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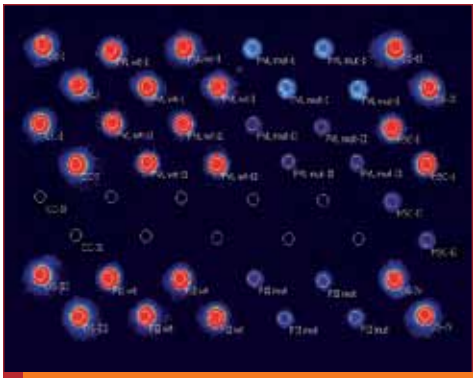

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Early detection of acute kidney injury in sepsis: how about NGAL?

Sepsis frequently results in acute kidney injury (AKI). Although AKI markedly contributes to mortality in sepsis, its diagnosis is frequently delayed due to limitations of current biomarkers of renal impairment. Neutrophil-gelatinase-associated lipocalin (NGAL) has been demonstrated to be a biomarker of early AKI. This review analyses the potential use of NGAL in sepsis.

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Pathophysiology, definition and epidemiology of sepsis

Sepsis is a clinical syndrome characterised by systemic inflammatory response to infection [1-2]. Incidence of sepsis has increased by a factor of four within the last three decades, with an estimated incidence of 650,000 cases per year in the USA. SIRS (systemic inflammatory response syndrome) describes a similar inflammatory reaction to non-infectious aetiologies such as poly-trauma, acute pancreatitis and burns. Apart from different aetiology, sepsis and SIRS share a common definition requiring two or more of four criteria of systemic inflammation (fever/hypothermia, tachycardia >90/min, tachypnoe >20/min or $paCO_2$<32mmHg and leukocytosis (>12G/L) or leukopenia (<4G/L) [Table 1], [1-2].

Pathophysiology of sepsis is mainly attributed to imbalanced and generalised release of pro-inflammatory mediators resulting in impaired circulation, tissue injury and organ failures up to multiple-organ-dysfunction-syndrome (MODS). Despite strong evidence for therapeutic efficacy of early causative therapy (treatment of infection source), antibiotics and several supportive strategies, mortality from severe sepsis and septic shock remained up to 20-50% in recent sepsis trials [1-2]. There is an ongoing debate on the benefits of supportive strategies such as hydrocortisone, intensified-insulin-therapy and immunomodulation. However, there is strong consensus about the paramount importance of early sensitive diagnosis and staging of sepsis (severe sepsis and septic shock) in order to initiate appropriate monitoring and therapy as early as possible. In general, patients with severe sepsis will require intensive care and haemodynamic monitoring to optimise circulation.

1.) SIRS: > 2 of the following four criteria	
a.)	Temperature > 38.3° or < 36°
b.)	Heart rate > 90/min
c.)	Respiratory rate > 30/min or paCO2 < 32mmHg
d.)	WBC count > 12 G/L or < 4 G/L
2.) Sepsis: SIRS AND documented infection and/or high clinical evidence of infection	
3.) Severe sepsis: Sepsis AND > 1 organ failure	
a.)	Encephalopthy, delirium, abrupt change in mental status
b.)	Coagulapthy: INR > 1.5. Platelet count < 100 G/L
c.)	Acute lung injury:paO2/FiO2 < 250 in the absence of pneumonia as infection source Acute lung injury paO2/FiO2 < 200 in the presence of pneumonia as infection source
d.)	Metabolic acidosis, base excess < -5mmol; Lactate > 2mmol/L
e.)	Renal dysfunction: urinary output < 0.5mL/kg*h for > 2h; RRT Serum creatinine > 2.0 mg/dL
f.)	Hepatic dysfunction: Bilirubin > 2.0mg/dL
g.)	Lactate > upper normal level
4.) Septic shock: Sepsis AND adequate fluid resuscitation AND absence of other causes of hypotension AND	
a.)	Systolic blood pressure < 90mmHg or
b.)	Mean arterial blood pressure < 70mmHg or
c.)	Decrease in diastolic blood pressure > 40 mmHg

Table 1. Definitions of SIRS, sepsis, severe sepsis and septic shock (1).

Diagnoses of severe sepsis and septic shock are mainly based on the evidence of organ failure and emphasize the impact of circulatory failure. The impact of different organ failures on outcome of ICU-patients is substantiated by numerous studies [1-5)]. Interestingly, renal and liver failure were among the organ failures with the most pronounced impact on outcome in several studies [3-5]. At first glance, this might be surprising. However, circulatory and respiratory failure can be easily detected at early stages of severe sepsis, and symptomatic therapy of these organ failures is the main target of intensive care. By contrast, renal and liver failure remain underrated and "late-stage-diagnosed losses of organ function" in the development of MODS. Difficulties in early detection of renal and hepatic failure by traditional markers has probably also resulted in their under-representation in scoring-systems:

Regarding renal failure, APACHE-II and the SOFA-score are mainly based on absolute serum creatinine values. However, the use of serum creatinine as a marker in these scores and particularly as an early marker of septic renal failure is limited by a number of drawbacks: serum levels of creatinine are dependent on age, gender, muscle mass and race. Furthermore, in case of impaired glomerular filtration, serum creatinine levels can be lowered by tubular secretion, which contributes to the phenomenon of the "creatinine-blind-range" of renal failure: glomerular-filtration rate (GFR) can decrease to about 50% with serum creatinine levels staying within the normal range. Numerous formulae for GFR estimation slightly improve this drawback. However, GFR formulae are neither part of sepsis definitions nor are they included in SAPS-II, SOFA- and APACHE-II-score. Even the more recent Acute-Kidney-Injury-Network (AKIN) definition of AKI rejected GFR, which has been included in the previous RIFLE-classification (RIFLE: Risk, Injury, Failure; Loss, End-Stage Renal Disease). RIFLE and AKIN as well as the new KDIGO-definition (KDIGO: Kidney Disease: Improving Global Outcomes) are

mainly based on changes in serum creatinine compared to baseline values, which are "known or presumed to have occurred within the prior seven days" [6]. Comparison with a baseline value which is not known in a substantial percentage of patients remains a major problem of these definitions. In general, their usefulness is substantiated as consensus definitions for acute changes in renal function within 2-7 days after the first measurement of serum creatinine rather than being highly sensitive for early AKI. This also relates to the fact that increased serum creatinine on ICU admission of a septic patient might result from constant chronic renal impairment as well as acute renal failure in a patient with previously normal renal function. Both, acute and chronic renal impairment have been demonstrated to significantly influence outcome, albeit to a different degree, with patients with AKI more frequently requiring mechanical ventilation [4, 7].

In the context of sepsis, specification of renal impairment is particularly important: acute septic renal impairment results in markedly worse prognosis, classification as severe sepsis and intensified monitoring in an ICU. By contrast, stable chronic renal impairment in a patients just fulfilling two of the four sepsis criteria would be a minor risk factor contributing to outcome similar to older age. Being a marker of function rather than of injury remains the major drawback of serum creatinine for differentiation of renal impairment.

Approaches to early detection of AKI

Systematic efforts have therefore been made to characterise markers of early renal injury. Using several established animal models of acute renal injury (e.g. ischaemia, nephrotoxic medication including contrast-medium), up-regulation of a number of potential genes has been demonstrated as a short-term reaction to experimental acute renal injury [8]. Among those up-regulated genes and a number of other biomarkers, NGAL, Kidney-Injury-Molecule-1 (KIM-1), interleukin-18 (IL-18) and cystatin C have been most intensively studied. Cystatin C provides characteristics most similar to creatinine: this marker is a cysteine proteinase inhibitor synthesised in all nucleated cells and freely filtered by the glomerulus. The major adavantages over serum creatinine are that cystatin C is not secreted by the tubulus and that it is not affected by age, gender, muscle mass and race. However, with increased levels of cystatin C resulting from accumulation due to decreased glomerular filtration, cystatin C remains as a marker of decrease in renal function rather than a biomarker of early kidney injury. Several studies suggest its slightly earlier (within 24h?) detection of AKI compared to serum creatinine.

Another "candidate molecule" for early detection of AKI is IL-18, a pro-inflammatory cytokine that is induced in the proximal tubule and detected in urine after AKI. In clinical settings, increase in urinary levels within 6h and peak-values within 12h have been demonstrated in cardiopulmonary bypass patients with AKI after 48h according to serum creatinine.

KIM-1 is a transmembrane protein that is markedly over-expressed in the proximal tubule after ischaemic or toxic AKI. A number of clinical studies suggest earlier detection of AKI by KIM-1 compared to serum creatinine, e.g. with elevated urinary KIM-1 levels 12h after paediatric cardiac surgery and prediction of renal replacement therapy (RRT) and mortality in AKI.

NGAL

The most promising biomarker for early acute kidney injury at present is NGAL, which is the profuct of one of seven genes markedly up-regulated in a ischaemia-reperfusion mouse model [8]. NGAL is a 178 amino-acids polypeptide expressed by neutrophils and other epithelial cells including the proximal tubule. NGAL provides several physiological functions including bacteriostatic (depriving bacteria of iron essential for growth), antioxidant (stops free and reactive iron from producing oxygen free radicals) and growth-factor properties (regulates cell proliferation, apoptosis, differentiation). Furthermore, there is a possible rescue role in other epithelia (breast, uterus), and NGAL also is overexpressed in some epithelial tumours.

Regarding its potential clinical use, NGAL has been validated as an early biomarker of AKI induced by cisplatin, contrast-media and cardiac surgery as well as a screening marker for patients at risk in the emergency department (ED) and ICU. In these settings, urinary and plasma levels of NGAL after 2h-12h were significant predictors of AKI defined by later increases in serum creatinine within 24-48h. Depending on setting and methodology, best predictive capabilities of NGAL were found for cut-off values between 50 and 150 µg/L. In a large ED study in 635 patients, urinary NGAL levels clearly differentiated between acute (markedly elevated NGAL) and chronic (not elevated NGAL) renal impairment, whereas there was substantial overlap of serum creatinine values for both groups ([9].

These abilities to discriminate between acute and chronic renal impairment might be particularly useful in patients with sepsis [Figures 1 and 2]. With assessment of renal function in ICU patients based on serum creatinine, normal creatinine levels might be "false negative" and will increase as late as after 48h. On

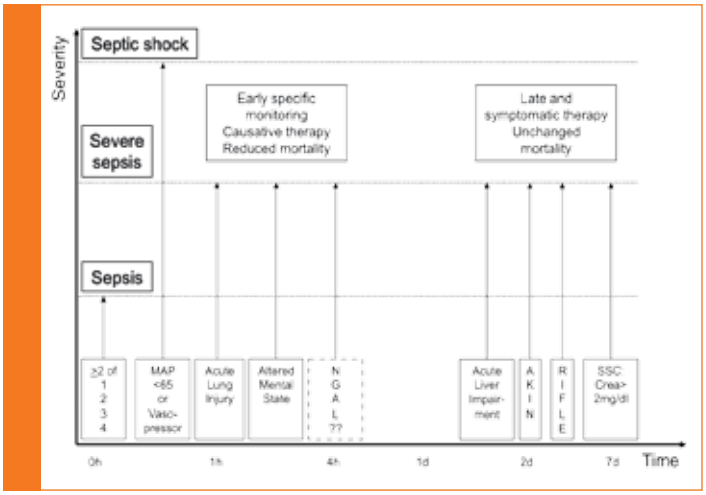

Figure 1. Time course and staging of sepsis according to different organ failures. SSC: Surviving Sepsis Campaign

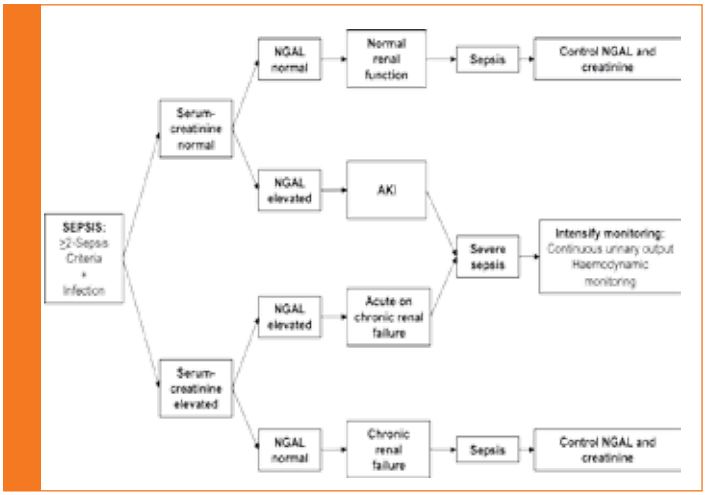

Figure 2. Algorithm for potential use of NGAL to classify sepsis and severe sepsis.

the other hand, increased values of creatinine can result from stable chronic renal impairment. Misinterpretation of these values - "false positive" for septic renal failure - might result in inappropriate allocation of resources, e.g. efficacy for most of the supportive measures in sepsis has been demonstrated predominantly for patients at high risk and with severe sepsis, whereas side effects might outweigh the benefits in patients with less pronounced sepsis.

A potential role for NGAL in sepsis has been suggested in several clinical studies. In 143 paediatric ICU patients Wheeler *et al.* demonstrated that septic shock, but not SIRS, resulted in a significant elevation of NGAL compared to controls [10]. Furthermore, NGAL on admission was significantly higher in children developing AKI within seven days after admission compared to children without AKI. Serum levels of NGAL and creatinine did not correlate on day one after admission.

A study in 971 ICU patients investigated the predictive capabilities of nine biomarkers on admission regarding severe sepsis within 72h. The best predictive capabilities were found for NGAL, whereas D-dimer, BNP and CRP were of limited use. A score based on NGAL, IL-1-receptor-antagonist and protein C levels significantly distinguished four groups of patients developing no sepsis, severe sepsis, septic shock and death [11]: the area under the curve for the score derived from these three biomarkers was 0.80 for severe sepsis, 0.77 for septic shock and 0.79 for death.

Another recent study found significantly elevated plasma NGAL levels within 4 hours after admission in septic as well as non-septic-patients with AKI according to RIFLE-criteria compared to patients without AKI [12]. Increases in NGAL were even more pronounced in septic compared to non-septic AKI patients. Similarly, urinary NGAL-levels were higher in septic compared to non-septic patients without AKI [13], suggesting that cut-off-values for NGAL to predict AKI might be higher than for non-septic patients.

In summary, clinical applications of NGAL in sepsis comprise early detection of AKI in patients with normal serum creatinine (NGAL+, crea-) compared to patients without renal impairment (NGAL-, crea-). Furthermore, NGAL might be useful to distinguish patients with stable chronic renal impairment (NGAL-, crea+) from patients with ongoing or "acute on chronic" renal injury (NGAL+, crea+) [Figure 1].

With regard to sepsis, more sensitive detection of AKI (NGAL+, crea-) would result in staging as "severe sepsis" instead of sepsis in patients without other organ failures [Figure 2]. Early detection of AKI might help to allocate additional causative (antimicrobial therapy, intervention) and supportive measures for sepsis as well as specific measures to prevent further renal damage. These attempts include intensified haemodynamic monitoring to optimise fluid load, avoidance of further nephrotoxic medications and procedures (contrast-application) or at least prophylactic approaches such as hydration or administration of theophylline or acetylcysteine [13]. Allocation of these resources according to significant predictors and avoidance of further renal impairment carries a high potential for cost effectiveness as emphasised by a number of studies.

Further studies are required to validate that early determination of NGAL improves diagnosis and outcome in septic and non-septic patients at risk of AKI. Future studies should also investigate if including NGAL into scoring (APACHE-II, SOFA, SAPS-II) systems improves their predictive capabilities.

Stage		RIFLE	AKIN	KDIGO
1 (R in RIFLE)	S-creatinine	Increased x1.5 (+50%) or GFR decrease > 25%	Increased x1.5 (+50%) or Increased > 0.3mg/dL	Increased x1.5-1.9 (+50-90%) or£Increased > 0.3mg/dL
	Urinary Output	< 0.5 mL/kg/h for 6-12h		
2 (I in RIFLE)	S-creatinine	Increased x2 (+100%) or GFR decrease > 50%	Increased x2 (+100%)	Increased x2.0-2.9 (+100-190%)
	Urinary Output	< 0.5 mL/kg/h for 12-24h		
3 (F in RIFLE)	S-creatinine	Increased x3 (+200%) or GFR decrease > 75% or S-creatinine > 4mg/dL (with acute rise of > 0.5mg/dL)	Increased x3 (+200%) or S-creatinine > 4mg/dL (with acute rise of > 0.5mg/dL) or initiation of RRT	Increased x3 (+200%) or S-creatinine > 4mg/dL (with acute rise of > 0.5mg/dL) or initiation of RRT or - in patients < 18 years decrease in GFR < 35mL/min/1.73m²
	Urinary Output	< 0.3 mL/kg/h for > 24h or anuria for > 12h		
4 (L in RIFLE)		Persistent ARF: complete loss of renal function for > 4 weeks	-	-
5 (E in RIFLE)		End Stage Renal Disease	-	-

Table 2. Comparison of definitions of AKI according to RIFLE, AKIN and KDIGO.

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