The role of serum angiopoietin-2 as a biomarker in sepsis induced acute kidney injury

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Background
Acute kidney injury (AKI) is a major complication of sepsis in ICU patients. The overall incidence of AKI in ICU patients ranges from 20 to 50% with lower incidence seen in elective surgical patients and higher incidence in sepsis patients. AKI represents a significant risk factor for mortality and can be associated with mortality greater than 50%. The aim of this study was to investigate the role of angiopoietin-2 as a biomarker in sepsis induced AKI.

Patients and methods
The study was conducted on 60 participants (20 patients with septic AKI, 20 patients with sepsis only without AKI and 20 healthy volunteers as the control group). Serum angiopoietin-2 levels were assessed by the ELISA technique. Clinical, biochemical, and therapeutic data were collected.

Results
High levels of serum angiopoietin-2 were detected in patients with septic AKI. These levels were significantly higher in relation to septic patients with no AKI and the control group. There was a statistically significant positive correlation between serum angiopoietin-2 level in the septic AKI group and serum creatinine, white blood cell count, erythrocyte sedimentation rate, C-reactive protein, and there was a statistically significant negative correlation between serum angiopoietin-2 level in the septic AKI group and the estimated glomerular filtration rate.

Conclusion
Serum angiopoietin-2 levels were significantly positive in patients with septic AKI. Serum angiopoietin-2 may be used as a biomarker in sepsis induced AKI.

Keywords:
AKI, angiopoietin-2, sepsis

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Introduction

The incidence of acute kidney injury (AKI) in the ICU has increased during the past decade because of the increased acuity and recognition. Early epidemiology studies were confounded by erratic definitions of AKI until recent consensus guidelines (RIFLE and AKIN) standardized its definition. The overall incidence of AKI in the ICU patients ranges from 20 to 50% with lower incidence seen in elective surgical patients and higher incidence in sepsis patients. AKI represents a significant risk factor for mortality and can be associated with mortality greater than 50% [1,2].

Sepsis is a systemic, deleterious host response to infection, leading to severe sepsis (acute organ dysfunction secondary to documented or suspected infection) and septic shock (severe sepsis plus hypotension not reversed with fluid resuscitation). Severe sepsis and septic shock are major healthcare problems, affecting millions of people around the world each year, killing one in four (and often more), and are increasing in incidence [3–7].

AKI occurs in ~20–25% of the patients with sepsis and 51% with septic shock. The combination of AKI and sepsis is associated with a 70% mortality, as compared with a 45% mortality among patients with AKI alone. Thus, the combination of sepsis and AKI constitutes a particularly serious medical problem [8].

In the current clinical practice, AKI is typically diagnosed by measuring serum creatinine (SCr). Unfortunately, creatinine is an unreliable indicator during acute changes in kidney function. The widespread availability of enabling technologies such as functional genomics and proteomics has accelerated the rate of novel biomarker discovery [9–11].

Sepsis-induced AKI is the most common form of AKI observed in critically ill patients. AKI mortality in septic critically ill patients remains high despite the increasing ability to support vital organ systems. This high mortality is partly because of poor understanding

of the pathophysiological mechanisms of sepsis-induced AKI. Recent experimental studies have suggested that the pathogenesis of sepsis-induced AKI is much more complex than isolated hypoperfusion because of decreased cardiac output and hypotension. In nonresuscitated septic patients with a low cardiac output, a decrease in renal blood flow (RBF) could contribute to the development of AKI. In resuscitated septic patients with a hyperdynamic circulatory state, RBF is unchanged or increased. However, in resuscitated septic patients, sepsis-induced AKI can occur in the setting of renal hyperemia in the absence of renal hypoperfusion or renal ischemia.

Alterations in the microcirculation in the renal cortex or renal medulla can occur despite normal or increased global RBF. Increased renal vascular resistance may represent a key hemodynamic factor that is involved in sepsis-associated AKI. Sepsis-induced renal microvascular alterations (vasoconstriction, capillary leak syndrome with tissue edema, leukocytes and platelet adhesion with endothelial dysfunction and/or microthrombosis) and/or an increase in intra-abdominal pressure could contribute to an increase in renal vascular resistance.

Further studies are needed to explore the time course of renal microvascular alterations during sepsis, and also the initiation and development of AKI.

Doppler ultrasonography combined with the calculation of the resistive indices may indicate the extent of the vascular resistance changes and may help predict persistent AKI and determine the optimal systemic hemodynamics required for renal perfusion.

The angiopoietin (Angpt)/Tie ligand-receptor system was first identified in the mid-1990s. It is the second class of transmembrane vascular-specific receptor tyrosine kinases (the first being the vascular endothelial growth factor/vascular endothelial growth factor-receptor system). Gaining attention as an important regulator in vessel maturation and remodeling, several studies demonstrated that the Angpt–Tie2 system not only regulates angiogenesis but also controls endothelial inflammation and permeability in no redundant manner. It consists of two transmembrane receptor tyrosine kinases, Tie1 and Tie2, and four corresponding ligands, Angpt-1–4. A functional Angpt–Tie2 system is critically important for the formation of blood and lymphatic vessels during embryogenesis. In healthy adults, its function shifts toward the maintenance of endothelial homeostasis [12,13].

This study aimed to evaluate the role of serum Angpt-2 as a serum biomarker in sepsis induced AKI and also study its relation with renal functions, inflammatory markers such as white blood cell (WBC) count, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP).

Materials and methods

The study was conducted on 60 participants recruited from the Internal Medicine and ICU departments of Alexandria Main University Hospital, and were divided into the following three groups: group I, consisting of 20 patients with septic AKI, eight (40%) of them were men and the remaining 12 (60%) were women, their ages ranging from 25 to 70 years with a mean of 45.70 ± 13.90 years; group II, consisting of 20 patients suffering from sepsis with no renal impairment, nine (45%) of them were men and 11 (55%) were women, their ages ranging from 25 to 72 years with a mean age of 43.35 ± 13.08 years; group III, consisting of 20 healthy volunteers as controls, seven (35%) of them were men and the remaining 13 (65%) were women, their ages ranging from 23 to 75 years with a mean of 51.90 ± 14.69 years.

All the participants were subjected to full history taking, thorough clinical examination, and laboratory investigations including complete blood picture, serum urea, serum creatinine, complete urine analysis, serum lipid profile, ESR, CRP and serum Angpt-2 by the ELISA technique.

Statistical analysis [14]

Data were fed into the computer and analyzed using the IBM SPSS software package version 20.0 [15]. Qualitative data were described using number and percent. Quantitative data were described using range (minimum and maximum), mean, SD, and median. The comparison between different groups regarding categorical variables was done using the χ^2-test. When more than 20% of the cells have expected count less than five, correction for χ^2 was conducted using the Monte Carlo correction. The distributions of quantitative variables were tested for normality using the Kolmogorov–Smirnov test, Shapiro–Wilk test and D'Agstino test; in addition, histogram and QQ plot were used for the vision test. If it revealed normal data distribution, parametric tests were conducted. If the data were abnormally distributed, nonparametric tests were conducted. For normally distributed data, the comparison between more than two independent populations was performed using the *F*-test (ANOVA) and the post-hoc test (LSD). Correlations between two quantitative variables were assessed using the Pearson coefficient. For abnormally distributed data,

comparison between more than two independent populations was done using the Kruskal–Wallis test, and the pair wise comparison was done using the Mann–Whitney test. Significance of the obtained results was judged at the 5% level.

Results

Serum Angpt-2 level was significantly higher in the septic AKI group in relation to the sepsis without AKI group and the control group (Figs 1 and 2 and Table 1).

There was a statistically significant positive correlation between serum Ang-2 and serum creatinine with *P* value less than 0.001 (Fig. 3).

There was a statistically significant negative correlation between serum Angpt-2 and estimated glomerular filtration rate (eGFR) with *P* value less than 0.001 (Fig. 4).

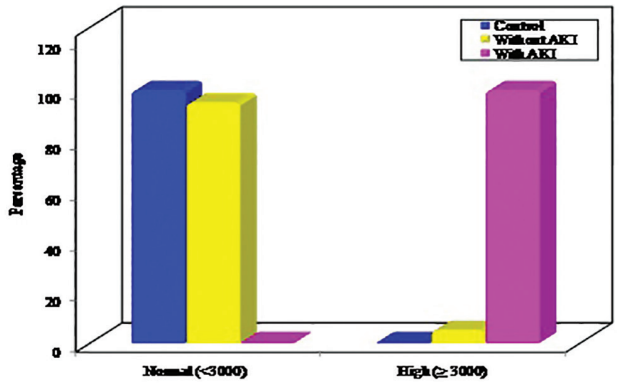
There was a statistically significant positive correlation between serum Angpt-2 and WBCs with *P* value less than 0.001 (Fig. 5).

Table 1 Correlation between angiopoietin-2 in sepsis with acute kidney injury group

Parameters	Angiopoietin-2	
	r	*P*
Creatinine	0.931*	<0.001
eGFR	−0.971*	<0.001
WBCs	0.829*	<0.001
ESR	0.752*	<0.001
CRP	0.974*	<0.001

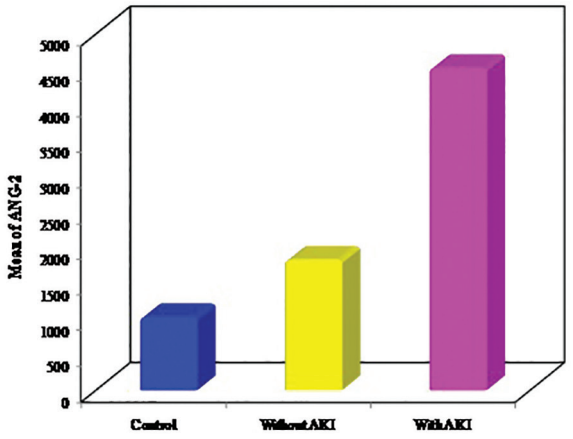
CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; ESR, erythrocyte sedimentation rate; *r*, Pearson's coefficient; WBC, white blood cell, *Statistically significant at $P \leq 0.05$.

Figure 1



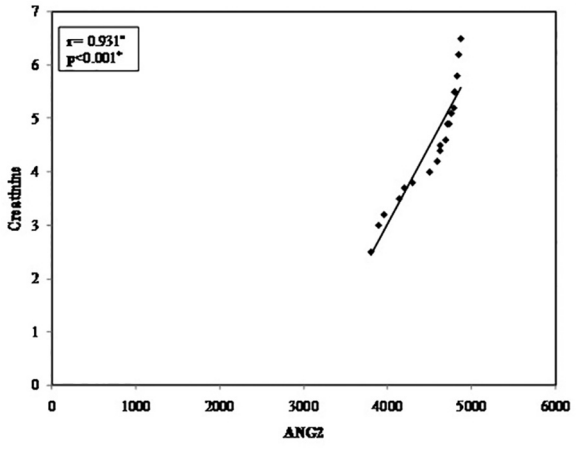
Comparison between the different studied groups according to angiopoietin-2. AKI, acute kidney injury.

Figure 2



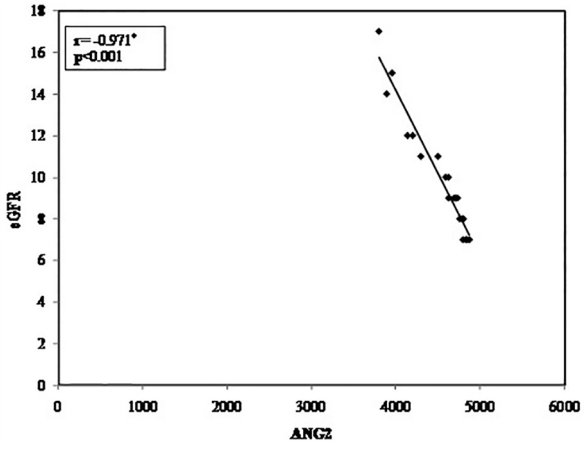
Comparison between the different studied groups according to angiopoietin-2.

Figure 3



Correlation between angiopoietin-2 (pg/ml) and creatinine (mg/dl) in the sepsis with acute kidney injury group.

Figure 4



Correlation between angiopoietin-2 (pg/ml) and estimated glomerular filtration rate (eGFR) (ml/min/1.73 m^2) in the sepsis with acute kidney injury group.

There was a statistically significant positive correlation between serum Angpt-2 and ESR with *P* value less than 0.001 (Fig. 6).

There was a statistically significant positive correlation between serum Angpt-2 and CRP with *P* value less than 0.001 (Fig. 7).

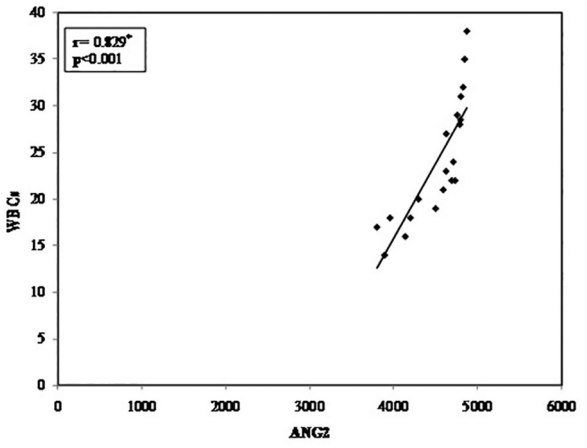
Discussion

In spite of the advances in the basic sciences, which uncovered the pathogenesis of AKI and paved the way for successful therapeutic approaches, effective therapeutic strategies in humans with AKI always yield disappointing results.

Most of the preventive measures for AKI, which are effective in the experimental settings, do not show comparable positive results in the clinical settings. This can be explained by the inability to identify the time of injury in the clinical settings. The injury begins by inducing molecular modifications that subsequently evolve into cellular damage. The cells start to produce biomarkers of injury and only subsequently does the clinical picture of the syndrome develop with typical signs and symptoms. Therefore, we could imagine that the molecular and cellular clocks always anticipate the clinical clock, which is always late. The biological clock of biomarkers displays an intermediate time in this progression, but it, most certainly, is reflective of an earlier stage when compared with the clinical clock. Thus, in the timeline of the AKI syndrome early biomarkers represent a unique possibility for a timely diagnosis and intervention to protect the kidney from further damage and to prevent the tissue damage from the existing risk factors. If we wait for the clinical clock to activate the alarm we will always be late. We need to diagnose AKI and treat it as soon as possible. However, in current clinical practice, the gold standard for identification and classification of AKI is dependent on serial serum creatinine measurements, which are especially unreliable during acute changes in kidney function.

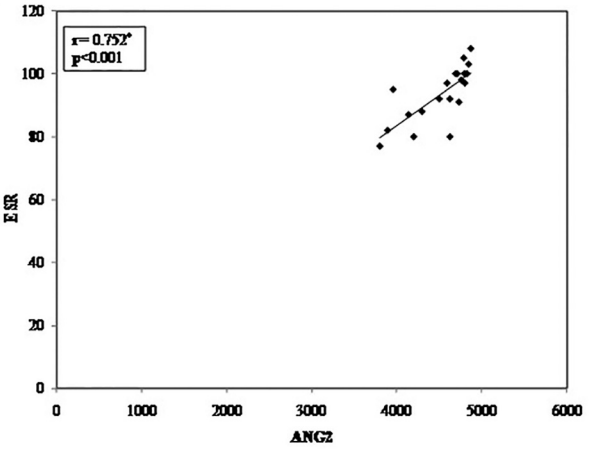
AKI is a common heterogeneous disease that complicates several medical and surgical conditions. AKI is caused by several risk factors and etiologic mechanisms contributing individually or in combination to the ensuing kidney tubular injury. Several pathophysiological mechanisms such as ischemia, hypotension, hypoperfusion, cytokine release, and atheroemboli are incriminated. Thus, it is very unlikely that a single biomarker would be sufficient for accurate and reliable diagnosis and risk stratification of AKI. In clinical medicine, a single

Figure 5



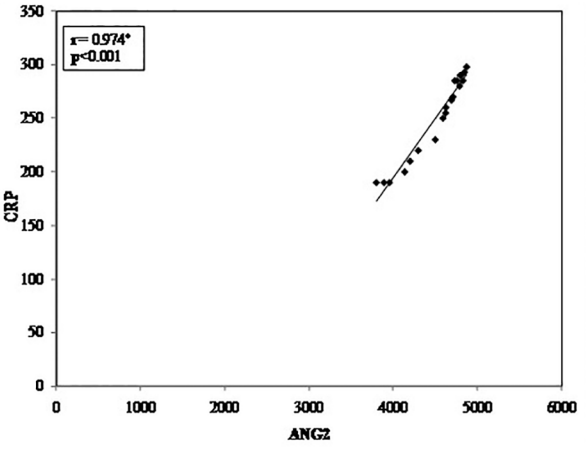
Correlation between angiopoietin-2 (pg/ml) and white blood cells (WBCs) (/mm^3) in sepsis with acute kidney injury group.

Figure 6



Correlation between angiopoietin-2 (pg/ml) and erythrocyte sedimentation rate (ESR) (mm/h) in sepsis with acute kidney injury group.

Figure 7



Correlation between angiopoietin-2 (pg/ml) and C-reactive protein (CRP) in sepsis with acute kidney injury group.

biomarker is seldom a stand-alone test. Several biomarkers representing different injury pathways would be necessary for screening and prognosis of kidney injury.

The present study aimed to evaluate the role of serum Angpt-2 as a biomarker for sepsis induced AKI.

Angpt-2, as a biomarker for early detection of AKI, was not studied in many researches involving humans, and was studied only in some researches involving animals. Therefore, Angpt-2 needed to be studied more in AKI, especially in septic patients.

In the present study, serum levels of Angpt-2 were studied in three groups: the first group included 20 patients with sepsis induced AKI; the second group consisted of 20 patients with sepsis and no renal impairment; and the third group included 20 healthy controls. It was found that the level of Angpt-2 was significantly higher in patients with sepsis induced AKI compared with septic patients with no renal impairment and with healthy controls.

These data coincide with the findings of Kümpers *et al.* [16]; they measured circulating Angpt-2 by the ELISA technique in 117 critically ill patients with AKI at inception of renal replacement therapy in the ICU. Mortality, length of stay and renal recovery were prospectively assessed during a study period of 28 days. They found that the circulating Angpt-2 levels were significantly higher in AKI patients with RIFLE category Injury or Failure, compared with patients with RIFLE category Risk. Angpt-2 concentrations were significantly higher in nonsurvivors than in survivors at day 0 and day 14 after initiation of renal replacement therapy. There was a strong independent prognostic impact of elevated Angpt-2 on patients 28 days after survival. The most interesting finding was that the Angpt-2 levels were not significantly affected by dialysis, although no indication as to the sieving coefficient was given, and thus it remained a predictor for outcome for this selected group of patients [16].

In addition, these data coincide with the data reported by David *et al.* [17]; they recently applied models of sepsis in mice lacking just one allele of Angpt-2 (+/-). Compared with wild-type littermates (+/+), these mice exhibited less tissue inflammation, less renal failure, less lung injury, and better survival chances.

The theory that may explain the increase of serum Angpt-2 in AKI is that the damaged glomerular endothelium may over secrete Angpt-2. This is supported by the finding that in normal mature glomeruli, Angpt-2 levels are low or undetectable, but are reported to be upregulated in certain disease models, including diabetic nephropathy and glomerulonephritis. Although such a scenario is more likely to occur in AKI, it would not explain the raise of Angpt-2 level after the unilateral nephrectomy without inducing kidney disease in the remaining healthy kidney described by David *et al.* [17].

In the present study, the serum level of Angpt-2 steadily increased in correlation with the level of renal function tests and also in correlation with the inflammatory markers including ESR, CRP, and WBC count.

In addition, in the present study the serum level of Angpt-2 steadily increased creating a negative correlation with the decrease in eGFR measured by the Cockroft–Gault equation.

Most of the available studies were conducted on chronic kidney disease (CKD) patients as shown by David *et al.* [17] who investigated the impact of Angpt-2 level on the outcome in CKD, prospectively studying 128 CKD patients (43 CKD stage 4, 85 CKD stage 5, 57 hemodialysis, 28 peritoneal dialysis) over a follow-up period of 4 years. They found that Angpt-2 values were significantly higher in CKD patients than in controls. Furthermore, Angpt-2 was significantly higher in dialysis than in stage 4 CKD patients and correlated with markers of vascular disease (cholesterol, high sensitivity CRP, osteoprotegerin) [17].

Zaki *et al.* studied the role of Angpt-2 in the progression of CKD, and it was found that Angpt-2 level was significantly elevated in higher stages of CKD (stage 4 or stage 5) than early stages of CKD (stage 2 or stage 3) and controls.

In a succeeding study, David *et al.* [17] showed that serum Angpt-2 levels steadily increase with progression of CKD, as shown by significant positive and negative correlations with serum creatinine and glomerular filtration rate (GFR), respectively. They further added power to the relation between CKD and Angpt-2 by investigating the effect of sudden loss of GFR after unilateral nephrectomy for kidney donation on the serum Angpt-2 levels. They detected a close inverse correlation between the mean changes (0–72 h) in Angpt-2 level and the decrease in GFR.

The theory explaining the increase in the Angpt-2 levels parallel to the loss of renal function is that there occurs a reduction of renal excretion of Angpt-2, either due to reduced GFR or diminished tubular

secretion with gradual accumulation of Angpt-2 in the circulation, but there are several observations that argue against this theory. Purified recombinant Angpt-2 protein exhibits predominant single bands of a molecular mass of ~62 kDa. Furthermore, *in-vivo* Angpt-2 exists mainly as a multimeric protein; therefore, its glomerular excretion is rather unlikely. Indeed, Angpt-2 is neither detectable in urine from apparently healthy individuals nor cleared by dialysis.

El-Banawy *et al.* [18] showed that the Angpt-2 levels were significantly increased in systemic lupus erythematosis (SLE) patients than in controls, and it was significantly higher in patients with lupus nephritis than in patients without it. Angpt-2 was significantly positively correlated with proteinuria and histological activity index, and was negatively correlated with C3 and eGFR.

Similarly, Kümpers *et al.* [19] showed that Angpt-2 levels were increased and Angpt-1 levels were decreased in patients with active SLE compared with healthy controls and that this tendency was still present in inactive SLE, although less pronounced. Angpt-2 concentrations correlated well with the SLE disease activity index (SLEDAI) score, proteinuria, double-stranded DNA titer and soluble vascular cell adhesion molecule-1 (sVCAM-1). In this study, renal involvement was the only independent predictor for an elevated Angpt-2 level, and serum Angpt-2 was identified as a strong predictor for disease activity [19].

David *et al.* [17] studied the Angpt levels in patients treated by means of dialysis followed by the kidney transplantation, the association of altered Angpt levels with atherosclerosis, and the changes in altered levels after renal transplantation. They found that the circulating Angpt-2 level was increased in patients treated with dialysis and its level correlated significantly with scores of coronary artery disease (CAD) and peripheral arterial disease (PAD). Indeed, Angpt-2 levels have been normalized three months after the kidney transplantation [17].

In our study, we found that the serum level of Angpt-2 steadily increased in correlation with the level of renal function tests and also in correlation with the inflammatory markers including ESR, CRP, and WBC count.

In addition, in the present study the serum level of Angpt-2 steadily increased creating a negative correlation with the decrease in eGFR measured by the Cockroft–Gault equation.

Conclusion

Serum Angpt-2 levels were significantly positive in patients with septic AKI. Serum Angpt-2 may be used as a biomarker in sepsis induced AKI.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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