Novel biomarkers in the diagnosis and prognosis of sepsis-related organ dysfunction

Doctoral (PhD) thesis

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I. Introduction

Early diagnosis and effective therapy of sepsis are still one of the biggest challenges at the intensive care unit (ICU) with adverse mortality rates despite of the availability of modern treatment modalities. Recent epidemiological studies show an increasing incidence with a slightly decreasing mortality rate. The first consensus definitions of sepsis (Sepsis-1) were created in 1992, while the Sepsis-2 definitions were established in 2001. The diagnosis of sepsis was based on the presence of systemic inflammatory response syndrome (SIRS). Based on latest the Sepsis-3 definitions created in 2016, sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection.

Early diagnosis and effective supportive therapy of sepsis are essential for a favorable outcome. Besides microbiological investigations and organ dysfunction scores (e.g. the Sequential Organ Failure Assessment (SOFA) score), serum procalcitonin (PCT) and high-sensitivity C-reactive protein (hs-CRP) are still the most widely used inflammatory markers in the clinical evaluation of sepsis. Besides hs-CRP and PCT, more than 200 novel (mostly serum) sepsis biomarkers have been evaluated to date, however, no single marker was sensitive or specific enough for accurately diagnosing sepsis. On the other hand, a multi-marker approach involving various promising sepsis biomarkers (e.g. presepsin, IL-6) may provide useful information regarding the extent or the source of organ dysfunction during sepsis, as the currently used clinical prediction scores (e.g. SOFA) may have limitations regarding this issue due to the heterogeneity of sepsis itself.

Numerous inflammatory markers (e.g. presepsin) and intracellular proteins (e.g. actin) can be released into the circulation – or into the urine – during sepsis.

Actin is a globular protein (MW = 42 kDa) existing in monomeric/globular (G-actin) and in polymeric/filamentous (F-actin) forms. In acute tissue injuries the released extracellular actin is found to be highly toxic in the circulation due to its spontaneous polymerizing tendency causing unfavorable effects on the coagulation system. Gelsolin and Gc-globulin are the most important proteins of the so-called actin scavenger system which is responsible for binding and depolymerizing extracellular actin in the circulation, thus making the urinary appearance of these protein complexes unlikely.

However, an earlier study indicates that actin could be detected in the urine of kidney transplant patients with sustained acute kidney injury (AKI).

Presepsin (PSEP) is the 13-kDa soluble N-terminal fragment originating from the 55-kDa cluster of differentiation (CD) marker protein CD14, which is the receptor for lipopolysaccharide (LPS) and LPS-binding protein complexes. The measurement of PSEP concentrations was deemed valuable for the early diagnosis of sepsis and the evaluation of sepsis severity in contrast to other conditions (e.g. trauma, burn injury, surgeries). According to several multicentric studies, the diagnostic cut-off levels of PSEP varied among 400 - 600 pg/mL for sepsis, while the measurement of PSEP levels was also useful for the prognosis of septic patients. Several studies reported increasing PSEP levels as kidney function decreases (e.g. during chronic kidney disease or sepsis-related AKI). However, PSEP – along with hs-CRP and PCT – could be removed from the circulation due to its relatively small size using different modalities of renal replacement therapy (RRT), thereby potentially causing falsely low biomarker levels.

Albumin has various physiological functions in the circulation by (e.g. maintaining the plasma oncotic pressure, transporting substances, scavenging free radicals). As albumin levels mostly decrease during severe catabolic conditions, elevated CRP:albumin ratios, PCT:albumin ratios and Presepsin:albumin ratios (calculated from inversely changing inflammatory markers and albumin levels) were observed in sepsis, sepsis-related AKI, pancreatitis, coronary artery disease and some types of malignancies. Gelsolin (GSN) is a multifunctional protein existing in three different isoforms. Secreted or plasma GSN (MW = 83 kDa) is an essential component of the so-called extracellular actin scavenger system. A growing body of experimental evidence indicates decreasing serum GSN levels in various clinical conditions (e.g. severe sepsis, multiple organ dysfunction syndrome (MODS), extensive trauma, acute liver failure, myocardial infarction).

As increasing ratios of inflammatory markers (e.g. CRP, PCT, PSEP) and scavenger proteins (e.g. albumin) were found to be useful markers in sepsis, the simultaneous measurement of PSEP and GSN levels could also yield valuable information regarding the diagnosis and prognosis of sepsis.

II. Aims

We hypothesized that elevated urinary actin (u-actin) concentrations might be useful regarding the early diagnosis of sepsis-related AKI as well as a predictive marker of the outcome. Unfortunately, there are no commercial diagnostic kits available yet for serum and/or urinary actin quantification.

Major objectives of our first study were:

- The development of a sensitive and specific Western blot method for the quantitative analysis of u-actin levels.
- Monitoring u-actin levels in control, septic and sepsis-related AKI patient groups. We hypothesized that u-actin could be an early sensitive biomarker of sepsis-related AKI, therefore we investigated u-actin levels:
    - in the diagnosis of sepsis-related AKI
    - in assessing the severity of sepsis and sepsis-related AKI
    - in the mortality prediction of sepsis

Since the increasing ratios of several inflammatory markers (e.g. CRP, PCT, PSEP) and scavenger proteins (e.g. albumin) were found to be useful markers in sepsis, we hypothesized that the simultaneous measurement of PSEP and GSN levels could also yield valuable information regarding the diagnosis and prognosis of sepsis and sepsis-related organ dysfunctions. Therefore, we investigated a new potential marker: the Presepsin:gelsolin (PSEP:GSN) ratio.

The main focuses of our second study were the followings:

- comparing PSEP:GSN ratios of control, non-septic and septic patients
- investigating PSEP:GSN ratios in septic patients with and without MODS
- analyzing the diagnostic performance of PSEP:GSN ratio in non-septic vs. septic and septic non-AKI vs. sepsis-related AKI patients
- investigating the diagnostic utility of PSEP:GSN ratio in sepsis-related hemodynamic instability based on the dosage and the duration of vasopressor requirement
- analyzing the diagnostic and prognostic performance of PSEP:GSN ratio in sepsis-related respiratory insufficiency based on the requirement for oxygen supplementation vs. mechanical ventilation

III. Materials and methods

III.1. Study design of urinary actin measurements

Patients with acutely confirmed sepsis or sepsis-related AKI were enrolled consecutively in our follow-up study conducted between January 2016 and December 2019 at the Department of Anesthesiology and Intensive Therapy (Medical School, University of Pécs, Hungary). All patients or their next-of-kin were given detailed information regarding our study protocol while written consent was also obtained from all. Exclusion criteria were patients with malignancies needing palliative care, end-stage renal disease with chronic dialysis or kidney transplantation, under 18 years of age or unobtainable consent. The study protocol was registered retrospectively at ClinicalTrials.gov (NCT04968262) was approved by the Regional Research Ethics Committee of the University of Pécs (no. 4327.316-2900/KK15/2011) in accordance with the 7th revision of the Helsinki Declarations (2013).

The diagnosis of sepsis and AKI were established using the Sepsis-3 definitions and KDIGO guidelines, respectively. Inclusion criteria for sepsis were signs of organ dysfunction shown in increased Sequential Organ Failure Assessment (SOFA) score (>2), elevated serum PCT levels (>2 ng/mL) and a suspected or microbiologically confirmed infection. Patients with elevated serum creatinine levels (≥1.5 - fold increase from the baseline in the last 7 days or ≥26.5 μmol/L increase within 48 hours) or with decreased urine output (<0.5 mL/kg/h for 6 hours) were regarded to have AKI. Management of sepsis and sepsis-related AKI followed the international guidelines of the 2016 Surviving Sepsis Campaign regarding vasopressor, respiratory, anticoagulation and hydrocortisone therapy, along with feeding, ulcer prophylaxis and sedation. All patients received adequate fluid resuscitation and ex juvantibus broad spectrum antimicrobial therapy guided by the clinical presentation for 24 to 72 hours, which was later modified based on the results of microbiological investigations. Defined end points were death or withdrawal of consent during the sample collection period.

Regarding disease severity and mortality, the first-day values of Simplified Acute Physiology Score II (SAPS II), Acute Physiology and Chronic Health Evaluation II (APACHE II) and SOFA scores were determined. Using 30-day mortality data, patients were divided into survivor and non-survivor groups.

Outpatients from the Department of Ophthalmology (Medical School, University of Pécs, Hungary) served as controls. Exclusion criteria for control patients were lack of consent, acute inflammation (hs-CRP >5 mg/L), infectious disease or kidney disease.

III.2. Study design of presepsin:gelsolin measurements

Besides control individuals, non-septic patients needing ICU hospitalization after major surgical interventions and acutely diagnosed septic patients were enrolled consecutively in our single center prospective observational study conducted between January 2018 and February 2020 at the Department of Anesthesiology and Intensive Therapy. The study protocol was registered retrospectively at ClinicalTrials.gov (NCT05060679) and was approved by the Regional Research Ethics Committee of the University of Pécs (no. 4327.316-2900/KK15/2011) conforming to the 7th revision of the Helsinki Declarations (2013).

The diagnosis of sepsis was determined after admission based on the Sepsis-3 definitions. Inclusion criteria for sepsis were the followings: a suspected or microbiologically confirmed infection and at least 1 vital organ dysfunction shown in increased SOFA score (>2). Non-septic patients could have also had increased admission SOFA scores, yet these patients' clinical presentation was not associated with the presence of an infection. The exclusion criteria, the defined end points and the diagnostic criteria for sepsis-related AKI were the same as in our first study (see above).

Regarding sepsis-related hemodynamic instability, patients were categorized based on low (≤0.1 μg/kg/min) and high (>0.1 μg/kg/min) dose vasopressor (mostly norepinephrine) requirement, while patients were also divided based on shorter (≤5 consecutive days) and longer (>5 consecutive days) vasopressor requirement during ICU stay. Patients were regarded to have septic shock if they had persisting sepsis-induced hypotension despite adequate fluid resuscitation, higher doses of vasopressor requirement and elevated plasma lactate levels (>2 mmol/L).

Regarding sepsis-related respiratory insufficiency, patients were categorized based on their requirement for oxygen supplementation (e.g. face mask (FiO2 ≥50%), high-flow nasal oxygen therapy) and mechanical ventilation (invasive ventilation after endotracheal intubation), while the latter group was also divided based on shorter (≤5 consecutive days) and longer (>5 consecutive days) requirement for mechanical ventilation during ICU stay. Patients needing mechanical

ventilation were further divided based on the development of (at least) moderate acute respiratory distress syndrome (ARDS) according to the Berlin definition (diffuse pulmonary infiltrates on chest radiographs, the absence of congestive heart failure, decreased lung compliance, Horowitz quotient <200 mmHg, positive end-expiratory pressure (≥5 cmH2O)).

As the majority of mechanically ventilated septic patients received propofol or dexmedetomidine sedation during the early stages of respiratory failure, we had limitations regarding the accurate assessment of the patients' level of consciousness using the Glasgow Coma Scale.

First-day values of SAPS II, APACHE II and SOFA scores were calculated for the assessment of disease severity. Septic patients were considered to have MODS if they developed at least 2 or more vital organ dysfunctions (e.g. hemodynamic instability, respiratory insufficiency, AKI, acute liver failure, thrombocytopenia, altered mental state) during follow-up based on significantly elevated SOFA scores. Patients were categorized as survivors and non-survivors using 10-day mortality data.

Control individuals were recruited as outpatients from the Department of Ophthalmology (Medical School, University of Pécs, Hungary). Exclusion criteria were lack of consent, infectious disease, kidney disease or acute inflammation (hs-CRP >5 mg/L).

III.3. Blood and urine sampling

Blood and urine samples were taken from septic patients at the ICU at three time points (T1-3): T1: within 24 hours after admission; T2: second day morning of follow-up; T3: third day morning of follow-up. Sampling points for non-septic patients were the first (T1) and third (T3) postoperative morning. Arterial blood (5 mL) was taken from every septic patient from central venous catheter into plastic blood collection tubes with accelerator gel (BD Vacutainer, Franklin Lakes, NJ, USA), while urine (6 mL) was obtained simultaneously from the bladder catheter using plain plastic tubes (Sarstedt AG, Nümbrecht, Germany). Not more than one sample (venous blood, midstream spot urine) was collected from control patients. Urine and clotted blood samples were centrifuged (10 min, 1500 g) and supernatants were treated with electrophoresis sample buffer then heated at 100 °C for 3 minutes. Native and denatured sample aliquots were stored at -70 °C until analysis.

III.4. Laboratory analysis

Serum parameters including total protein (se-TP), albumin, bilirubin, kidney function markers (se-urea, se-creatinine) along with plasma lactate, platelet count (PLT), and inflammatory parameters (white blood cell count (WBC), hs-CRP, PCT) and basic urinary markers (total protein (u-TP), u-albumin, u-creatinine) were measured using automated routine procedures at our accredited laboratory (Department of Laboratory Medicine, Medical School, University of Pécs, Hungary; NAH-9-0008/2021). Serum GSN, urinary orosomucoid (u-ORM) and urinary cystatin-C (u-CysC) were measured by an automated immune turbidimetric assay (Cobas 8000/c502 module (Roche Diagnostics GmbH, Mannheim, Germany)) developed and validated in our laboratory.

III.5. Determination of urinary actin

Serum and urine samples were separated with a 10% SDS-polyacrylamide gel electrophoresis according to Laemmli. Serum actin (se-actin) levels were determined by a quantitative enhanced chemiluminescence (ECL) Western blot based on the work of Lee et al. and Horváth-Szalai et al. This method was optimized and adapted for the determination of u-actin concentrations. A polyclonal pan-anti-actin primary antibody (1:1000 dilution; Rabbit Anti-Human Actin, N-terminal, ref.no: A2103, Sigma-Aldrich, St. Louis, MO, USA) and a horseradish peroxidase (HRP)-labeled secondary antibody (1:4000 dilution; Goat Anti-Rabbit IgG, Cat. #31460, Thermo Scientific, Rockford, IL, USA) were used. The calibration of Western blot was done by running a dilution series of a purified rabbit skeletal muscle extract of known G-actin concentration (Department of Biophysics, Medical School, University of Pécs, Hungary). A 3-part dilution series of a serum sample from a healthy individual was applied in every gel as an internal standard which had been quantified by the mentioned rabbit skeletal muscle extract calibrator. Therefore, actin concentrations could be calculated in each serum and urine sample based on the light signals being directly proportional to the internal standard series applied in every gel.

III.6. Determination of presepsin and presepsin:gelsolin ratio

PSEP concentrations were measured using an automated Point of Care instrument (PATHFAST; LSI Medience Corporation, Tokyo, Japan) based on a chemiluminescent enzyme immunoassay technique with a detection range of 20 – 20,000 pg/mL. Tests were performed according to the

manufacturer's instructions and the performance of the method was checked by bi-level controls. PSEP:GSN ratio was calculated as the ratio of PSEP to GSN concentrations.

III.7. Statistical analysis

The IBM SPSS Statistics for Windows, Version 22 (IBM Corp., NY, USA) software was used for statistical analysis. Since our data did not show normal distribution by the Kolmogorov-Smirnov and Shapiro-Wilk tests, we performed non-parametric tests. The control, non-septic and septic patient groups were compared using Chi-square or Fischer's exact test for qualitative data and Mann-Whitney U or Kruskal-Wallis tests for quantitative data. Friedman's ANOVA with post hoc Dunn tests along with Wilcoxon tests were carried out to compare the quantitative data of different time points in every patient group. Diagnostic and prognostic performance of laboratory and clinical parameters were evaluated by receiver operating characteristic (ROC) curves. Spearman's rank correlation test was applied for investigating relationships between quantitative variables. Quantitative data were presented as medians and interquartile ranges (IQR) while qualitative data as frequencies and percentages (%). Values of $p<0.05$ were considered as statistically significant.

IV. Results

IV.1. Urinary actin in sepsis-related acute kidney injury

IV.1.1. Clinical and laboratory data

Control, septic and sepsis-related AKI patients' demographic and laboratory data

In the present study, 24 control individuals, 17 septic patients and 43 patients with sepsis-related AKI were enrolled. A moderate difference ($p<0.05$) was found between the patient groups regarding age, gender and the majority of comorbidities. A significant difference ($p<0.05$) was found between the control and septic patient groups in se-TP, se-albumin, se-CK, WBC, PLT, hs-CRP, u-CysC and u-ORM levels. Admission values of se-creatinine, u-TP, u-albumin, and u-actin/u-creatinine were significantly higher ($p<0.05$) in the septic and sepsis-related AKI groups compared with the control patients. Se-PCT ($p<0.05$), u-actin ($p<0.05$) and u-actin/u-TP ($p<0.05$) levels were also significantly higher between the septic and sepsis-related AKI patients. (Table 6. in the original Ph.D. thesis)

Septic and sepsis-related AKI patients' clinical data

Major differences in therapeutic requirements of 60 septic patients are the following: 38 needed mechanical ventilation, 22 needed oxygen supplementation (median Horowitz quotient: 221.2), 51 needed vasopressor support, 12 had liver failure, 13 had thrombocytopenia, 30 had elevated lactate levels (>2 mmol/L) and 47 needed hydrocortisone supplementation. Moreover, 41.9% of sepsis-related AKI patients required some kind of renal replacement therapy (RRT). Regarding the clinical prediction scores, sepsis-related AKI patients had considerably higher values (APACHE II: $p<0.05$; SAPS II: $p<0.05$; SOFA: $p<0.05$) compared with the sepsis group while multiple organ dysfunction syndrome (MODS) was also a more common complication in the former group (69.8%) than in the latter (53.0%) (Table 7. in the original Ph.D. thesis).

IV.1.2. Urinary actin in control, septic, and sepsis-related AKI patients

No considerable difference was observed in serum actin levels between the control and septic patient groups by comparing the first (median: 0.75 vs. 0.8 mg/L, $p=0.757$), second ($p=0.584$) and third ($p=0.608$) day serum samples during follow-up. However, a significant increase in u-actin levels was discovered between the control and septic patients during the first (median: 0.78 vs.

3.98 µg/L, p<0.001), second (p<0.001) and third (p<0.001) day of follow-up (Fig 1A). This tendency was also explicit between the septic and sepsis-related AKI groups at the first day (median: 1.27 vs. 9.52 µg/L, p<0.001), but this difference was not statistically significant by the second (p=0.368) and third (p=0.220) day of follow-up (Fig 1B). U-actin levels were also in good agreement with the severity of AKI stages regarding the first day (median: 3.16 vs. 10.78 vs. 11.55 µg/L, p<0.01), while this change was also statistically significant on the second (p<0.01) and third day (p<0.05) of follow-up (Fig 2A). This tendency remained the same among sepsis-related AKI patients when referring u-actin values to u-creatinine on the first (median: 0.47 vs. 3.74 vs. 6.16 µg/mmol, p<0.05), second (p<0.05) and third day (p<0.05) of follow-up (Fig 2B).

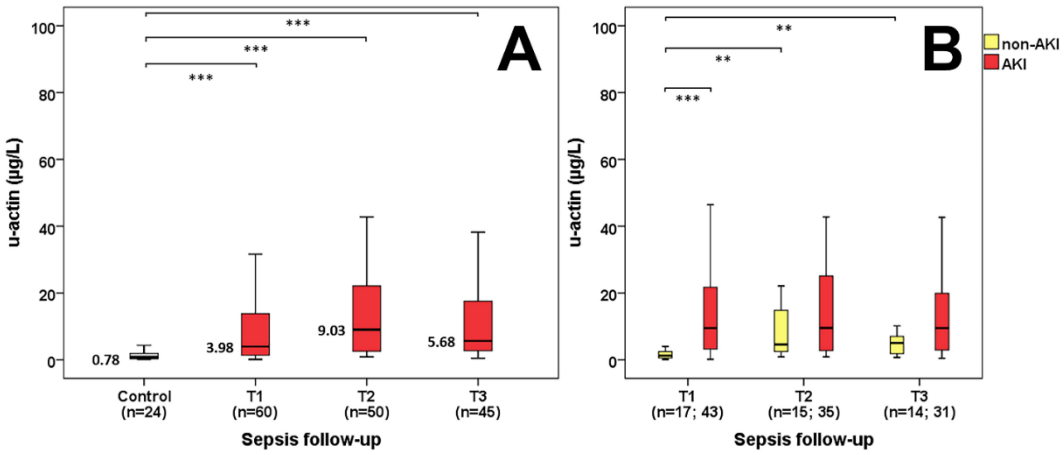

Fig 1. U-actin levels of control and septic patients (A) along with sepsis and sepsis-related AKI patients (B) during follow-up. n: sample count. **p<0.01; ***p<0.001.

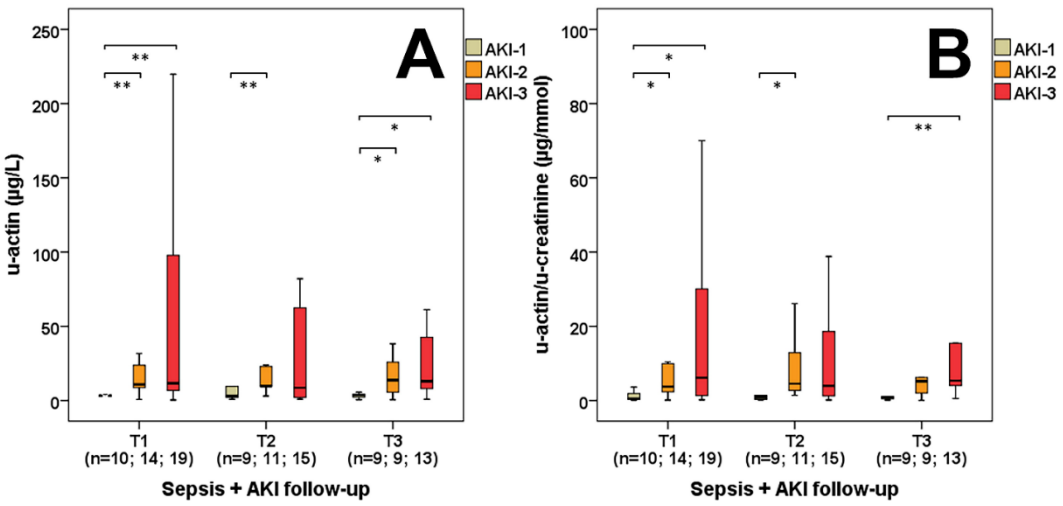

Fig 2. U-actin (A) and u-actin/u-creatinine (B) levels of the individual sepsis-related AKI stages during follow-up. n: sample count. *p<0.05; **p<0.01.

IV.1.3. Survival data and distinctive power of urinary actin in sepsis

No significant difference was found in u-actin levels between survivors and non-survivors based on 30-day mortality data during follow-up (Fig 3A). The diagnostic performance of first-day u-actin levels in sepsis-related AKI was assessed using ROC analysis (Fig 3B). For discerning all sepsis-related AKI from septic patients without AKI, area under the curve (AUC) values were found to be the following: u-actin: 0.876 (p<0.001); se-creatinine: 0.875 (p<0.001); Derived cut-off values were: u-actin 2.63 µg/L (sensitivity: 86.0%, specificity: 82.4%); se-creatinine: 111 µmol/L (sensitivity: 90.7%, specificity: 76.5%).

Correlations

In our study, data from all sample collection time points were used for calculating correlations, while the absolute u-actin levels were presented in most cases due to their strong correlation to u-actin/u-creatinine (ρ=0.898, p<0.001). U-actin levels showed moderate correlation (p<0.001) with u-TP (ρ=0.489), u-albumin (ρ=0.617), se-creatinine (ρ=0.371), u-CysC (ρ=0.434) and u-ORM (ρ=0.367). Weak correlations (p<0.01) were observed between u-actin and se-actin (ρ=0.272) and WBC (ρ=0.223), respectively. No further associations were found with other inflammatory or clinical parameters.

IV.2. Presepsin:gelsolin ratio in sepsis and sepsis-related organ dysfunction

IV.2.1. Clinical and laboratory data

Control, non-septic and septic patients' demographic and laboratory data

In the present study, 126 patients were enrolled consecutively (23 control individuals, 38 non-septic and 65 septic patients). A moderate difference ($p<0.05$) was found between the patient groups regarding age and some of the listed of comorbidities. A significant difference ($p<0.05$) was observed between the control, non-septic and septic patient groups in se-TP, se-albumin, hs-CRP, PSEP and GSN levels along with PSEP:GSN ratios. Admission values of se-urea, se-creatinine, WBC and PLT were significantly different ($p<0.05$) as well in the non-septic and septic groups compared with those of the controls. APACHE II, SAPS II and SOFA scores along with PCT levels were considerably higher ($p<0.05$) in septic patients than in non-septic patients. Major therapeutic requirements of the non-septic group were the followings: all patients received adequate fluid resuscitation, yet 23 patients also had temporary low dose vasopressor requirement; 36 patients needed oxygen supplementation, 2 patients needed temporary mechanical ventilation and no patients required RRT during follow-up (Table 8. in the original Ph.D. thesis).

Comparing patients with sepsis and sepsis-related organ dysfunction

Major therapeutic requirements of 65 septic patients were the followings: 54 needed vasopressor support, 48 needed mechanical ventilation, 17 needed oxygen supplementation (median Horowitz quotient: 184 mmHg), 53 needed hydrocortisone supplementation. Mechanically ventilated patients received propofol or dexmedetomidine sedation during the early stage of severe respiratory failure. Furthermore, only 11 septic patients developed liver failure, while also 11 septic patients had thrombocytopenia.

Septic patients were further divided based on the occurrence of MODS (at least 2 or more vital organ dysfunctions). A significant difference ($p<0.05$) was observed between the two patient groups regarding the length of ICU stay, 10-day mortality, the majority of therapeutic requirements (e.g. vasopressor support and mechanical ventilation), clinical prediction scores (APACHE II; SAPS II; SOFA) and most of the laboratory markers including se-albumin, se-creatinine, se-bilirubin, PCT, PSEP and GSN levels along with PSEP:GSN ratios (Table 9. and Table 10. in the original Ph.D. thesis).

IV.2.2. Presepsin:gelsolin ratio in control, non-septic and septic patients

A considerable difference was found in PSEP:GSN ratios between the control, non-septic and septic patients (T1 median: 1.7 vs. 9.9 vs. 105.9 ng/mg, p<0.001), while there was no significant change in the kinetics of PSEP:GSN ratios during follow-up regarding the non-septic (T1, T3) and septic (T1, T2, T3) patient groups (Fig 3A). The diagnostic performance of first-day PSEP:GSN ratios in sepsis was assessed using ROC analysis. For distinguishing all non-septic patients from septic patients, area under the curve (AUC) value of first-day PSEP:GSN ratio (p<0.001) was found to be equally as good or slightly better than that of SOFA (p<0.001), PCT (p<0.001) and PSEP (p<0.001) (Fig 3B) (Table 11. in the original Ph.D. thesis).

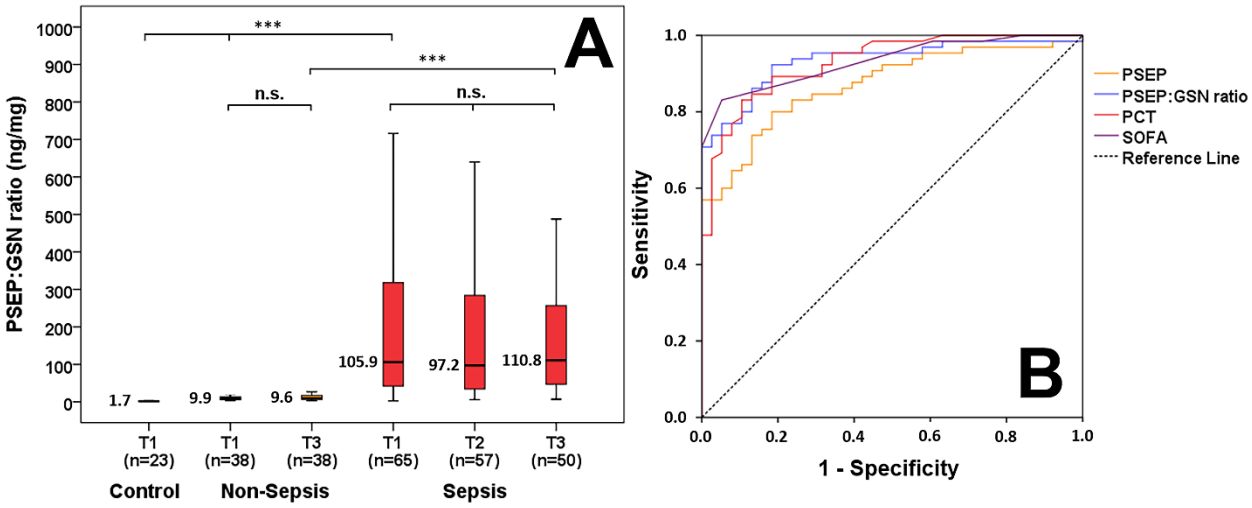

Fig 3. PSEP:GSN ratios of control, non-septic and septic patients during follow-up (A). Receiver operating characteristic (ROC) curves of admission laboratory parameters for distinguishing non-sepsis from sepsis (B). n: sample number. n.s.: not significant. ***p<0.001.

Monitoring presepsin:gelsolin ratio in septic non-AKI and sepsis-related AKI patients

Sepsis-related AKI patients had significantly higher PSEP:GSN ratios than septic non-AKI patients at T1 (median: 43.6 vs. 176.1 ng/mg, p<0.001), T2 (p<0.001) and T3 (p<0.05) as well. PSEP:GSN ratios were also in good agreement with the severity of AKI stages regarding the first (median: 85.8 vs. 111.1 vs. 419.5 ng/mg, p<0.05) and second (p<0.05) day of follow-up. From 20 AKI-3 stage septic patients, 15 (75.0%) patients required some form of RRT: 6 (40.0%) patients received intermittent hemodialysis (IHD) and 9 (60.0%) patients received continuous renal replacement therapy (CRRT). For discerning all sepsis-related AKI patients from septic patients

without AKI, AUC values of first-day se-creatinine ($p<0.001$) were superior to PSEP ($p<0.001$), PSEP:GSN ratio ($p<0.001$) and PCT ($p<0.05$).

Usefulness of presepsin:gelsolin ratio regarding mortality prediction in sepsis

PSEP:GSN ratios were significantly lower in survivors compared with non-survivors at T1 (median: 80.6 vs. 322.7 ng/mg, $p<0.01$), T2 ($p<0.05$) and T3 ($p<0.01$) as well. Regarding 10-day mortality prediction, AUC values of first-day PSEP:GSN ratio ($p<0.01$) and PSEP ($p<0.05$) were slightly lower compared with APACHE II ($p<0.001$), SAPS II ($p<0.01$) and SOFA ($p<0.01$) scores. AUC values for PCT (0.511) were not statictically significant.

IV.2.3. Presepsin:gelsolin ratio in sepsis-related organ dysfunction

Presepsin:gelsolin ratio in sepsis based on requirements of vasopressor support

In contrast to septic patients with no vasopressor requirement, proportionately higher PSEP:GSN ratios were found in septic patients with lower and higher doses of norepinephrine requirement at T1 (median: 17.4 vs. 70.9 vs. 307.1 ng/mg, $p<0.001$), T2 ($p<0.01$) and T3 ($p<0.001$) (Fig 4A). Thus, patients with septic shock showed significantly increased PSEP:GSN ratios compared with septic patients without septic shock at T1 (median: 59.2 vs. 317.8 ng/mg, $p<0.001$), T2 ($p<0.001$) and T3 ($p<0.001$) (Fig 4B). Furthermore, septic patients needing vasopressor support longer than 5 consecutive days had significantly higher PSEP:GSN ratios than septic patients with shorter ($\leq$5 days) vasopressor requirement at T1 (median: 66.7 vs. 247.4 ng/mg, $p<0.001$), T2 ($p<0.001$) and T3 ($p<0.001$) as well (Fig 4C). For distinguishing all patients with septic shock from patients without septic shock, AUC values of first-day PSEP:GSN ratio ($p<0.001$) were slightly superior to SOFA ($p<0.001$), PSEP ($p<0.001$) and PCT ($p<0.05$) (Fig 4D). For discerning septic patients needing shorter ($\leq$5 days) and longer (>5 days) vasopressor support, AUC values of first-day PSEP:GSN ratio ($p<0.001$) were superior to PSEP ($p<0.001$) and SOFA ($p<0.05$), while PCT (0.468) did not satisfy the criteria for statistical significance (Fig 4E) (Table 12. in the original Ph.D. thesis).

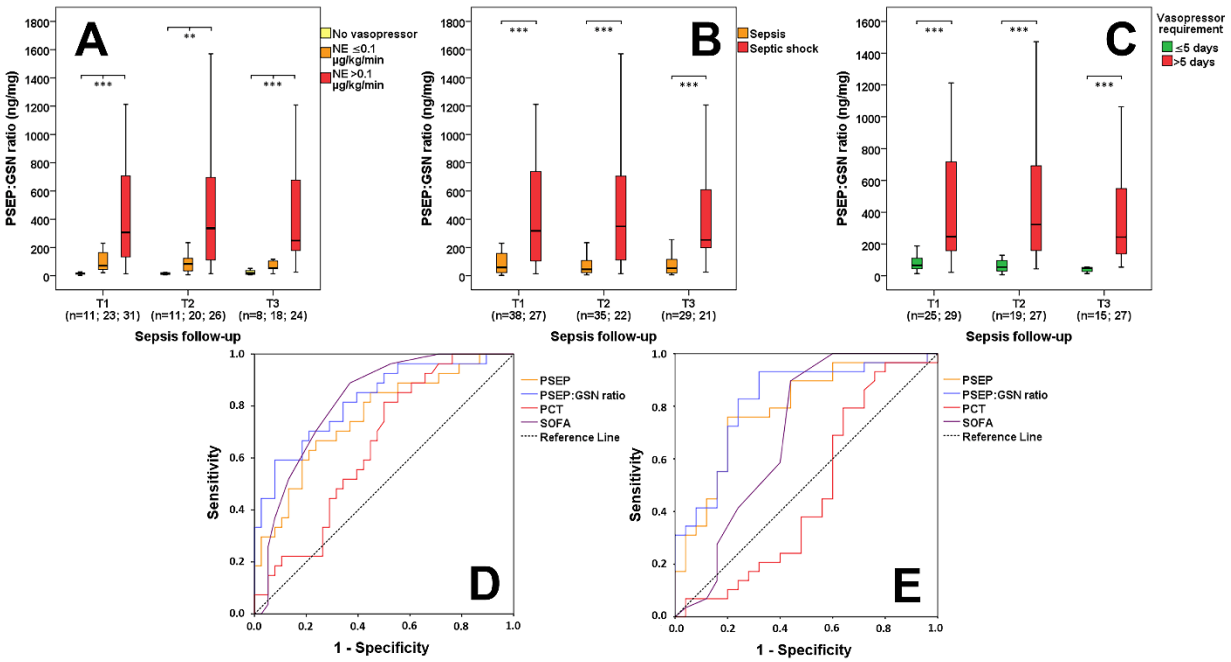

Fig 4. PSEP:GSN ratios of septic patients with different doses of vasopressor requirement during follow-up (A). PSEP:GSN ratios of patients with sepsis and septic shock (B) during follow-up. PSEP:GSN ratios of septic patients needing shorter (≤5 days) and longer (>5 days) vasopressor support during follow-up (C). Receiver operating characteristic (ROC) curves of admission parameters for distinguishing sepsis from septic shock (D) along with discerning septic patients' shorter (≤5 days) and longer (>5 days) vasopressor requirement (E). NE: norepinephrine; n: sample number. n.s.: not significant. **p<0.01; ***p<0.001.

Presepsin:gelsolin ratio in sepsis based on requirements of respiratory support

Septic patients needing mechanical ventilation showed significantly increased PSEP:GSN ratios compared with septic patients needing oxygen supplementation at T1 (median: 26.9 vs. 173.2 ng/mg, p<0.001), T2 (p<0.001) and T3 (p<0.001) (Fig 5A). In contrast to septic patients with requirement of oxygen supplementation, considerably higher PSEP:GSN ratios were found in septic patients needing mechanical ventilation, especially if they also had moderate or severe stage ARDS at T1 (median: 26.9 vs. 94.2 vs. 554.8 ng/mg, p<0.01), T2 (p<0.01) and T3 (p<0.01) (Fig 5B). Furthermore, septic patients needing mechanical ventilation longer than 5 consecutive days showed significantly higher PSEP:GSN ratios than septic patients with shorter (≤5 days) mechanical ventilation requirement at T1 (median: 63.2 vs. 230.4 ng/mg, p<0.01), T2 (p<0.001) and T3 (p<0.001) as well (Fig 5C). For distinguishing all patients needing oxygen supplementation

from patients needing mechanical ventilation, AUC values of first-day PSEP:GSN ratio ($p<0.001$) were slightly superior to SOFA ($p<0.01$) and PSEP ($p<0.05$), while PCT (0.488) did not satisfy the criteria for statistical significance (Fig 5D). For discerning all septic patients needing shorter (≤5 days) and longer (>5 days) mechanical ventilation, AUC values of first-day PSEP:GSN ratio ($p<0.01$) were superior to PSEP ($p<0.01$) and SOFA ($p<0.05$), while PCT (0.480) did not satisfy the criteria for statistical significance (Fig 5E).

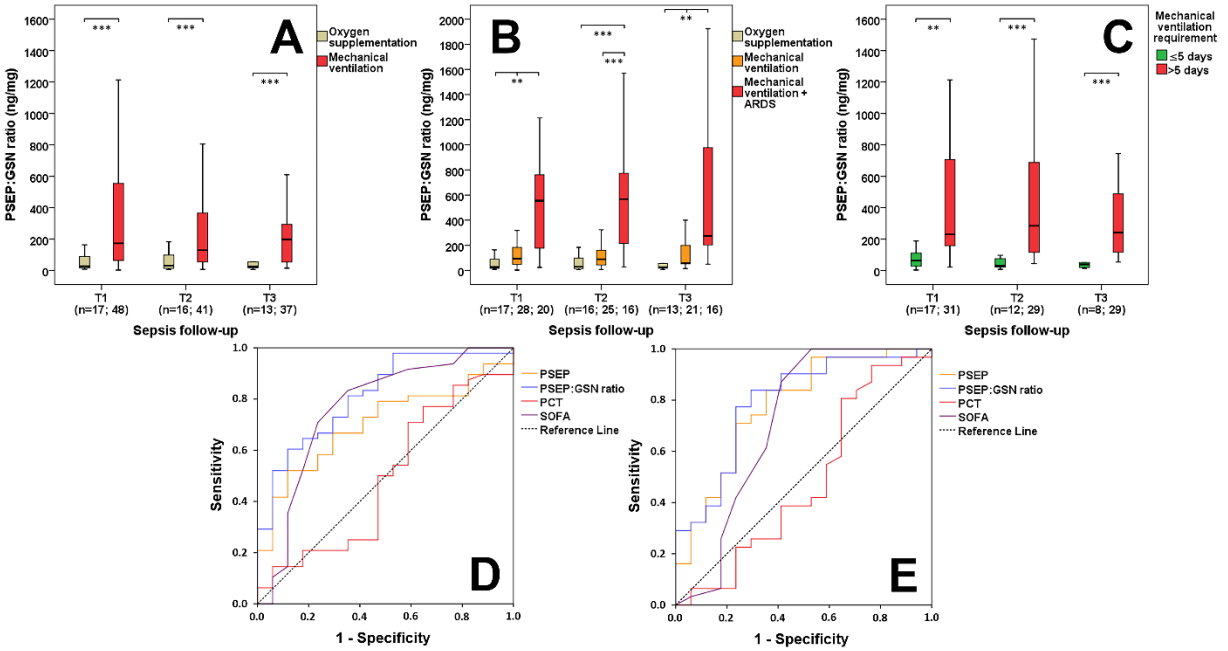

Fig 5. PSEP:GSN ratios of septic patients with requirements of oxygen supplementation and mechanical ventilation (A), with the latter group having ARDS (B) during follow-up. PSEP:GSN ratios of septic patients having shorter (≤5 days) and longer (>5 days) requirement of mechanical ventilation during follow-up (C). Receiver operating characteristic (ROC) curves of admission parameters for distinguishing septic patients needing oxygen supplementation from mechanical ventilation (D) along with discerning septic patients' shorter (≤5 days) and longer (>5 days) requirement of mechanical ventilation (E). n: sample number. n.s.: not significant. **$p<0.01$; ***$p<0.001$.

Correlations

Quantitative data from all sample collection time points were used for calculating correlations. PSEP:GSN ratio showed strong correlation ($p<0.001$) with PSEP ($\rho=0.924$). Moderate correlations ($p<0.001$) were found between PSEP:GSN ratio and se-urea ($\rho=0.720$), se-creatinine ($\rho=0.611$),

hs-CRP (ρ=0.573), PCT (ρ=0.576) and WBC (ρ=0.452), along with APACHE II (ρ=0.759), SAPS II (ρ=0.743) and SOFA (ρ=0.741) clinical scores. PSEP:GSN ratio showed negative correlations ($p<0.001$) with se-TP (ρ=-0.439), se-albumin (ρ=-0.667), and GSN (ρ=-0.853). No further associations were observed with other inflammatory or clinical parameters.

V. Discussion

V.1. Urinary actin in sepsis-related acute kidney injury

The early diagnosis and effective therapy of sepsis and sepsis-related AKI are essential for a successful recovery. However, the currently used biomarkers (sepsis: PCT, hs-CRP; AKI: se-creatinine, urine output) show several limitations regarding the diagnosis and prognosis of sepsis and AKI, hence a multi-marker approach involving novel laboratory markers may prove to be beneficial in achieving a favorable outcome.

There is a growing body of evidence indicating the importance of decreased actin scavenger protein concentrations and/or elevated se-actin levels in various clinical conditions (e.g. trauma, acute liver failure, myocardial infarction, sepsis). Our previous study showed that the increase of se-actin was inversely proportional to the amounts of actin scavenger proteins in the circulation which was associated with increased mortality rate.

Since urine is noninvasively available, it is becoming a popular source for disease biomarker studies. Several studies have already examined actin in the circulation, however, the importance of u-actin has not yet been extensively investigated. Only one study carried out by Kwon et al. investigated u-actin as a potential AKI marker. They found increased u-actin/u-creatinine ratios in patients after cadaveric kidney transplantation (u-actin/u-creatinine levels were significantly higher in patients with sustained AKI compared to patients recovering from AKI). However, the origin of this considerable u-actin release has not been clarified, the ischemic injury of renal allografts was assumed to be the main cause of AKI.

One of the main focuses of our study was to develop a sensitive method for detecting actin in the urine samples of septic patients. In accordance with previous findings, se-actin levels were slightly elevated in our septic patients compared with controls. In contrast to the study conducted by Kwon et al., we could also detect low concentrations of u-actin in each control sample as well. This might be due to our improved actin-detecting technique. Significantly higher u-actin levels were found in the urine samples of septic patients compared with the control group, yet this increase was also explicit between the sepsis and sepsis-related AKI groups, especially in sepsis-related AKI patients needing renal replacement therapy (RRT).

During the 3-day follow-up period, no significant difference was found in u-actin levels between survivors and non-survivors. These findings suggest that u-actin may not be a suitable marker for sepsis or sepsis-related AKI mortality prediction. Moreover, no correlation was observed between u-actin and the conventional inflammatory markers (hs-CRP, PCT) or clinical scores (APACHE II, SAPS II, SOFA). Therefore, our results contradict the data available from large multi-center clinical trials indicating better performance of currently used sepsis markers regarding mortality prediction.

In our research, the absolute u-actin levels are shown in most of our results due to their strong correlation to u-actin/u-creatinine. We are aware of the practice that novel urinary AKI biomarkers have to be normalized to u-creatinine concentrations in order to control variations in urine flow rate. However, there is a growing body of evidence challenging the importance of u-creatinine normalization in AKI since the excretion rates of u-creatinine and other novel urinary AKI biomarkers may be affected differently during an acute severe condition.

A moderate correlation with u-TP, u-albumin and se-creatinine suggest that elevated u-actin levels could be the consequence of severe glomerular injury potentially caused by sepsis-related systemic cellular damage with an excessive release of free extracellular actin. However, the increase of u-actin might not be the direct result of excessive free actin release due to a weaker correlation with se-actin and a moderate correlation with u-CysC indicating a tubular dysfunction as well during the development of AKI. Se-CK did not show significant correlations with se-actin or u-actin suggesting the cause of increased u-actin is not extensive muscle damage. Since renal ischemia is common during sepsis, it could also contribute to the development of sepsis-related AKI, as postulated by Kwon et al.

As actin is bound to gelsolin or Gc-globulin in the circulation, it is unlikely to appear in the urine. However, u-actin could appear in the urine due to both severe glomerular or tubular injury, so it seems that the elevation of u-actin assumes severe kidney injury. As KIM-1 or NGAL are known examples of damage biomarkers in AKI according to recent reviews, u-actin may also be classified into this group. We suspect that u-actin might provide more accurate information regarding the extent of kidney injury compared with serum creatinine. Therefore, a multi-marker approach including u-actin and the measurement of various damage biomarkers (e.g. urine Cystatin C, KIM-

1, NGAL, IL-18) may provide valuable information regarding the more accurate staging of sepsis-related AKI.

Since different forms of RRT – especially continuous renal replacement therapy (CRRT) – represent a better modality for the management of severe sepsis-related AKI, there is no clear evidence demonstrating the adequate time point of initiation of RRT in sepsis-related AKI. Since actin is either bound to the actin scavenger proteins in the circulation or immediately starts forming filaments during severe tissue injury, it could not be removed from the circulation by RRT, yet the increase of u-actin could yield valuable information regarding the extent of kidney damage and the need for the early initiation of RRT in sepsis-related AKI.

V.2. Presepsin:gelsolin ratio in sepsis-related organ dysfunction

The early diagnosis and effective therapy of sepsis are essential for a successful recovery. However, the currently used biomarkers (hs-CRP, PCT) show several diagnostic and prognostic limitations, hence a multi-marker approach including novel biomarkers may contribute to achieving a more favorable outcome.

One of the main focuses of our study was to describe the time course of PSEP:GSN ratio among non-septic and septic patients. Compared with controls, significantly elevated PSEP:GSN ratios were found in non-septic and septic patients. First-day PSEP:GSN ratios showed good performance compared with SOFA score, PCT and PSEP levels regarding the diagnosis of sepsis. Compared with septic non-AKI patients, PSEP:GSN ratios were even higher in sepsis-related AKI patients, especially in AKI-3 stage septic patients needing RRT. This tendency was the same when investigating PSEP levels among control, non-septic and septic patients. In accordance with previous studies, our results show a similarly increasing tendency of PSEP levels in sepsis and in sepsis-related AKI.

However, first-day se-creatinine had better performance than PSEP and PSEP:GSN ratio in the diagnosis of sepsis-related AKI. As se-creatinine only reflects the decreased glomerular filtration rate, our results suggest that PSEP:GSN ratio provides a more complex information regarding the patients condition and the overall organ dysfunction during sepsis. To the best of our knowledge, this is the first study to examine PSEP:GSN ratio in sepsis, therefore we did not have any other

study for reference in this field. Additional investigation with increased case numbers may clarify the usefulness of PSEP:GSN ratio regarding the diagnosis of sepsis-related AKI.

There is a growing body of evidence indicating the further increase of PSEP levels as kidney function decreases. Like several other inflammatory markers (e.g. IL-6, PCT), PSEP also does not bind to carrier proteins in the circulation, thus it can be removed from the circulation by different forms of RRT. Based on our results, a slightly decreasing tendency of PSEP:GSN ratio was observed during follow-up in sepsis-related AKI patients needing RRT. We are aware of the evidence that these procedures can effectively reduce the blood concentrations of numerous inflammatory markers, thereby potentially causing falsely low biomarker levels during RRT, thus limiting the prognostic value of these molecules in sepsis-related AKI.

Regarding sepsis-related hemodynamic instability, increasing PSEP:GSN ratios were in good agreement with the dosage and the duration of vasopressor requirement in septic patients. Moreover, PSEP:GSN ratios were also significantly higher in patients with septic shock than in septic patients without shock. First-day PSEP:GSN ratios also showed superior performance compared with SOFA score and PSEP levels regarding the diagnosis of septic shock and the length of vasopressor requirement in sepsis. Regarding sepsis-related respiratory insufficiency, considerably elevated PSEP:GSN ratios were observed in patients needing mechanical ventilation compared with patients needing oxygen supplementation. This increase was even more explicit in mechanically ventilated patients with moderate ARDS, while higher PSEP:GSN ratios were also associated with prolonged mechanical ventilation requirement in septic patients. First-day PSEP:GSN ratios showed better performance than SOFA score and PSEP levels regarding the requirement and duration of mechanical ventilation in sepsis. Our results suggest that PSEP:GSN ratio could be a useful marker besides the routinely used SOFA score concerning the more accurate assessment of hemodynamic and respiratory insufficiency in septic patients.

Moderate correlations were observed between PSEP:GSN ratio and the conventional inflammatory markers (hs-CRP, PCT). Regarding 10-day mortality data, PSEP:GSN ratios were considerably elevated in non-survivors compared with survivors during follow-up, while the prognostic performance of PSEP:GSN ratio was similar to widely used clinical scores (APACHE II, SAPS II, SOFA). These results suggest that PSEP:GSN ratio could also be a useful marker regarding the short-term mortality prediction of sepsis, yet the prognostic performance of PSEP levels was

markedly inferior compared with the conventional clinical prognostic scores. Therefore, our results somewhat contradict the data available from several larger multi-center studies indicating better performance of PSEP levels regarding mortality prediction.

As albumin has a variety of essential physiological functions in the circulation (e.g. maintaining the plasma oncotic pressure, providing buffer capacity, transporting substances, scavenging free radicals), decreasing albumin levels are often associated with malnutrition or inflammation. Elevated CRP:albumin ratios, PCT:albumin ratios and Presepsin:albumin ratios – found mostly due to hypoalbuminemia and increased inflammatory marker levels – were detected in several catabolic conditions including sepsis, sepsis-related AKI, pancreatitis, coronary artery disease and different types of malignancies. Since GSN also has a protective role by being an actin scavenger protein, several studies reported decreasing serum GSN concentrations in various clinical conditions (e.g. trauma, acute liver failure, myocardial infarction, sepsis). Our previous study showed that the increase of serum actin was inversely proportional to the amounts of GSN in the circulation which was associated with increased mortality rate. As a result, we also found significantly elevated PSEP:GSN ratios in septic patients, especially in sepsis-related organ dysfunctions (AKI, hemodynamic instability, respiratory insufficiency, MODS).

V.3. Limitations

As far as we know, before our study nobody investigated u-actin or PSEP:GSN ratio in sepsis or in sepsis-related AKI, thus we aimed to be the first to explore this promising and interesting field by conducting a small sample size pilot study to establish some baseline data. Since our studies were performed as single center studies (16 bedded central ICU), we had very limited capacities for consecutive patient recruitment, hence the patient groups were different regarding age and gender, while the studies took a longer time period to achieve these relatively small sample sizes. Septic patients with severe organ dysfunction were more frequently admitted to our ICU being a regional center for critical care. Differences in sample size between control, non-septic and septic groups might reduce the power of comparison, although non-parametric tests (e.g. Mann-Whitney U test) could be used well with unequal sample sizes. There was a slightly variable time interval (mostly within 12 hours) in the actual first day sample collection, as most patients were admitted at night or in the late afternoon before taking the first sample on the next morning. We are aware

of the concern that outpatients are a difficult control group for ICU patients, yet we aimed to establish a reference range for u-actin and PSEP:GSN ratio in patients without inflammation.

In the future, we should increase the number of critically ill patients due to the heterogeneity of sepsis while also extending the sample collection period to 5-10 days in order to draw more accurate conclusions. Exploring other patient groups with different kinds of kidney disease (e.g. CKD, glomerulonephritis) might provide additional information regarding the appearance of actin in the urine. As this is the first study investigating the importance of u-actin in sepsis-related AKI, there are no commercially available diagnostic kits for serum and/or urinary actin quantification. For this reason, we developed a highly sensitive Western blot method evaluated by an ECL technique. Since Western blot is a quite expensive and time-consuming laboratory technique, its routine clinical utility is questionable, hence the development of a more rapid and efficient laboratory method (e.g. ELISA) is necessary. As there are no commercially available rapid diagnostic kits for GSN quantification as well, the development of an efficient Point of Care test would facilitate the prompt determination of PSEP:GSN ratio in routine clinical practice.

VI. Summary, novel findings

In our first study, we analyzed the diagnostic and prognostic utility of u-actin in sepsis and sepsis-related AKI. Our results may contribute to the more accurate assessment of kidney damage in sepsis-related AKI.

The summary of our results regarding our first study are the following:

- We adapted a sensitive method for the quantitative analysis of u-actin levels.
- Compared with controls, significantly elevated u-actin levels were found in septic and septic patients.
- U-actin levels were even more elevated in patients with sepsis-related AKI and were also in good agreement with the severity of AKI stages, especially in patients needing RRT.
- A moderate correlation with u-TP, u-albumin and se-creatinine suggest that elevated u-actin levels could be the consequence of severe glomerular injury, while a weaker correlation with se-actin and a moderate correlation with u-CysC indicating a tubular dysfunction as well during the development of AKI. We suspect that u-actin could be useful regarding the more accurate assessment of kidney injury compared with se-creatinine.
- The increase of u-actin could yield valuable information regarding the extent of kidney damage and the need for the early initiation of RRT in sepsis-related AKI.
- U-actin determination could aid in the diagnosis and follow-up of AKI in sepsis but an automated method (e.g. ELISA) should be worked out in the future instead of the applied Western blot technique.

We investigated a novel marker, the PSEP:GSN in control, non-septic and septic patients. PSEP:GSN ratio could be a useful marker besides the routinely used SOFA score regarding the more accurate assessment of sepsis-related hemodynamic and respiratory dysfunction.

The most important results of our second study are the followings:

- The measurement of PSEP is currently available using commercially available Point of Care test, while GSN levels can also be determined in our institute using an automated immuneturbidimetric assay, hence the rapid measurement of the PSEP:GSN ratio is readily accessible for research purposes.

- Compared with controls, significantly elevated PSEP:GSN ratios were found in non-septic and septic patients.
- PSEP:GSN ratios were also elevated in patients with sepsis-related AKI and were in good agreement with the severity of AKI stages as well, yet the diagnostic performance of the marker was inferior to se-creatinine.
- Increasing PSEP:GSN ratios were in good agreement with the dosage and the duration of vasopressor requirement in septic patients. Moreover, PSEP:GSN ratios were significantly higher in patients with septic shock than in septic patients without shock.
- Compared to septic patients needing oxygen supplementation, considerably elevated PSEP:GSN ratios were observed in septic patients needing mechanical ventilation, especially in patients with (at least) moderate ARDS, while higher PSEP:GSN ratios were also associated with prolonged mechanical ventilation requirement in septic patients.
- The diagnostic and prognostic performance of PSEP:GSN ratio was superior in differentiating oxygen supplementation vs. mechanical ventilation requirement and sepsis vs. septic shock compared to other routinely used markers and scores (e.g. PSEP, PCT, SOFA) among septic patients.

U-actin may serve as a complementary diagnostic biomarker to se-creatinine in sepsis-related AKI while higher u-actin levels also seem to reflect the severity of AKI. As the optimal time point for the initiation of RRT in sepsis-related AKI is still controversial, u-actin might provide valuable information regarding this issue.

The measurement of PSEP is widely available using commercially available Point of Care tests, thus it can yield rapid and accurate results regarding the early diagnosis of sepsis. However, PSEP:GSN ratio was shown to be a potentially useful biomarker regarding the severity and prognosis of sepsis. The comprehensive evaluation of each patient's clinical parameters is still essential in achieving a correct diagnosis, yet increasing PSEP:GSN ratios could also yield valuable information regarding the often-perceivable imbalance of excessive inflammatory response and simultaneous depletion of the patient's scavenger capacity during sepsis.

VII. List of publications

Publications related to this thesis:

Ragán D, Kustán P, Horváth-Szalai Z, Szirmay B, Bugyi B, Ludány A, Miseta A, Nagy B, Mühl D. Urinary actin, as a potential marker of sepsis-related acute kidney injury: A pilot study. PloS One. 2021;16(7):e0255266. **IF: 3,240**

Ragán D, Horváth-Szalai Z, Szirmay B, Mühl D. Novel damage biomarkers of sepsis-related acute kidney injury. E-JIFCC. 2022;33(1):11-22. **IF: -------**

Ragán D, Kustán P, Horváth-Szalai Z, Szirmay B, Miseta A, Woth G, Mühl D, Kőszegi T. Presepsin:gelsolin ratio, as a promising marker of sepsis-related organ dysfunction: a prospective observational study. BMC Infectious Diseases. **Under review**

Ragán D, Kustán P, Németh Á, Ludány A, Mühl D, Kőszegi T. A presepsin, mint a szepszis új, ígéretes biomarkere – előtanulmány. Aneszteziológia és Intenzív Terápia 2019; 49(2):35-39. **IF: -------**

Kustán P, Horváth-Szalai Z, Németh B, Török C, **Ragán D,** Kőszegi T, Mühl D: A szepszis diagnózisa napjainkban. Magyar Epidemiológia. 2015;12(1-2):59-66. **IF: -------**

Presentations/abstracts related to this thesis:

Ragán D, Kustán P, Ludány A, Mühl D, Kőszegi T. Actin, as a potential urinary marker of sepsis-related acute kidney injury. Clinica Chimica Acta. 2019;493:S10. **IF: 2,615**

Ragán D, Kustán P, Ludány A, Mühl D, Kőszegi T. Presepsin, as a novel sepsis biomarker. VIII. Interdiszciplináris Doktorandusz Konferencia; Pécs, 2019.05.24.

Ragán D, Kustán P, Mühl D, Ludány A, Kőszegi T. Urinary actin, as a novel marker of sepsis induced acute kidney injury. SepsEast 2018; Budapest 2018.11.15-17.

Ragán D, Kustán P, Ludány A, Mühl D, Kőszegi T. A presepsin, mint potenciális szepszis biomarker. MEDPécs – Medical Conference for PhD Students and Experts of Clinical Sciences; Pécs, 2018.10.27.

Ragán D, Kustán P, Ludány A, Mühl D, Kőszegi T. Actin is a promising urinary marker of sepsis-induced acute kidney injury. Clinical Chemistry and Laboratory Medicine. 2018;56(9):eA151-2. **IF: 3,638**

Ragán D, Kustán P, Ludány A, Mühl D, Kőszegi T. Urinary actin, as a potential marker of sepsis induced acute kidney injury (AKI). VII. Interdiszciplináris Doktorandusz Konferencia; Pécs, 2018.05.17.

Ragán D, Kőszegi T, Ludány A, Mühl D, Kustán P. A vizelet-aktin, mint potenciális marker szepszis indukálta veseelégtelenségben. DKK17 – Doktoranduszok a Klinikai Kutatásokban; Pécs, 2017.10.28.

Ragán D, Kustán P, Mühl D, Ludány A, Kőszegi T. A vizelet-aktin, mint potenciális marker szepszis indukálta veseelégtelenségben. XXXIII. Országos Tudományos Diákköri Konferencia Orvos- és Egészségtudományi Szekció; Pécs, 2017.04.18-21.

Ragán D, Kustán P, Mühl D, Ludány A, Kőszegi T. A vizelet-aktin, mint potenciális marker szepszis indukálta veseelégtelenségben. PTE ÁOK Tudományos Diákköri Konferencia; Pécs, 2016.04.14-15.

Ragán D, Kustán P, Mühl D, Ludány A, Kőszegi T. A vizelet-aktin, mint az akut vesekárosodás potenciális markere szepszisben. VIII. Nemzetközi és XIV. Országos Interdiszciplináris Grastyán Konferencia; Pécs, 2016.03.10.

Presentations/abstracts not related to this thesis:

Kanizsai P, Molnár T, Papp I, **Ragán D,** Tőkés-Füzesi M. Comparison of monocyte distribution width (MDW) with different sepsis markers in emergency care. Clinical Chemistry and Laboratory Medicine. 2021;59(9):eA60. **IF: 3,694**

Ragán D, Wahler I, Kőszegi T, Mühl D, Molnár T. Melatonin, as a potential anti-inflammatory mediator in sepsis. MEDPécs – Medical Conference for PhD Students and Experts of Clinical Sciences; Pécs, 2019.11.09.

Ragán D, Wahler I, Kőszegi T, Mühl D, Molnár T. Vizeletben detektált melatonin metabolit jelentősége szepszisben. IV. Mediterrán Intenzíves Randevú (MIRA); Pécs, 2019.09.20-21.

Kustán P, Szirmay B, Horváth-Szalai Z, **Ragán D,** Ludány A, Mühl D, Kőszegi T. Novel urinary protein markers in sepsis. Clinical Chemistry and Laboratory Medicine. 2016;54(10):eA199-200. **IF: 3,432**

Kustán P, Szirmay B, Horváth-Szalai Z, **Ragán D,** Ludány A, Mühl D, Kőszegi T. Monitoring of novel urinary protein markers in sepsis. 4th Joint EFLM-UEMS Congress; Varsó, 2016.09.21.

Cumulative impact factor of publications related to this thesis:	**3,240**
Cumulative impact factor of publications not related to this thesis:	**13,379**
Cumulative impact factor of all publications:	**16,619**

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