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IL6 Plasma Concentrations in Patients with Sepsis Receiving SLED and Antibiotics: A Predictor for Survival

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Abstract. *Background: The present study evaluated interleukin-6 (IL6) as a predictor of mortality in patients and sepsis with acute kidney injury (AKI) receiving sustained low-efficiency dialysis (SLED) and antibiotic therapy. Patients and Methods: Seven patients with sepsis receiving antibiotics and SLED for AKI were studied. Blood was obtained at baseline prior to SLED and antibiotics, during SLED, and then after stopping SLED. IL6 concentrations were measured using an enzyme-linked immunosorbent assay (ELISA). Results: Mean plasma IL6 concentrations ranged between 700 and 900 pg/ml for the first 8 h after starting SLED but was significantly lower after discontinuation of SLED (200-250 pg/ml) (p=0.0044). Three out of seven patients survived to be discharged from the hospital and all three had significantly lower concentrations of IL6 during the first 8 h compared to those who died in the hospital (p<0.0001). Conclusion: The combination of SLED and antibiotic therapy was unable to lower the initial high plasma IL6 concentrations, and high initial IL6 concentrations predicted in-hospital mortality.*

Sepsis and septic shock are mediated, at least in part, by inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL6) and interleukin-8 (IL8), all of which have a molecular weight of less than 25 kDa and are thus of a size that might be modified by certain forms of dialysis (1, 2). Multiple investigators have reported a correlation between plasma IL6 concentrations and mortality in patients with sepsis and severe sepsis (3-5). The critical time of IL6 elevation and concentration after the onset of sepsis is not entirely clear but early sustained elevations in IL6 may be particularly damaging (3). Early initiation of antibiotic therapy is recommended to improve outcome in sepsis and dialysis may be beneficial in lowering inflammatory cytokine concentrations and improving survival (6).

The lowering of cytokine concentrations by continuous renal replacement therapy (CRRT) has been reported (7). Intermittent hemodialysis with standard hemodialysis membranes does not reduce cytokine concentrations but when high molecular weight membranes are used, plasma cytokine concentrations may be reduced (8). Because sustained low efficiency dialysis (SLED) is a hybrid method of CRRT and intermittent hemodialysis, the effect of plasma cytokine concentrations is not predictable and has not been well described.

In the following study, we evaluated plasma IL6 concentrations as a predictor of mortality in patients with sepsis and AKI receiving SLED and antibiotics. Data from this small study suggest that a high plasma IL6 concentration in the early septic period increased the risk of mortality. The combination of SLED and antibiotics appeared to have no effect during the potentially critical early elevation of IL6.

Patients and Methods

Patients. This study was conducted in the medical, surgical and cardiac intensive care units at The Nebraska Medical Center and was approved by the University of Nebraska Medical Center Institutional Review Board (482-07-FB). All patients gave their consent, were over the age of 19 years, with AKI, and had urine output of less than 200 ml/day. To be eligible for inclusion in the study, patients were anticipated to require SLED for at least 8 h and were prescribed at least one of the following antibiotics: ciprofloxacin, imipenem-cilastatin, linezolid, piperacillin-tazobactam, or vancomycin. The antibiotic selection was a result of an ongoing study investigating the pharmacokinetics of these antibiotics during SLED. The mean Acute Physiologic and Chronic Health Evaluation (APACHE II) scores were calculated for each patient (9).

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Table I. *Characteristics of patients receiving sustained low-efficiency dialysis.*

UPN	Age (gender)	Diagnosis	Antibiotics	APACHE II	Outcome (days)
02	62 (F)	Sepsis, heart block	V, I, M	36	A (29)
03	77 (M)	Septic shock	V, Z, C	46	D (46)
04	53 (M)	Cellulitis, line infection	V, CEF, CL, C, O	39	D (11)
05	62 (F)	Cirrhosis, infection	C, V, CEF	24	D (29)
06	50 (M)	Liver transplant, infection	C, I, V	35	A (92)
09	55 (M)	Heart failure, peritonitis	V, Z	24	A (34)
10	58 (F)	Cirrhosis, infection	L, Z	24	D (11)

F: Female, M: male, V: vancomcyin, I: imipenem/cilastatin, CEF: cefipime, C: ciprofloxacin, Z: zosyn, CL: clindamycin, M: metronidazole, O: ofloxacin, A: alive and discharge day, D: death and hospital stay.

Renal replacement therapy. Vascular access for SLED was determined by the attending physician. A Fresenius F-50 dialyzer containing a CRRT chip and a F50 NR polysulfone filter was used in all patients. Blood flow and dialysate flow were standardized and set at 200 ml per minute. Dialysate composition was determined by the Nephrology Service and individualized to the patient. Either 2% trisodium citrate anticoagulation or heparin anticoagulation was used. The minimum dialysis time was 8 hours.

Blood draws. All blood samples were collected through a central venous catheter. Blood samples were collected in heparinized vacutainers prior to the start of antibiotics and the start of SLED then at 1, 2, 4, and 8 hours after completion of antibiotic infusion. There was a variable length of dialysis time between the 8-hour blood draw and stopping SLED. Blood samples were then collected at the end of SLED and 30 minutes, and at 1, 3, 8, and 24 hours after stoppage.

Sample handling and IL6 analysis. Blood was centrifuged at 1000×g for 10 minutes and plasma collected and aliquots stored at −70˚C until analyzed. Plasma IL6 concentrations were measured in patients using a commercially available sandwich type ELISA (R&D Systems, Minneapolis, MN , USA). Each sample was assayed in duplicate and values averaged. The lower limit of detection for the assay was 0.7 pg/ml, with a dynamic range of 0.7 to 300 pg/ml. Concentrations greater than 300 pg/ml were diluted 10:1 and re-analyzed. Intra-assay and inter-assay coefficient of variation were below 5%.

Statistical analysis. Time courses were analyzed for statistical significance using a repeated measures analysis of variance. Comparison of plasma IL6 concentrations in patients who survived *versus* those who did not were compared using Student's *t*-test. Statistical significance was defined at $p<0.05$.

Results

A total of 10 patients agreed to participate in this study, one patient died prior to completion of the study and two others had incomplete blood samples, making these three patients ineligible for inclusion in the analysis. Table I contains the demographic, treatment, and outcome data for the seven evaluable patients. The average age of the cohort in Table I was 60 years, and the mean APACHE II score was 32.5. The cohort is best described as a middle-aged acutely-ill group of patients with sepsis requiring SLED for oliguric renal failure and antibiotic therapy for sepsis.

IL6 concentrations were significantly higher during the first 8 h of SLED compared to the concentrations measured at 3, 8, and 24 h after SLED was stopped. Three out of the seven patients survived to be discharged from the hospital; calculated in-hospital mortality was 57%. IL6 concentrations were significantly higher in patients who died during their hospitalization ($p<0.0001$) (Figure 1). The three patients who survived hospitalization had lower IL6 concentrations at each of the studied time points, with the most striking difference being lower baseline levels in surviving patients (Figure 2).

Discussion

IL6 is a 21-kDa protein with pleiotropic activity, and its elevated levels are associated with sepsis and severe sepsis (10,11). IL6 has significant angiogenic activity and elevations may cause significant pathology based on inappropriate activation of vascular endothelium. Inflammation and activation of vascular endothelial cells can lead to increased risk of thrombosis and subsequent ischemic organ damage (12, 13).

Various investigators have reported a relationship between elevated plasma IL6 concentrations and mortality. However, the effect of lowering IL6 on mortality has not been well-described, in large part due to limited methods for reproducibly lowering plasma IL6 concentrations. Antibiotics and dialysis may be able to lower IL6 plasma concentrations, with unclear clinical benefit (14, 15). In the present study, we found no lowering of plasma IL6 concentrations with antibiotics and SLED.

In the present study, four out of the seven patients presented with very high plasma concentrations of IL6 and concentrations were not lowered during the first 8 h of SLED

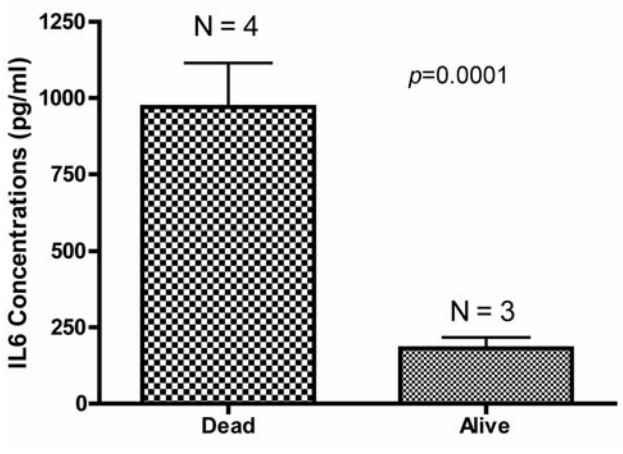

Figure 1. *Comparison of mean interleukin (IL6) concentrations in survivors and non-survivors.*

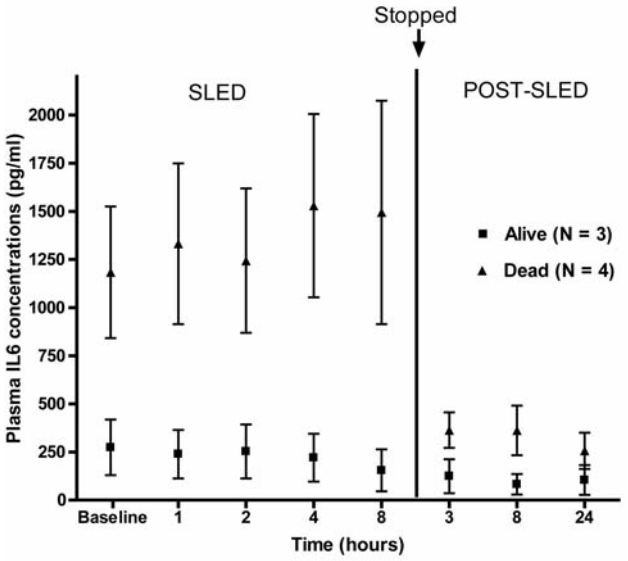

Figure 2. *Time course of mean (±SEM) plasma IL6 concentrations in patients alive at discharge (N=3) and patients who died during hospitalization (N=4).*

and antibiotic therapy; all these patients died in hospital despite later post-SLED lowering of IL6 concentration. Because of the small size of this study (N=7), conclusions regarding plasma IL6 concentrations and survival must be interpreted cautiously. However, despite the small patient numbers, a highly significant relationship between plasma IL6 concentration and survival suggests that IL6 is a robust predictor of outcome.

There are several limitations to our data. Firstly, the effect of SLED on plasma IL6 concentrations could not be separated from the effect of antibiotics and other supportive care measures used on the same timeline. Secondly, blood samples were available only for the first 8 h of SLED then post-SLED. During much of the time SLED was being utilized, IL6 concentrations could not be measured. Thirdly, because of dilution, we were unable to measure IL6 in dialysate due to concentrations below the limit of detection. Future studies will address several of these limitations, including a more complete sampling for cytokine analysis, performing assays on other cytokines relevant to outcomes in sepsis, and developing methods to concentrate dialysate for cytokine analysis which do not degrade these proteins.

Conclusion

In conclusion, while there are limitations associated to this study, our results demonstrate that a high systemic IL6 concentration shortly after diagnosis of sepsis predicts mortality. This report demonstrates the potential importance of IL6 as a biomarker and prognostic factor for sepsis.

Conflicts of Interest

The Authors have no financial or non-financial competing interests.

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