre-Existing Type 2 Diabetes Mellitus in Critically Ill Patients (2019). J Clin Med. 2019:8(5):661. http://dx.doi.org/10.3390/jcm8050661. Published.

Yagmur E, Otto S, Koek GH. Weiskirchen R, Trautwein C, **Koch A***, Tacke F*. Decreased CTRP3 Plasma Concentrations Are Associated with Sepsis and Predict Mortality in Critically Ill Patients (2019). Diagnostics (Basel) 2019:9(2):63. http://dx.doi.org/10.3390/diagnostics9020063. Published.

Yagmur E, Sckaer JH, Koek GH, Weiskirchen R, Trautwein C, **Koch A***, Tacke F*. Elevated MR-proANP plasma concentrations are associated with sepsis and predict mortality in critically ill patients (2019). J Transl Med. 2019:17(1):415. http://dx.doi.org/10.1186/s12967-019-02165-2. Published.

Wirtz TH, Buendgens L, Weiskirchen R, Loosen SH, Haehnsen N, Puengel T, Abu Jhaisha S, Brozat JF, Hohlstein P, Koek GH, Eisert A, Mohr R, Roderburg C, Luedde T, Trautwein C, Tacke F, **Koch A***. Association of Serum Calprotectin Concentrations with Mortality in Critically Ill and Septic Patients (2020). Diagnostics (Basel) 2020: 10(11):990. http://dx.doi.org/10.3390/diagnostics10110990. Published.

Kurt B, Buendgens L, Wirtz TH, Loosen SH, Schulze-Hagen M, Truhn D, Brozat JF, Abu Jhaisha S, Hohlstein P, Koek G, Weiskirchen R, Trautwein C, Tacke F, Hamesch K, **Koch A***. Serum Perilipin 2 (PLIN2) Predicts Multiple Organ Dysfunction in Critically Ill Patients. Biomedicines. 2021:13;9(9):1210. http://dx.doi.org/10.3390/biomedicines9091210. Published.

Yagmur E*, Abu Jhaisha S*, Sapundzhieva N, Brozat JF, Hohlstein P, Wirtz TH, Koek GH, Weiskirchen R, Trautwein C, Tacke F*, **Koch A*.** Clusterin plasma concentrations in critically ill patients. Submitted.

Addendum

Acknowledgements

I would like to express my sincere thanks to Dr. Ger Koek and Prof. Dr. Frank Tacke for the excellent supervision of my PhD thesis and the successful scientific collaboration.

My special thanks also go to my clinical and scientific teachers, Prof. Dr. Christian Trautwein and emeritus Prof. Dr. Siegfried Matern for their great support and encouragement of my scientific and medical work.

I am very grateful to the nursing and medical team of the internal intensive care unit IM08 of the University Hospital Aachen for the excellent, collegial clinical cooperation and the long-standing support of our scientific work.

I would especially like to thank my wife, Dr. Mechthild Koch, my children Lars, Peter and Miriam, as well as my parents, without whose support this scientific work would not have been possible.

Danksagung

Sehr herzlich bedanke ich mich bei Herrn Dr. Ger Koek und Herrn Prof. Dr. Frank Tacke für die exzellente Betreuung meiner PhD-Arbeit und die erfolgreiche wissenschaftliche Zusammenarbeit.

Mein besonderer Dank gilt auch meinen klinischen und wissenschaftlichen Lehrern, Herrn Prof. Dr. Christian Trautwein und Herrn Emeritus Prof. Dr. Siegfried Matern für die große Unterstützung und die Förderung meiner wissenschaftlichen und ärztlichen Tätigkeit.

Sehr dankbar bin ich dem pflegerischen und ärztlichem Team der internistischen Intensivstation IM08 des Universitätsklinikums Aachen für die exzellente, kollegiale klinische Zusammenarbeit und die langjährige Unterstützung unserer wissenschaftlichen Arbeit.

Ganz besonders herzlich bedanke ich mich bei meiner Ehefrau, Frau Dr. Mechthild Koch, meinen Kindern Lars, Peter und Miriam und meinen Eltern, ohne deren Rückhalt und Unterstützung diese wissenschaftliche Arbeit nicht möglich gewesen wäre.

Addendum

Curriculum Vitae

Prof. dr. med. Alexander Michael Koch, MHBA
Date of birth 10/08/1967, place of birth Nuremberg, Germany

Education and academic career

1987 - 1994	Degree in Medicine and Medical Sciences at the Medical Faculty of the University RWTH Aachen, Germany
1994 - 2002	Medical fellow in the Department of Gastroenterology at the University Hospital Aachen, Germany
02/25/1999	MD thesis degree (Dr. med.) from the Medical Faculty of RWTH University Aachen, Germany, Department of Radiology. Scientific topic: “Comparison of quality and dose effects between modern helical CT-Scan systems and conventional computertomography”
08/31/2000	Board Exam in Internal Medicine
05/22/2002	Board Exam in Intensive Care Medicine
since 2002	Senior Physician in the Department of Gastroenterology at the University Hospital Aachen, Germany
11/24/2004	Board Exam in Gastroenterology
since June 2006	Full Permission for educating Intensive Care Medicine, (Ärztekammer Nordrhein, Germany)
since April 2009	Vice Director Department of Gastroenterology at the University Hospital Aachen, Germany (Director: Univ.-Prof. Dr. med. Christian Trautwein)
since April 2011	Head of Department of Intensive Care and Emergency Medicine at the University Hospital Aachen, Germany
04/03/2012	Habilitation (Venia legendi, licence to teach) for Internal Medicine at the Medical Faculty of RWTH Aachen, Germany. Scientific topic: “Inflammation and metabolism: new prognosic biomarkers in critically ill patients”

December 2014	Master degree (MHBA, Master of Health Business Administration) from Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU), Germany. Scientific topic: “Nutritional Intervention in malnourished patients - a critical cost-benefit analysis on the basis of an interventional controlled clinical pilot-study”
January 2017	Awarded a professorship at the Medical Faculty of RWTH University Aachen, Germany
since 2018	PhD-student at Maastricht University. Project title “Identification of novel biomarkers in critically ill patients”. Project supervision by Dr. Ger H. Koek (Maastricht University, Maastricht UMC+, Department of Internal Medicine, Section of Gastroenterology and Hepatology, Netherlands) and Prof. Dr. Frank Tacke (Charité University Medical Center Berlin, Department of Hepatology and Gastroenterology, Germany).
11/27/2020	Board Exam in Transplantation Medicine
06/17/2021	Board Exam in Nutritional Medicine
since April 2021	Full Permission for educating Nutritional Medicine, (Ärztekammer Nordrhein, Germany)

University Hospital Aachen painted by Peter Koch (at the age of 6 years)