

# Nonconventional markers of sepsis

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## ARTICLE INFO

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### Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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## ABSTRACT

Sepsis still remains a challenging healthcare problem with high mortality rate. To improve outcome, early diagnosis and monitoring of sepsis is of utmost importance. In this process objective laboratory parameters are the most helpful.

Procalcitonin and C-reactive protein are the most commonly used and recommended markers of sepsis however, more than 200 sepsis biomarkers have already been published. This mini review focuses on nonconventional novel possibilities for the recognition of sepsis severity. Presepsin, actin and actin scavenger proteins (gelsolin and Gc-globulin) and orosomuroid are discussed. Besides serum parameters, the urinary levels of these markers are also elaborated, since urinary biomarkers of sepsis provide new diagnostic implications and are helpful for monitoring both the kidney function and the septic process.

Increasing serum actin levels and decreasing levels of actin binding proteins seem to be associated with sepsis severity and outcome. Actin can be detected in the urine samples of septic patients as well, and strongly elevated levels of it were found in sepsis-related acute kidney injury. Both serum and urinary

orosomuroid might be able to indicate sepsis, however urinary orosomuroid is a more sensitive inflammatory marker.

Novel laboratory tests can provide rapid help for clinical decision making because the key point in successful treatment lies in the early diagnosis of sepsis.



## INTRODUCTION

Although sepsis is one of the oldest syndromes in medicine it is a challenging healthcare problem even nowadays. In spite of the era of modern antibiotics and intensive therapy sepsis is still one of the leading causes of morbidity and mortality (1).

Sepsis is a heterogeneous and complex syndrome with various etiology, severity and prognosis. To our present knowledge the inflammatory response is the key role in the pathophysiology of sepsis however; a kind of uncertainty exists regarding the factors most likely to lead to increased lethality. In spite of the uncertainties one fact is obvious: the earlier the diagnosis of sepsis is raised, the more favorable outcome may be predicted (2, 3).

Based on the novel results and advances of pathobiology, management and epidemiology of sepsis, the definitions of the syndrome have been changed recently. Sepsis-3 consensus defines sepsis as a life-threatening organ dysfunction caused by a dysregulated host response to infection (4).

The diagnosis of sepsis is most often not easy especially in newborns or in patients whose immune response is not adequate. Therefore, it is of utmost importance to introduce diagnostic biomarkers which can predict or verify systemic inflammation as early as possible. These tests should also be applicable for monitoring of the disease progression and efficacy of therapy as well.

Microbiological identification of pathogens is essential for efficient therapy of sepsis, because the clinical signs are nonspecific. Gold standard microbiological culturing methods require quite a long time (days), but new molecular biological techniques, polymerase chain reaction and mass spectrometric methods can shorten pathogen identification in the bloodstream (5). However, these methods can not differentiate between colonization and infection, moreover they need a well trained and equipped laboratory.

The diagnosis and monitoring of sepsis is of utmost importance, in this regard objective laboratory tests may provide rapid information for proper decision making. Up to now, more than 200 sepsis biomarkers have already been studied, most of them belonging to the inflammatory mediators' family (acute phase proteins, cytokines, chemokines, CD markers, adhesion molecules, etc.) (6, 7).

This mini review discusses classical sepsis biomarkers as well but the major focus will be on some of novel interesting nonconventional markers of sepsis.

## CONVENTIONAL SEPSIS MARKERS: SERUM PCT AND CRP

The diagnostic and therapeutic guidelines of sepsis management recommend the use of procalcitonin (PCT) and C-reactive protein (CRP) measurements for early recognition of the syndrome (2, 8).

Blood levels of PCT rise 4-6 hours after the onset of systemic infection and PCT's half-life is about one day. Procalcitonin concentrations showed good correlation with the severity of sepsis, higher PCT levels correlated with higher risk of mortality (9). Massive tissue damage could also provoke elevated serum PCT values without infection, but fungal and viral infections do not elevate the PCT concentrations (10).

Monitoring of PCT kinetics is recommended because delta PCT is a better marker of infection than absolute levels and furthermore, early PCT kinetics could indicate the efficacy of antibiotic therapy (11, 12).

CRP is a non-specific inflammatory marker, therefore it increases in many acute and chronic diseases (tissue injury, autoimmune disorders, malignancies), however in sepsis management, CRP could supplement PCT measurements. After infections serum CRP reaches its maximum within 48-72 hours. Strongly elevated CRP levels were found to be severity and mortality predictors in sepsis (13). The measurement of high sensitivity CRP (hsCRP) is recommended.

Since both biomarkers have some limitations, promising other possibilities should be searched for and in fact, are available nowadays.

### PRESEPSIN

CD14 molecule is a pattern recognition receptor existing in two forms: as a membrane-bound type (mCD14) and a soluble form (sCD14). Both forms play a role in recognition of LPS and in cell activation. Soluble CD14 subtype (sCD14-ST) also called as presepsin elevates significantly during inflammation and seems to be usable in differentiating between bacterial and nonbacterial infections (14).

Presepsin is normally present in very low concentrations in the serum of healthy individuals. In response to bacterial infections, its concentration increases within 2 hours, according to the severity of the disease (15). Studies have been reported with various diagnostic cut-off levels for sepsis between 400–600 pg/ml (16, 17). Preliminary studies showed that plasma presepsin is a highly sensitive and specific marker of sepsis, and its concentration significantly correlates with the severity of the disorder and in-hospital mortality of patients suffering from severe sepsis and septic shock (18). A novel

point of care test is available on the market for rapid presepsin determination, which can help clinicians in rapid decision making.

Due to its 13 kDa molecular weight, presepsin is filtered through the glomeruli, then reabsorbed, and catabolized within proximal tubular cells (19). There is increasing evidence, that presepsin levels are affected by kidney function. Elevated presepsin levels were found in patients with decreased renal function and inverse correlation was described between presepsin and GFR as well (19, 20). Therefore, presepsin levels should be interpreted more attentively in patients with kidney disease.

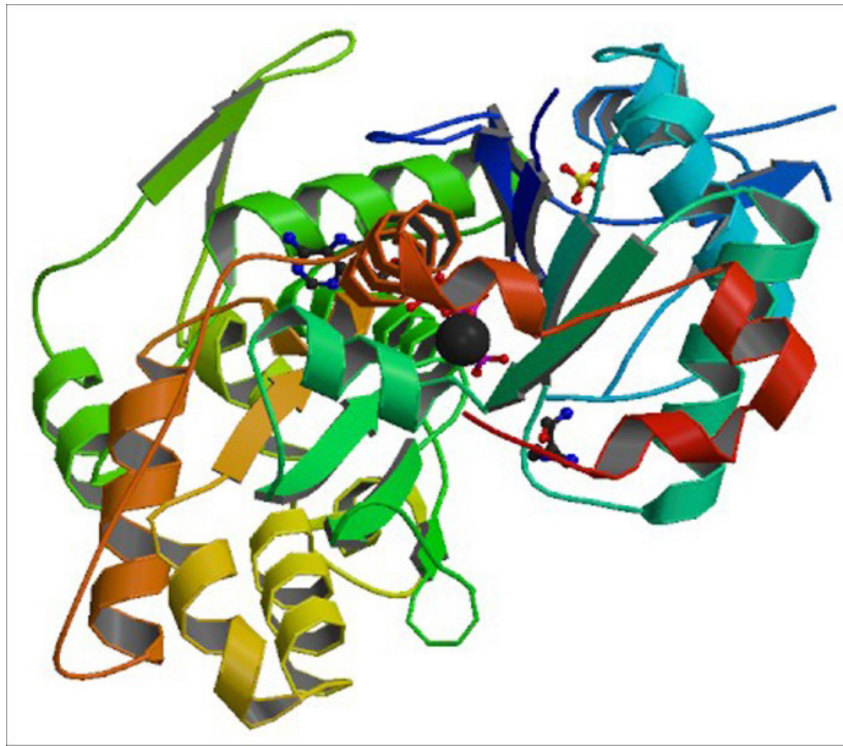
### ACTIN

Actin is a multifunctional 43 kDa protein which is present in all eukaryotic cells in monomeric/globular (G-actin) and in polymeric/filamentous (F-actin) form (Figure 1). The two forms dynamically change due to the very rapid polymerization and depolymerization of the molecule. Actin takes a pivotal part in many cellular processes (building up microfilamental cytoskeleton, motility, moving, division, junctions) and in muscle contraction, too (22).

As actin is one the most abundant intracellular protein, during massive cell injury and catabolic conditions high amounts of actin can release into the circulation. Free extracellular actin has toxic effects, since actin filaments are thought to increase blood viscosity, to activate platelets, and cause endothelial cell damage and small blood vessel obstructions. Therefore, high amounts of extracellular actin may contribute to the development of multiple organ failure (23, 24).

The so called actin scavenger system is responsible for the protection of the body from actin toxicity; however the capacity of this defense system can be overwhelmed by massive tissue injury (25).

**Figure 1** The structure of native G-actin



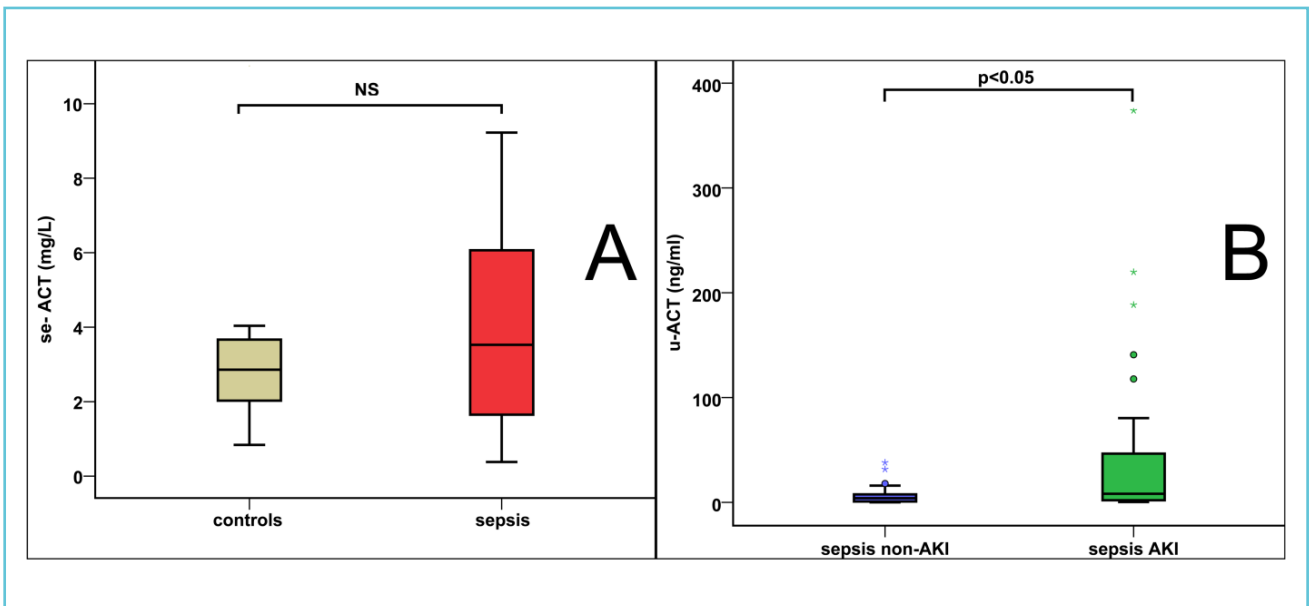
*G-actin contains 4 subdomains, and includes one ATP molecule and one Ca<sup>2+</sup> ion located in the nucleotide cleft, which exists in a closed state (21).*

In healthy individuals the major source of extracellular actin is most probably the skeletal muscle with its large mass and high actin content. Circulating actin levels might provide clinically relevant information on disease severity, serum actin (se-ACT) levels were found to be higher in septic patients (3.5 (1.6-6.1) mg/L) than in controls (3.0 (2.1-3.7) mg/L) however did not meet criteria for statistical significance (Figure 2A). The cause of increased se-ACT levels in systemic inflammation and in sepsis might be the extensive tissue injury and detritus of blood cells (26). There is only scarce data on urinary appearance of actin, however due to its molecular weight free actin could be filtrated through the glomeruli. Recently, our research group has observed the presence of actin in urine samples of septic patients in contrast, actin could not be detected

in urine specimens from healthy individuals (27). Urinary actin (u-ACT) levels were determined by quantitative western blot, as in serum. Significantly higher urinary actin was measured in samples of patients with sepsis-related acute kidney injury (AKI, Figure 2B) compared to non-AKI patients (8.17 (2.09-45.53) ng/mL vs. 4.03 (0.91-10.21) ng/mL). Dialyzed patients showed extremely high u-ACT levels (36.02 (4.7-176.56) ng/ml). U-ACT correlated significantly ( $p < 0.01$ ) with kidney function markers (serum creatinine: 0.315, urinary albumin: 0.704) but no correlation was found with se-ACT levels (27).

Previously Kwon et al. found increased u-ACT levels as predictors of kidney failure after ischemic injury in renal allografts (u-ACT/u-Cr were  $1095.6 \pm 729.6$  ng/mg in cadavers with sustained acute renal failure and  $355.0 \pm 247.0$  ng/mg in

**Figure 2** Serum and urinary actin levels in septic patients



Based on (26), (27).

cadavers recovering from acute renal failure;  $p < 0.05$ ) (28). However the appearance of actin in urine has not been clarified, u-ACT excretion may reflect overall cellular damage in the kidneys, thus it might provide novel possibility for early diagnosis of AKI, which is the most severe complication of sepsis.

### ACTIN-BINDING PROTEINS

In order to protect the body from overwhelming actin toxicity, there are two major extracellular actin-binding proteins called gelsolin (GSN) and Gc-globulin (group specific component, also called vitamin D-binding protein) (Figure 3). Both plasma proteins are essential actin scavengers working in concert. GSN severs and depolymerizes actin filaments originating from disrupted cells, and Gc-globulin frees GSN from actin monomers and sequesters them. The bound actin filaments and monomers are finally cleared from the circulation by the reticulo-endothelial system (31). Furthermore, both GSN and Gc-globulin could modulate inflammatory processes. Under physiological conditions, the

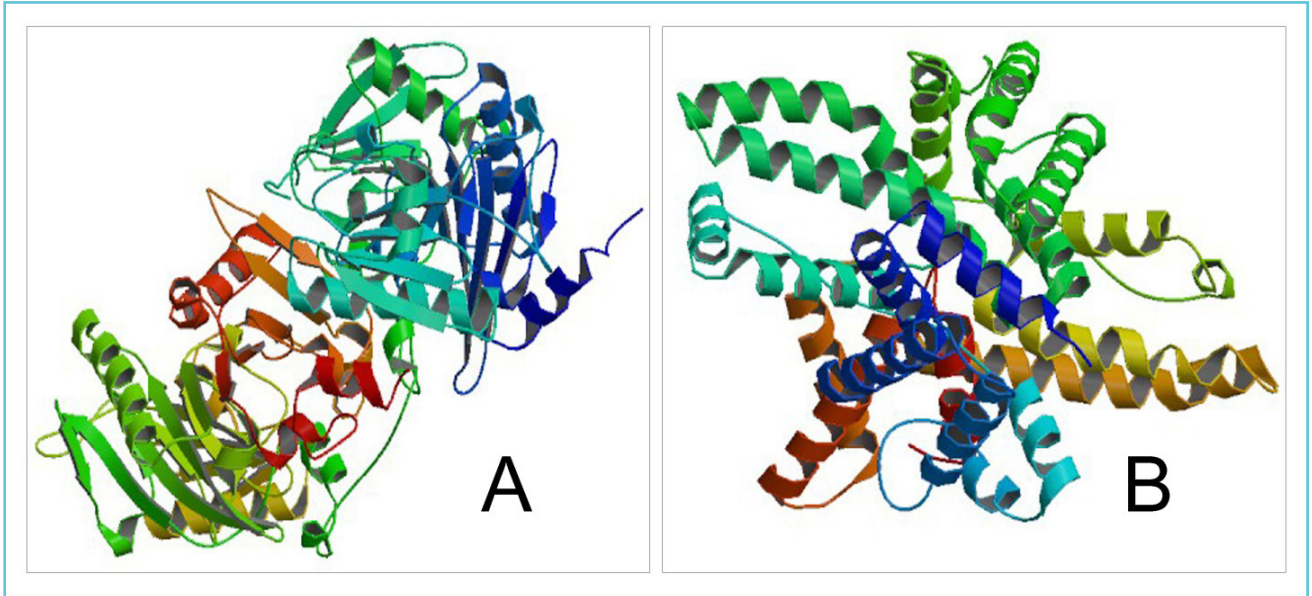
concentration of actin in the blood is far less than that of actin binding proteins. Interestingly, in case of severe systemic inflammation, due to excessive tissue injury the excessive amount of extracellular actin and the pro-inflammatory mediators exceed the binding capacity of the scavenger proteins, so the plasma concentration of these drops significantly (25). Both actin-binding proteins are cleared from the circulation by the reticulo-endothelial system, however urinary levels of them are also studied (31).

### GELSOLIN

Gelsolin is a ubiquitous, multifunctional protein. Three different isoforms exist in humans, two cytoplasmic forms and one circulatory isoform (32). Circulatory GSN is mainly secreted by muscle tissue (26). Circulatory GSN is a 93 kDa  $\text{Ca}^{2+}$ -dependent protein and its plasma values range between 190-300mg/L (but these are highly method-dependent) (31,32). Besides actin, plasma GSN may also be able to bind to bioactive molecules (lysophosphatidic acid, sphingosine 1-phosphate, fibronectin and platelet activating



**Figure 3** Crystal structures of calcium-free human gelsolin and that of uncomplexed Gc-globulin



A: GSN consists of six domains (G1-G6) indicated by different colors. In the Ca-free, inactive form of GSN, the six similarly folded domains adopt a compact globular structure held together by extensive noncovalent interactions of G2 with both G6 and the C-terminal tail (29).

B: Gc-globulin is built up of 3 three homologous  $\alpha$ -helical domains. Domains I and II can be subdivided further into two structurally related subdomains (30).

factor), pro-inflammatory mediators and bacterial wall components (lipoteichoic acid and lipopolysaccharides).

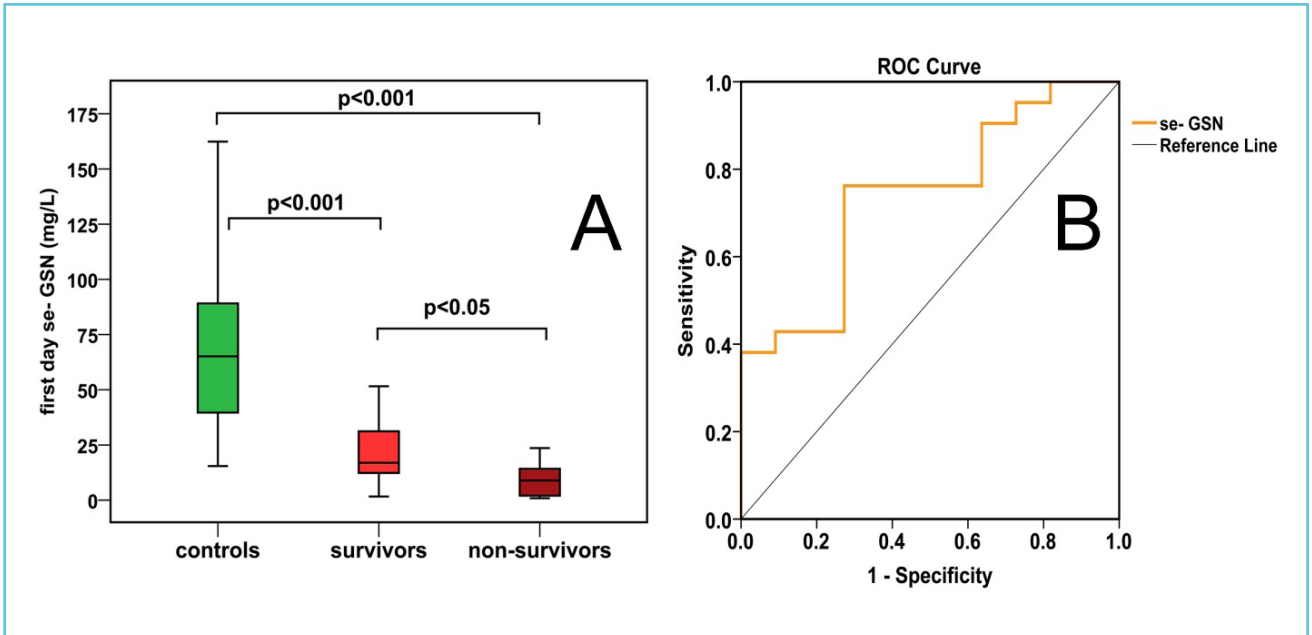
In follow-up studies, first-day GSN levels were proven to have a significant distinguishing ability regarding the septic and the non-septic states furthermore, GSN also predicted the outcome of sepsis (26, 34-36). Non-survivor septic patients showed lower levels of serum GSN (Figure 4). Recently, our research group introduced a new promising marker besides GSN, the serum actin/GSN ratio (derived from the same patients' actin and GSN levels) which had similar prognostic value as APACHE II clinical scores regarding intensive care unit mortality (26). One limiting factor is the lack of a rapid detection method for actin and GSN, which is the current focus of our research.

Higher plasma GSN levels seem to have good prognostic value in sepsis, moreover the protective role

of GSN have been proven by administration of exogenous gelsolin to rodents with septicemia and severe injury yielding reduction in mortality (37).

Studies regarding urinary GSN (u-GSN) levels in sepsis have been scarcely performed. Ferreira et al. (38) described u-GSN as a discriminating protein regarding cisplatin- and gentamicin-induced AKI in rats. Another study of Maddens et al. (39) reported increased u-GSN levels in septic mice. Both of these observations based on Western blot analyses indicated that u-GSN originates from the blood by glomerular filtration. In addition, u-GSN seems to be a possible diagnostic marker in patients suffering from type I diabetes mellitus (40). Interestingly, decreased u-GSN levels were found in rheumatoid arthritis patients (41), however they did not offer any predictive value. So far, all studies regarding u-GSN are promising starting points and should be further validated.

**Figure 4** A: First-day serum GSN levels in septic survivor and non-survivor patients based on 7-day mortality  
B: Receiver operating characteristic curve of serum GSN for predicting 7-day mortality of sepsis



AUC: 0.74, cut-off value: 11.38 mg/L (sensitivity: 76.2%, specificity: 72.7%). Based on (26).

## GC-GLOBULIN

Plasma Gc-globulin (52 - 59 kDa) is a member of the albuminoid superfamily. Gc-globulin is mainly produced by the liver (serum level: 300-600 mg/L) owning 3 major isoforms (Gc1f, Gc1s, Gc2) (42). Gc-globulin seems to act as an acute-phase protein after injury. Also, important function of Gc-globulin is binding and transporting 25-OH-D and 1,25-(OH)<sub>2</sub>D<sub>3</sub> vitamin metabolites. Furthermore it enhances neutrophil chemotaxis and could modulate T cell responses (42).

Admission plasma concentration of Gc-globulin below 134 mg/L (determined by immune nephelometry) was found to be associated with organ dysfunction (hematologic or respiratory failure) and sepsis after traumatic injury (43). Jeng et al. found an association between critical illness and lower 25-OH-D and Gc-globulin levels in critically ill patients when compared to healthy controls (44).

Gc-globulin is filtered freely through the glomeruli because of its low molecular weight. In the kidney, Gc-globulin is involved in the vitamin D biosynthesis process. Under normal circumstances, Gc-globulin is reabsorbed and catabolized by proximal tubular epithelial cells resulting only in a trace urinary excretion (42). Therefore, acute tubular injury is expected to result in exaggerated urinary Gc-globulin excretion. Recently, urinary Gc-globulin (u-Gc-globulin) was reported as a promising novel biomarker of major contrast material induced nephropathy-associated events (u-Gc-globulin/u-Cr in patients developing major adverse renal events (MARE) vs. those without MARE were 125.68 ± 211.62 vs. 14.99 ± 38.10 ng/ml/mmol/l; p < 0.001) (45). Shoukry et al. have determined increased u-Gc-globulin levels by ELISA in diabetic patients as an early diagnostic marker of diabetic nephropathy. Urinary Gc-globulin/u-Cr levels were

more than 10 times higher in macroalbuminuric patients compared to controls ( $1516.3 \pm 228.6$  ng/mg vs.  $123.4 \pm 28.2$  ng/mg;  $p < 0.001$ ) (46). Investigating the association between sepsis-induced acute kidney injury and Gc-globulin in urine still remains an interesting challenge.

## OROSOMUCOID

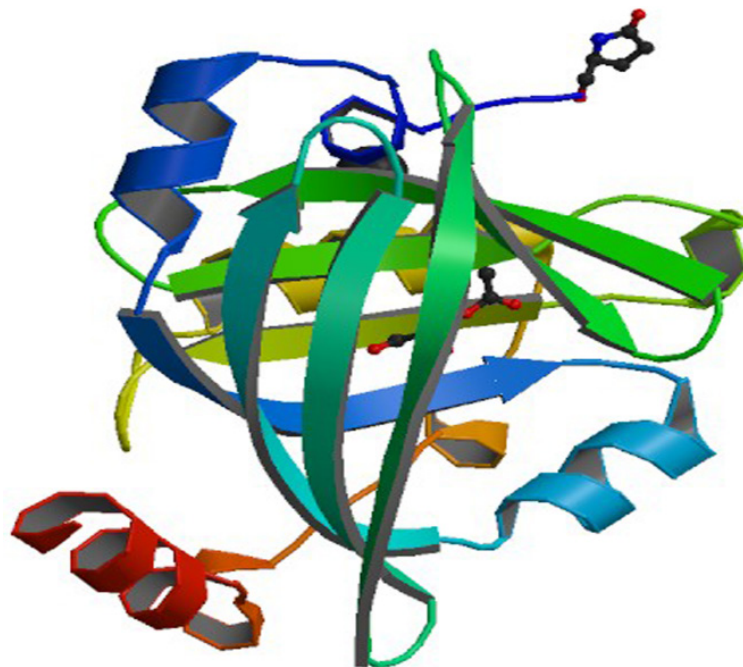
Orosomuroid (ORM) or  $\alpha$ -1-acid glycoprotein is a positive acute phase protein. ORM is a 41-43kDa heavily glycosylated protein (Figure 5) with several transport and immunomodulatory function (47). ORM has been described as part of the non-specific defense system against excessive inflammatory response (48). ORM has anti-neutrophil and anti-complement activity, it can inhibit apoptosis, macrophage activation, lymphocyte proliferation, superoxide generation, and platelet aggregation as well (49). Its protective role was

demonstrated also in several rodent models of shock, inflammation and sepsis (50-52).

The normal orosomuroid concentration in human serum ranges between 0.5-1.2 g/L and it can rise during acute and chronic inflammatory diseases (53). In spite of the well-known fact that serum orosomuroid (se-ORM) is a non-specific inflammatory marker, recently it has been described as a potential diagnostic and prognostic biomarker of sepsis. Significantly higher levels were found in sepsis than in SIRS and admission se-ORM levels showed a good prognostic accuracy for sepsis mortality if combined with SOFA score (AUC ROC: 0.878) (54).

ORM is also present in urine, but with much lower concentrations than in serum, normally ORM accounts for about 1-5 % of total proteins in urine (<3 mg/L) (55, 56). Previous studies described slightly elevated u-ORM levels in

**Figure 5** Crystal structure of human orosomuroid (alpha1-acid glycoprotein)



ORM contains a typical lipocalin fold with an eight-stranded beta-barrel. This structure is responsible for diverse ligand-binding. Furthermore, ORM structure contains five N-linked glycosylation sites (47).



diseases associated with chronic inflammatory activation, like autoimmune diseases, diabetes mellitus and cancer (57-60). U-ORM excretion can be elevated after acute inflammatory stimuli as well. Recently published data suggest that u-ORM could be a promising non-invasive marker for diagnosis of sepsis (61). About 100-times higher levels were found in sepsis than in controls, and SIRS patients showed 10-fold higher u-ORM levels than controls. U-ORM was referred to urinary creatinine levels and a cut off value at 6.75 mg/mmol with great sensitivity and specificity (94.7% and 90.0%, respectively) has been described for diagnosis of sepsis. The diagnostic accuracy of u-ORM for sepsis (AUC ROC: 0.954) was similar to PCT and higher than se-ORM. Furthermore, u-ORM levels correlated well with conventional inflammatory parameters. In this study, extremely elevated u-ORM levels were found in septic patients with dialysis requirement (61). Another paper demonstrated u-ORM above 40 mg/L as an early predictor for acute kidney injury after cardiac surgery in children (AUC ROC 0.87).

U-ORM values were found to be strongly associated with severity of AKI (62).

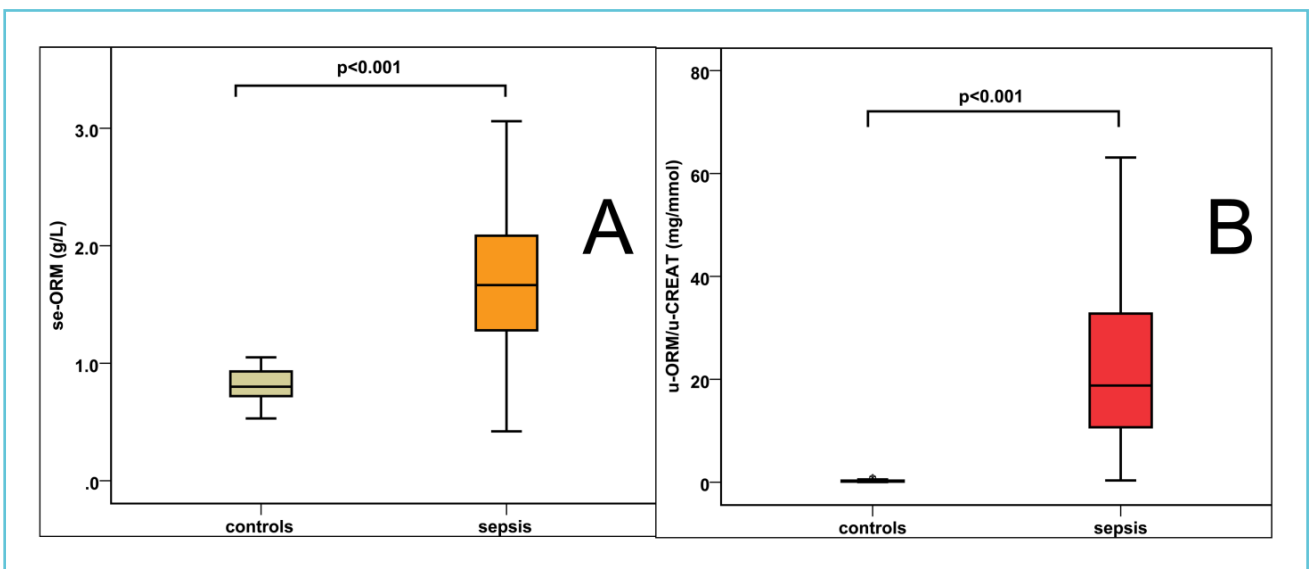
In spite of the promising data, the exact mechanism of u-ORM elevation is not well explored. Local renal processes due to systemic inflammation could play a crucial role, since extrahepatic gene expression of ORM (leukocytes, endothelial cells, kidney, etc.) has been described (63). Furthermore, glomerular and tubular dysfunction also may have a pivotal part.

U-ORM seems to be a more sensitive marker of sepsis than se-ORM (Figure 6), providing clinically relevant information for real-time monitoring of inflammatory activation in a non-invasive manner.

## CONCLUSION

The outcome of sepsis largely depends on early diagnosis and the earliest possible beginning of a consecutive adequate antibiotic therapy. For definitive diagnosis, identification of pathogens is still the gold standard however this approach quite often requires several hours or days leading to a delay in decision making.

**Figure 6** Serum orosomuroid (A) and urinary orosomuroid (B) levels in sepsis



Urinary orosomuroid levels are referred to urinary creatinine and expressed in mg/mmol. Based on (61).

Therefore, measurement of fast responding protein biomarkers of sepsis has gained a major focus in the last decades. Unfortunately, most of the protein biomarkers do not have proper specificity even if they possess better sensitivity. For the assessment of overall tissue damage, monitoring of the actin-scavenger system is a promising new entity. Urinary markers provide a non-invasive tool for real-time monitoring of septic processes. Orosomucoid determination in urine might be a novel possibility for the early recognition of systemic inflammation. Since sepsis is a heterogeneous clinical syndrome and not a definitive disease a single marker alone should never be satisfactory. Multi-marker approach and complex evaluation of the clinical signs and biomarkers should improve patient management at the bedside.

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