Identification of novel biomarkers in critically ill patients

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.