Actin-binding proteins in sepsis

Doctoral (PhD) Thesis

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I. INTRODUCTION

1. Epidemiology and conventional laboratory diagnostics of sepsis

Diagnosis of sepsis still remains one of the major challenges in medicine. Global estimates suggest 19 million hospitalized individuals with sepsis per year (1). Since sepsis is a multifaceted syndrome rather than a disease, it is difficult to raise objective diagnostic criteria. Sepsis was defined by the first international panel as a systemic inflammatory response to infection (2). Improved knowledge in the pathophysiology of the syndrome led to the refinement of its definition and is recently characterized as a life-threatening organ dysfunction caused by a dysregulated host response to infection. Despite of improving trends in acute mortality, lethality of septic shock can still be as high as 40–55% (3, 4).

Unfortunately, standardized microbial culturing methods are time-consuming and own limited efficiency (5). Therefore, besides the heterogeneous clinical symptoms (e.g. respiratory, cardiovascular, neurological dysfunctions), laboratory parameters (coagulation, liver and renal function tests) and protein markers are mandatory for early recognition and treatment of this life-threatening condition.

Currently, serum procalcitonin (PCT) is the most widely used marker in sepsis. PCT may accurately differentiate systemic inflammatory response syndrome (SIRS) from sepsis moreover, promising studies came to light regarding PCT-guided antibiotic therapy (5-7). A frequently investigated acute-phase protein in sepsis is high-sensitivity C-reactive protein (hsCRP) however, even local infections could trigger its synthesis (5). Hoping to find additional potential markers in sepsis, we elucidated the predictive value of serum actin and that of its binding proteins.

2. Serum actin and actin-binding proteins

Actin (molecular weight [MW]: 42 kDa) is a ubiquitous, conserved protein expressed in all eukaryotic cells. It exists in two main (globular [G]/monomeric and filamentous [F]/polymeric) forms (8, 9). Actin release from cells to the plasma could occur in inflammatory processes as a result of apoptosis/necrosis. The conditions of ionic strength, composition and pH in the plasma promote actin microfilament formation. Excessive amounts of free circulating actin could have

damaging effects, as microfilament formation could lead to the production of microthrombi and endothelial damage. In order to avert the potential deleterious effects of filament formation, an extracellular actin scavenging system (EASS) is evolved which is built on two proteins, gelsolin (GSN) and Gc (group-specific component)-globulin (10-15).

GSN is a calcium-dependent protein and has two main isoforms in mammals: cytoplasmic and plasma GSN. Plasma GSN (MW: 83 kDa) is mainly synthetized by skeletal muscle cells. Its concentration varies between 150-300 mg/L in healthy individuals, but it is highly method dependent (16). Plasma GSN has the ability to sever plasma filamentous actin into short oligomers in addition one molecule of GSN can also bind 2 molecules of G-actin. Plasma GSN could also adsorb lipopolysaccharide from Gram-negative bacteria with high affinity moreover, it binds to sphingosine 1-phosphate, and can inhibit platelet activating factor-mediated inflammatory responses (10-14, 16).

Gc-globulin (MW = 52–59 kDa) is a sparsely glycosylated α_2-globulin. It is mainly synthetized by hepatocytes and its serum concentration ranges between 200 and 600 mg/L in healthy individuals (17). Gc-globulin works in concert with GSN to scavenge free actin from the circulation, where acting as a potent monomer-trapping protein. Other key role of the molecule is the transport of vitamin D metabolites thereby functioning as the major vitamin D carrier. Gc-globulin can bind and inhibit endotoxins, and it is suggested to have co-chemotactic activity for C5a in inflammatory processes (17, 18).

In severe systemic inflammation and in tissue injury, the extracellular actin scavenging system gets overwhelmed by excessive amounts of intravascular actin. GSN and Gc-globulin complexed with actin are suggested to be cleared more rapidly by the reticuloendothelial system than the free proteins (10). So far, reduced serum GSN levels have been found in intensive care-related disorders, where declined serum GSN levels were associated with increased length of hospital stay, severe complications and increased mortality rate (19-22). Reduced serum Gc-globulin levels have been observed in trauma patients, in those suffering from sepsis, and in acute liver failure (23-28). In these cases, declined Gc-globulin levels predisposed to the development of organ dysfunctions, sepsis, and an increased risk of mortality.

II. OBJECTIVES

1. So far, the time-dependent changes of both serum actin and gelsolin levels in human sepsis have not been investigated. We aimed to monitor changes and predictive values of serum levels of actin, gelsolin and of a recently defined new marker: actin/gelsolin ratio in SIRS and in severe sepsis.

2. Even up to now, a rapid automated measurement of gelsolin has still remained a challenge. Therefore, our second task was to develop and validate a fast immune turbidimetric assay for serum gelsolin that would be suitable for possible routine clinical use. Our further objective was to adapt a rapid immune turbidimetric assay for serum Gc-globulin.

3. Simultaneous, rapid determination of the two main serum actin scavenger proteins in sepsis has not been investigated yet. Since rapid immune turbidimetric assays became available for the determination of both serum gelsolin and Gc-globulin, we aimed to investigate their predictive values together in sepsis.

III. PATIENTS AND METHODS

1. Patient categorization

Our study protocol was authorized by the Regional Research Ethical Committee of the University of Pécs (4327.316-2900/KK15/2011) and was performed according to the ethical guidelines of the 2003 Helsinki Declaration.

In our first investigation, patients with established diagnosis of SIRS or severe sepsis from the Department of Anesthesiology and Intensive Therapy (University of Pécs, Hungary) were enrolled in our follow-up study from January 2013 till December 2014. SIRS (n=12) and severe sepsis (n=32) were defined according to the Sepsis-2 criteria (29). Defined end points were the withdrawal of consent or death during the study period. The control group consisted of age- and gender- matched ambulatory ophthalmologic patients with comorbidities (n=28). Intensive care

patients were excluded if they were under 18 years of age or where it was not possible to obtain patient consent or consultee approval. Control patients under the age of 18 years and those suffering from acute inflammation or infectious disease were excluded from the control group. Both 7-day (non-survivors: n=11; survivors: n=21) and overall ICU mortalities were investigated. In our second clinical investigation, patients were enrolled in our follow-up study from January 2013 till August 2016. Intensive care unit patients were retrospectively categorized as stated by the Sepsis-3 definitions (3) into non-septic (n=28), septic (n=33) and septic shock (n=13) groups. As controls we used age- and gender- matched ambulatory patients with comorbidities (n=35). Among septic patients, 14-day mortality (non-survivors: n=18; survivors: n=28) was investigated. Intensive care unit (ICU) patients were excluded if they suffered from any autoimmune disorders, pre-existing hepatic failure or were under 18 years of age. Control patients under the age of 18 years and those with symptoms of acute inflammatory diseases or suffering from autoimmune disorders were also excluded from the study. Patients were followed in both studies during their ICU stay where serum samples were obtained on day 1, 2, 3 and 5 after clinical diagnosis.

2. Blood sampling

Venous blood (7.5 mL) was drawn from every patient using a closed blood sampling system (BD Vacutainer®). After 30 minutes, clotted blood samples were centrifuged for 10 min at 1500 g and sera were immediately analyzed or stored at −80°C.

3. Determination of serum actin and gelsolin levels by Western blot

Using 10% SDS-PAG electrophoresis by Laemmli (30), serum actin and GSN levels were determined by quantitative chemiluminescence Western blot based on the work of Lee et al. (21). Polyclonal primary antibodies (Rabbit Anti-Human Actin, N-terminal, ref. no: A2103, Sigma-Aldrich Co. LLC; Rabbit Anti-Human Gelsolin, ref.no: A0146, Dako A/S, Glostrup, Denmark) and horseradish peroxidase-labeled secondary antibodies (Swine Anti-Rabbit Immunoglobulins, ref.no: Z0196, Dako A/S) were applied. For the quantification of Western blot highly purified G-actin standard obtained from rabbit skeletal muscle and recombinant human GSN expressed in *Escherichia coli* (His-8) were used.

4. Immune turbidimetric assay for serum gelsolin

The immune turbidimetric assay for se-GSN measurement was performed on an open developmental channel of the c502 module of a Cobas 8000 analyzer (Roche Diagnostics GmbH, Mannheim, Germany). Because of the unavailability of any commercial GSN calibrator for immune turbidimetric assay, we applied recombinant human GSN expressed in *Escherichia coli* (His-8). Dilution series of the calibrator were prepared using fetal bovine serum (FBS, ref. no. Ph. Euro. 2262, PAN Biotech, Aidenbach, Germany). Pooled human serum from healthy volunteers served as an "in-house" control, due to the lack of commercially available quality control material. We used Polyclonal Rabbit Anti-Human GSN antibody in the assay (ref. no. A0146, Dako A/S) pre-diluted (1:4) with Dilution Buffer (ref. no. S2005, Dako A/S); and Reaction Buffer (ref. no. S2007, Dako A/S), based on the previous work of Christensen et al. (31) with modifications. The second edition of Eurachem guidelines (32) was applied for the validation.

5. Immune turbidimetric assay for serum Gc-globulin

The immune turbidimetric assay for serum Gc-globulin measurement was executed on an open developmental channel of the c502 module of a Cobas 8000 analyzer (Roche Diagnostics GmbH, Mannheim, Germany). Serum Gc-globulin levels were measured according to the modified protocol of Bangert (33) by using Polyclonal Rabbit Anti-Human Gc-Globulin antibody (ref. no. A0021, Dako A/S) pre-diluted (1:5) with Dilution Buffer (ref. no. S2005, Dako A/S); Reaction Buffer (ref. no. S2007, Dako A/S); Human Serum Protein Calibrator (ref. no. X0908, Dako A/S) and Human Serum Protein Low Control (ref. no. X0939, Dako A/S). The second edition of Eurachem guidelines (32) was applied to test the performance of the Gc-globulin assay.

6. Determination of routine laboratory parameters and clinical scores

All other laboratory parameters were determined by automated routine laboratory techniques. Acute Physiology And Chronic Health Evaluation (APACHE) II, Simplified Acute Physiology Score (SAPS) II and Sequential Organ Failure Assessment (SOFA) clinical scores were

calculated for the first day of intensive care treatment. Quick SOFA (qSOFA) scores were assessed based on ICU admission parameters. Mean arterial pressure (MAP) was assessed by intra-arterial blood pressure monitoring in the ICU.

7. Statistical analysis

For statistical analysis IBM SPSS Statistics for Windows, Version 22 and Origin Pro 8 softwares were used. Distribution of data was evaluated by Shapiro-Wilk test. Non-parametric tests and regression analyses were used for investigating differences between patient groups and for determining the predictive value of our markers. Bland-Altman plot was used for method comparison regarding GSN assay. Changes in the results were considered to be statistically significant at $p<0.05$.

IV. RESULTS

1. Serum actin, gelsolin levels and actin/gelsolin ratios in SIRS and in sepsis

Clinical and laboratory parameters

The majority of the patients (63.6%) were admitted to the ICU after surgical interventions and 36.4% of them after other medical events (e.g. acute respiratory failure). Among the first-day routine laboratory and clinical parameters, we observed significantly higher serum PCT ($p<0.001$), hsCRP levels ($p<0.001$), APACHE II ($p<0.001$), SAPS II ($p<0.001$) and SOFA ($p<0.05$) scores in septic compared with SIRS patients.

Non-survivor sepsis patients regarding 7-day mortality exhibited significantly ($p<0.05$) higher PCT levels and clinical scores than survivors. Common organ dysfunctions in sepsis were acute renal failure (65.6%) and acute lung injury (50%), in 21.8% of the septic patients we found thrombocytopenia, in 12.5% acute hepatic failure also developed. Hemoculture was positive in 18.8% of the septic patients, in 53.1% of the patients pathogens were detected in other specimen sources (e.g. bronchoalveolar lavage).

Serum actin, gelsolin levels and actin/gelsolin ratios in critically ill patients

Significantly ($p<0.01$) higher first-day GSN levels were observed in SIRS compared with sepsis, and the highest values were obtained in controls (Figure 1A). The highest first-day serum A/GSN were observed in sepsis, significantly lower A/GSN ratios were obtained in SIRS ($p<0.05$) and the lowest values were found in controls ($p<0.001$) (Figure 1B, D).

Survivor septic patients showed significantly ($p<0.05$) higher first-day GSN levels in their sera than non-survivors (Figure 1C). Furthermore, serum GSN levels were found to be higher in survivors compared with non-survivors on day 3 (22.95 vs. 3.69 mg/L; $p<0.05$), too. Higher median values of serum actin levels were observed in non-survivors than in survivors during the follow-up, although not being statistically significant. Patients who failed to survive sepsis had significantly higher 2nd day's A/GSN ratios than survivors (median: 2.18 vs. 0.19; $p<0.05$).

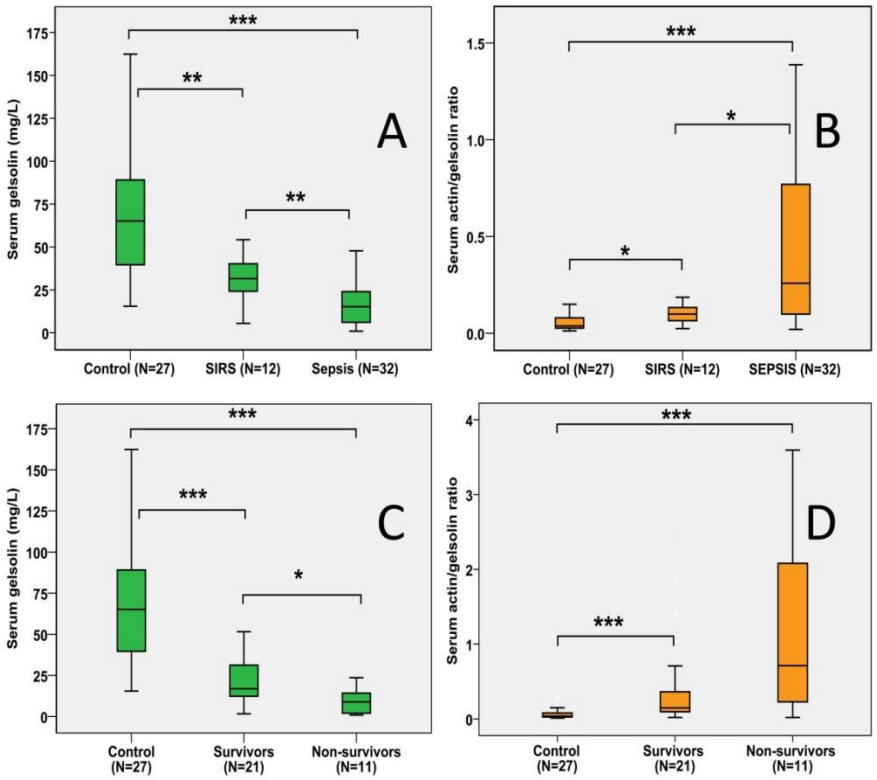

Figure 1. First-day serum GSN levels and A/GSN ratios in septic, SIRS and control patients (A, B), and in septic survivors, non-survivors based on 7-day mortality (C, D). *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$. SIRS: systemic inflammatory response syndrome

Spearman's correlation analysis

Serum GSN levels were found to correlate inversely with PCT (ρ= -0.38, $p<0.05$), hsCRP levels (ρ= -0.65, $p<0.01$), SAPS II (ρ= -0.37, $p<0.05$), SOFA clinical scores (ρ= -0.35, $p<0.05$) and positively with serum albumin levels (ρ=0.43, $p<0.01$). A/GSN ratios positively correlated with hsCRP concentrations (ρ=0.43, $p<0.01$) and SOFA clinical scores (ρ=0.32, $p<0.05$).

Receiver operating characteristics (ROC) and COX regression analyses

For differentiating patients with sepsis from those with SIRS, ROC area under the curve (AUC) values were 0.95 for serum PCT, 0.84 for hsCRP, 0.77 for GSN and 0.70 for A/GSN ratios, respectively (both significant at $p<0.05$).

Regarding 7-day mortality in sepsis, AUC value for PCT was found to be 0.75, for GSN it was 0.74 (both significant at $p<0.05$). AUC values for A/GSN (0.70) and for hsCRP (0.66) did not meet criteria for statistical significance.

We also determined the predictive values of the studied markers regarding overall ICU mortality. COX regression analysis showed that only first-day APACHE II scores (hazard ratio (HR)=1.208; 95% confidence interval (CI)=1.083 – 1.347; p=0.001) and A/GSN ratios (HR=1.172; 95% CI=1.079 – 1.273; $p<0.001$) were able to predict the outcome of sepsis.

2. Methodological developments

2.1. Immune turbidimetric assay for serum gelsolin

Validation data and stability studies

Figure 2A represents a cumulative graph of 9 independent calibrations. Limit of blank (LOB), limit of detection (LOD), and limit of quantification (LOQ) were found to be 0.47 mg/L, 0.72 mg/L and 1.99 mg/L, respectively. Coefficient of variation remained below 5% in most of the cases during the intra- and inter-assay variability measurements. Recovery varied between 84.56-

93.52% when investigating 4 different ranges. Linearity study gave an appropriate coefficient by the linear regression analysis (r^2= 0.998) after comparing calculated and measured GSN concentrations at 10 different concentrations (Figure 2B).

GSN levels remained almost unchanged (96.70-117.36%) during the 10-day stability period, and no considerable differences were observed even throughout five repeated freezing-thawing cycles.

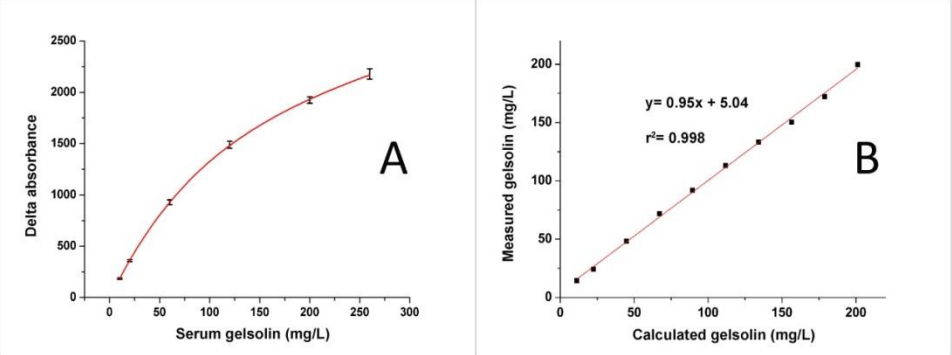

Figure 2. Validation results. A: Summarized graph of a 6-point calibration curve of GSN assay in the range of 10–260 mg/L by exponential graph fitting (n=9). B: Linearity of serum GSN assay. Dots represent means. R^2: regression coefficient.

Comparison of previous Western blot and recent immune turbidimetric results

Bland-Altman plot defined a bias of 0.26 and an agreement range from −0.79 to 1.09 units when comparing GSN levels of septic, SIRS and control patients measured by previous Western blot method and by the new turbidimetric assay.

2.2. Immune turbidimetric assay for serum Gc-globulin

Performance data and stability studies

LOB, LOD, LOQ were found to be 0.43 mg/L, 0.66 mg/L and 1.85 mg/L, respectively. Intra-assay imprecision was found to be between 1.38 – 1.64% of CV, while inter-assay imprecision was estimated to be 5.03% of CV. The method for Gc-globulin determination was found to be

linear (r^2=0.995) when investigating 7 different dilutions (8 – 332 mg/L) of a serum sample. No considerable differences of Gc-globulin concentration were noticed during the 6-day stability period and even throughout five repeated freezing-thawing cycles.

3. Assessment of predictive values of both gelsolin and Gc-globulin in sepsis

Main clinical and laboratory results

In the intensive care patients' groups qSOFA scores did not alter significantly. First-day APACHE II scores differed ($p<0.05$) in all three ICU groups, while SAPS II scores were higher ($p<0.01$) in sepsis and in septic shock when compared with the non-septic group. SOFA scores were more increased ($p<0.01$) in septic shock compared with sepsis and non-sepsis patients. Classic inflammatory markers (serum hsCRP, PCT) were significantly ($p<0.001$) higher in septic shock and in sepsis patients compared with non-septic ICU patients. Microbiological cultures gave positive results in 76.8% of the cases.

Serum gelsolin and Gc-globulin levels in septic shock, septic, and non-septic patients

First-day serum levels of both actin-binding proteins were significantly ($p<0.001$) higher in the control population than in the ICU patients (Figure 3A, B). Non-septic ICU patients exhibited higher ($p<0.001$) first-day serum GSN concentrations than patients with sepsis and septic shock (Figure 3A). First-day Gc-globulin levels were significantly ($p<0.001$) higher in non-septic critically ill patients and in septic patients ($p<0.01$) when compared with those suffering from septic shock (Figure 3B).

Patients suffering from sepsis had significantly ($p<0.01$) higher serum Gc-globulin levels during the 5-day follow-up when compared with those seen in septic shock (Figure 3D). Significantly ($p<0.05$) increased 2nd and 5th day's serum Gc-globulin levels were detected in septic patients when compared with the 1st day concentrations. The two investigated patient groups did not differ significantly regarding serum GSN levels during the 5-day follow-up (Figure 3C).

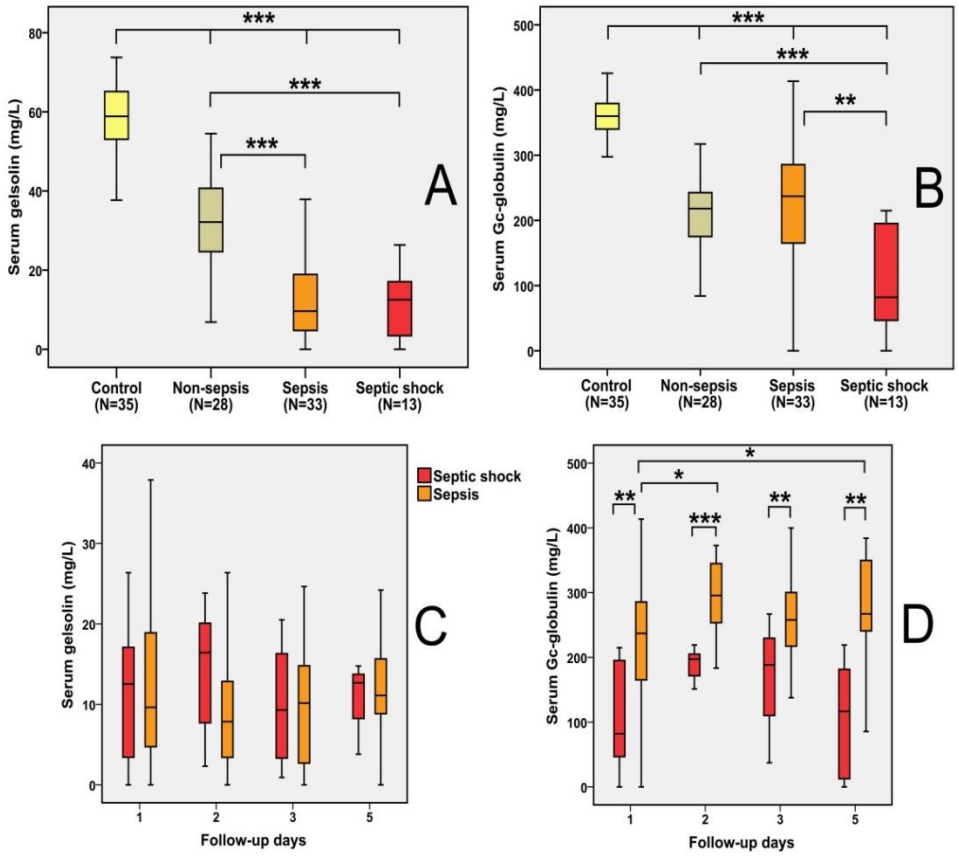

Figure 3. First-day serum gelsolin (A) and Gc-globulin (B) levels in septic shock, septic, non-septic ICU and control patients, and follow-up of serum gelsolin (C) and Gc-globulin (D) concentrations where patients are divided into septic and septic shock groups. *p<0.05, **p<0.01, ***p<0.001.

Serum gelsolin and Gc-globulin levels in septic survivors vs. non-survivors regarding 14-day mortality

First-day serum GSN levels were higher in survivor than in non-survivor septic patients (median: 12.9 vs. 6.9 mg/L; $p<0.05$). No further significant differences or changes were observed in serum GSN concentrations during the 5-day time course of sepsis.

There was a trend ($p<0.05$) towards increasing Gc-globulin levels, when comparing the 1st with the 2nd day's (median: 212.8 vs. 271.9 mg/L), and the 1st with the 3rd day's (median: 212.8 vs. 235.2 mg/L) Gc-globulin levels in survivors. Similar tendency ($p<0.05$) was seen in non-survivors when comparing the 1st with the 2nd day's serum Gc levels (median: 155 vs. 267.1 mg/L).

Spearman's correlation results

Serum GSN and Gc-globulin positively correlated with each other (ρ= 0.48, $p<0.01$), in addition, both of them positively correlated with serum albumin (GSN – albumin: ρ= 0.54; Gc – albumin: ρ=0.61, $p<0.01$) and negatively with hsCRP (GSN – hsCRP: ρ= -0.68; Gc – hsCRP: ρ= -0.43, $p<0.01$). Gc-globulin inversely correlated with plasma lactate (ρ= -0.64, $p<0.01$), with PCT (ρ= -0.34, $p<0.01$), and with clinical scores (Gc – SAPS II: ρ= -0.49, $p<0.01$; Gc – APACHE II: ρ= -0.35, $p<0.05$; Gc – SOFA: ρ= -0.52, $p<0.01$).

Results of ROC and logistic regression analyses

In the differentiation of septic patients from other patients suffering from non-infective diseases requiring ICU treatment, besides serum PCT (AUC: 0.98, $p<0.001$) and hsCRP (AUC: 0.80, $p<0.01$), GSN also had significant discriminative value (AUC: 0.88, $p<0.001$) with a cut-off point of 22.29 mg/L (sensitivity: 83.3%, specificity: 86.2%) (Figure 4A, B).

The discriminative function of plasma lactate regarding septic shock/sepsis states was proven to be the highest (AUC: 0.99, $p<0.001$), in addition, Gc-globulin (AUC: 0.76) and MAP (AUC: 0.74) also had significant ($p<0.05$) diagnostic values (Figure 4C, D). The optimal cut-off point for Gc-globulin was 116.5 mg/L (sensitivity: 78.3%, specificity: 60%).

For predicting 14-day mortality in sepsis, SOFA clinical scores (AUC: 0.88, $p<0.001$) and serum GSN (AUC: 0.71, $p<0.05$) proved to be useful as discriminating factors regarding surviving/non-surviving states (Figure 4E, F). The calculated cut-off value for GSN was 8.7 mg/L (sensitivity: 71.4%, specificity: 58.3%).

Including the investigated parameters into the logistic regression model, SOFA scores (ß= 0.53; p= 0.03; OR=1.70; 95% CI: 1.03 – 2.79) and serum GSN (ß=-0.15; p= 0.04; OR=0.87; 95% CI: 0.75 – 0.99) showed predictive capacity regarding 14-day mortality in sepsis.

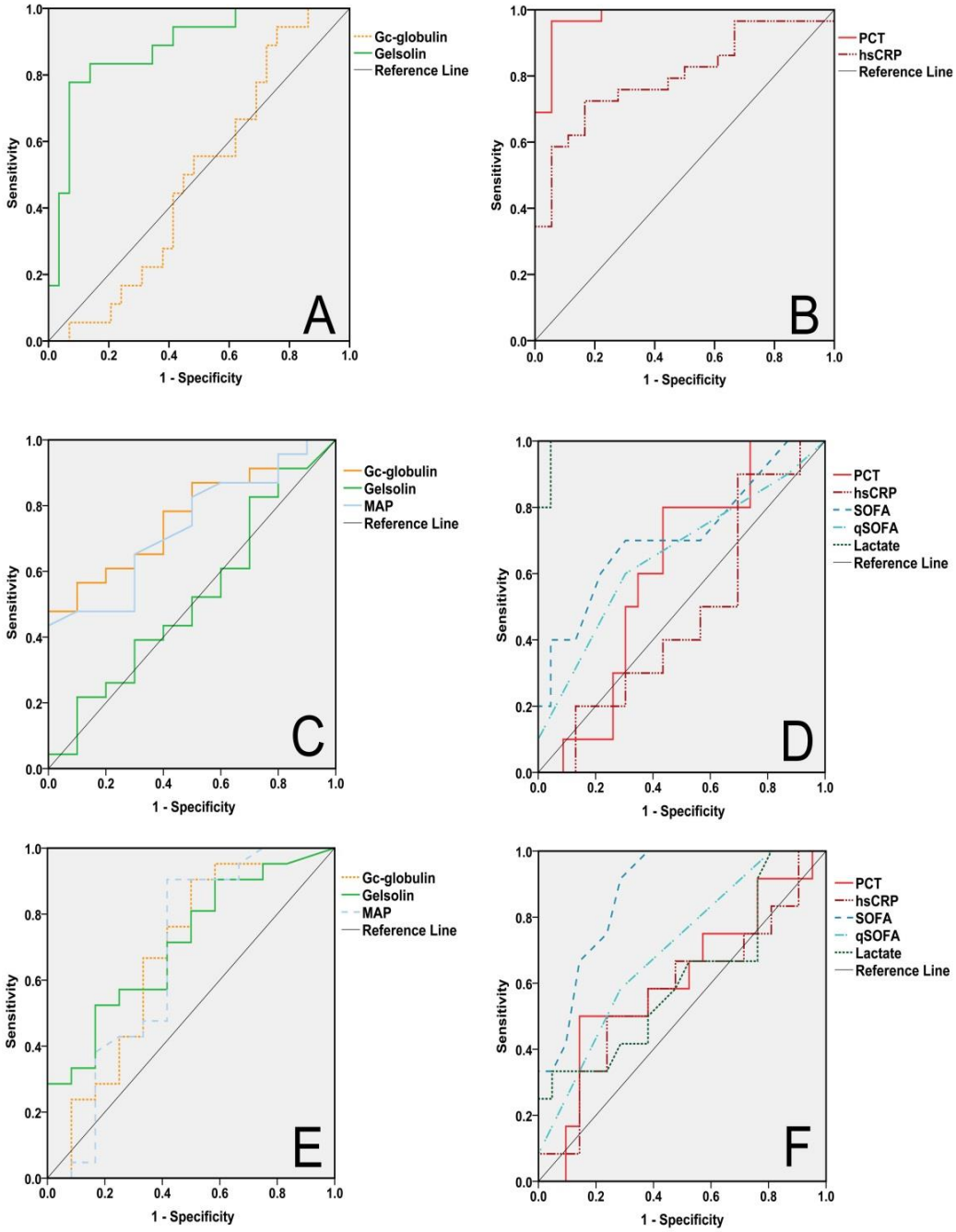

Figure 4. Receiver operating characteristic curves of first-day laboratory and clinical parameters for differentiating non-sepsis from sepsis (A, B), for distinguishing septic shock from sepsis (C, D) and for predicting 14-day mortality in sepsis (E, F). hsCRP: high sensitivity C-reactive protein; MAP: mean arterial pressure; PCT: procalcitonin; qSOFA: quick Sequential Organ Failure Assessment; SOFA: Sequential Organ Failure Assessment

V. DISCUSSION

1. Serum actin, gelsolin levels and actin/gelsolin ratios in SIRS and in sepsis

In our first study we elucidated the predictive values of serum GSN and of the newly defined A/GSN ratios quantified by Western blot. We emphasize that in our study septic patients' first-day GSN levels were found to be significantly lower in non-survivors than in survivors based on 7-day mortality, similar to the observation of Lee et al. (20, 21), but in contrast to Wang et al. (22).

Opposite to the studies performed by Lee et al. (20, 21) and Wang et al. (22), we did observe significant difference between septic surviving and non-surviving patients regarding serum albumin levels. Mounzer et al. (19) also reported a positive correlation between serum albumin and GSN levels in trauma patients.

We observed – in accordance with Mounzer et al. (19) – lower amounts of actin in the sera of septic patients than suggested by Lee et al. (21). Interestingly, Lee et al. identified actin only in 81% of plasma samples from septic patients and in none of normal volunteers, whereas we detected actin in all serum samples of septic and control patients. This could be attributed to our more sensitive actin-detecting method.

In contrast to Lee et al. (21), who examined only healthy and younger patients as controls compared with septic patients, we investigated age- and gender matched controls vs. septic patients.

Similarly to Belsky et al. (15), we did not observe any significant changes regarding serum actin levels during the follow-up period. In conjunction with Lee et al. (20), but contrary to Mounzer et al. (19) and to Wang et al. (22), we observed that GSN levels for all patients in the septic group did not show inter-day significant differences.

2. Methodological developments

In our methodological study, we introduced a fast, accurate immune turbidimetric method requiring a very low sample volume for the determination of se-GSN levels, partially based on

the previous work of Christensen et al (31). To our best knowledge, no other similarly fast assay for se-GSN is currently available.

Former study of Christensen et al. (31) presented an immune turbidimetric assay for se-GSN on Cobas Mira Plus which offered a slightly lower (4%) total imprecision when compared with our method and a detection limit of 2.7 U/L. However, due to unexpected GSN calibrator and control stability problems, this former assay is no longer available. Study of Dahl et al. (24) presented an immune nephelometric measurement for plasma GSN, but they did not offer any validation data about the assay.

We found se-GSN as a stable protein maintaining its concentration constant at +4°C for 10 days and even throughout 5 repeated freezing-thawing cycles. However, we do not consider EDTA (ethylenediamine tetraacetic acid)-containing tubes proper for sample collection when measuring GSN levels because plasma GSN drops significantly in a short time (data not shown). One possible explanation to this might be that EDTA binds Ca^{2+} ions more strongly than sodium citrate, in addition, it chelates other metal ions too (34), which results in the destabilization of the GSN domains.

In concert with Hamashima et al. (35), we found lower LOD regarding serum Gc-globulin assay when compared with Bangert et al. (33) which possibly arises from different blank samples. Regarding intra- and inter-assay imprecision we observed no significant differences when comparing the measuring system of Bangert et al. with ours. Hamashima et al. detected similar intra-assay but lower inter-assay imprecision than we obtained in our study. Immune turbidimetric assay of Hamashima et al. required higher sample volume than the assay of Bangert et al. and that of ours.

3. Predictive values of gelsolin and Gc-globulin in critically ill conditions

Our study revealed that serum GSN provides valuable complementary data for the rapid diagnosis of sepsis. Previous studies performed by Lee et al. also indicated that first-day GSN levels are significantly higher in survivors than in non-survivors of sepsis (20, 21).

For Gc-globulin, we did not find any association between first-day levels and mortality of ICU patients, similarly to the studies of Leaf et al. (26, 27) and Gressner et al. (28).

We did not find any significant differences in first-day's serum Gc-globulin levels when comparing sepsis with non-sepsis patients, contrary to the expectations of Jeng et al. (25). Similarly to the observations of Dahl et al. (24), we also noticed a slight increase in Gc-globulin concentrations during the 5 – day follow-up period regarding sepsis patients. That phenomenon can be attributed to the increased synthesis of Gc-globulin as an acute phase reactant after severe injury (23). Opposite to Gc-globulin, no significant increment was noted during the short-term observation period regarding GSN, similarly to the work of Lee et al. (20) and to our previous findings, which could be explained by the lack of newly induced synthesis in the muscle cells after injury (16).

Based on literature data of previous studies, we seem to be the first to measure serum GSN and Gc-globulin levels simultaneously by rapid immune turbidimetry during the course of sepsis. We demonstrated a significant positive correlation between the two actin-binding proteins, which supports the hypothesis that they act in concert in the intravascular space. In contrast to Gressner et al. (28), we found significant negative correlations between Gc-globulin, hsCRP and PCT, furthermore, between Gc-globulin and clinical scores, too. Similarly to our previous data, GSN negatively correlated with hsCRP. Also, similarly to our previous study, we investigated age-, gender- matched control patients with comorbidities therefore the comparisons of patient groups regarding serum GSN or Gc-globulin levels were not affected by chronic underlying diseases.

Apart from sepsis, decreased se-GSN levels were found after parenchymal tissue damages including acute lung injury, major trauma, myonecrosis, and acute liver failure, too (16). Depressed serum Gc-globulin levels were found also in patients with hepatic failure and in trauma patients with shock (17). These findings indicate that none of these proteins are specific for sepsis. However, since sepsis is a syndrome rather than a disease, none of the biomarkers would offer 100% specificity (4). The magnitude of the decrease in serum GSN and Gc-globulin levels is of utmost importance, therefore, appropriate cut-off values have to be set.

PCT is the gold standard serum marker of sepsis, with great sensitivity and specificity for the diagnosis of this clinical syndrome. However, we suggest that serum GSN, A/GSN ratios and Gc-globulin give important additional information regarding the outcome and the immune status of the septic patients.

VI. SUMMARY, NOVEL FINDINGS

- We developed a sensitive Western blot method for the detection of serum actin, which serves as a promising starting point for further methodological developments.

- We simultaneously examined the time-dependent changes of both serum actin and gelsolin levels in human sepsis, which is a novelty.

- The introduced new serum actin/gelsolin ratio proved to have a promising predictive capacity regarding overall ICU mortality in sepsis, similarly to that of APACHE II scores.

- We validated a new, rapid and accurate immune turbidimetric method requiring low sample volume for detecting serum gelsolin. The new, rapid gelsolin assay may prove to be useful in the clinical area, especially in the field of intensive care (sepsis) or even internal medicine (chronic inflammatory disorders).

- We proved that serum gelsolin and Gc-globulin act in concert and both of them negatively correlate with inflammatory parameters in SIRS and in sepsis, thereby also supporting their immunomodulatory roles.

- Serum gelsolin may serve as a complementary diagnostic and predictive marker in sepsis. Critically low serum Gc-globulin concentration reflects the potential development of septic shock.

- Immune turbidimetric measurement of gelsolin and Gc-globulin levels gives the possibility to obtain important additional information on sepsis severity within a short turnaround time.

VII. REFERENCES

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VIII. LIST OF PUBLICATIONS

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