Kidney International, Vol. 65 (2004), pp. 1357–1365

CLINICAL NEPHROLOGY – EPIDEMIOLOGY – CLINICAL TRIALS

Plasma cytokine levels predict mortality in patients with acute renal failure

EDITH M. SIMMONS, JONATHAN HIMMELFARB, M. TUGRUL SEZER, GLENN M. CHERTOW, RAVINDRA L. MEHTA, EMIL P. PAGANINI, SHARON SOROKO, STEPHANIE FREEDMAN, KAREN BECKER, DANIEL SPRATT, YU SHYR, and T. ALP IKIZLER, FOR THE PICARD STUDY GROUP

Vanderbilt University Medical Center, Nashville, Tennessee; Maine Medical Center, Portland, Maine; Maine Medical Center Research Institute, Scarborough, Maine; University of California San Francisco, San Francisco, California; University of California San Diego, San Diego, California; and Cleveland Clinic Foundation, Cleveland, Ohio

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Background. Critically ill patients with acute renal failure (ARF) experience a high mortality rate. Animal and human studies suggest that proinflammatory cytokines lead to the development of a systemic inflammatory response syndrome (SIRS), which is temporally followed by a counter anti-inflammatory response syndrome (CARS). This process has not been specifically described in critically ill patients with ARF.

Methods. The Program to Improve Care in Acute Renal Disease (PICARD) is a prospective, multicenter cohort study designed to examine the natural history, practice patterns, and outcomes of treatment in critically ill patients with ARF. In a subset of 98 patients with ARF, we measured plasma proinflammatory cytokines [interleukin (IL)-1β, IL-6, IL-8, tumor necrosis factor-α (TNF-α)], the acute-phase reactant C-reactive protein (CRP), and the anti-inflammatory cytokine IL-10 at study enrollment and over the course of illness.

Results. When compared with healthy subjects and end-stage renal disease patients on maintenance hemodialysis, patients with ARF had significantly higher plasma levels of all measured cytokines. Additionally, the proinflammatory cytokines IL-6 and IL-8 were significantly higher in nonsurvivors versus survivors [median 234.7 (interdecile range 64.8 to 1775.9) pg/mL vs. 113.5 (46.1 to 419.3) pg/mL, $P = 0.02$ for IL-6; 35.5 (14.1 to 237.9) pg/mL vs. 21.2 (8.5 to 87.1) pg/mL, $P = 0.03$ for IL-8]. The anti-inflammatory cytokine IL-10 was also significantly higher in nonsurvivors [3.1 (0.5 to 41.9) pg/mL vs. 2.4 (0.5 to 16.9) pg/mL, $P = 0.04$]. For each natural log unit increase in the levels of IL-6, IL-8, and IL-10, the odds of death increased by 65%, 54%, and 34%, respectively, corresponding to increases in relative risk of approximately 30%, 25%, and 15%. The presence or absence of SIRS or sepsis was not a major determinant of plasma cytokine concentration in this group of patients.

Conclusion. There is evidence of ongoing SIRS with concomitant CARS in critically ill patients with ARF, with higher levels of plasma IL-6, IL-8, and IL-10 in patients with ARF who die during hospitalization. Strategies to modulate inflammation must take into account the complex cytokine biology in patients with established ARF.

Key words: acute renal failure, cytokine, interleukin, inflammation, systemic inflammatory response syndrome, intensive care unit.

Received for publication March 28, 2003
and in revised form September 5, 2003, and October 8, 2003
Accepted for publication October 28, 2003

Severely ill patients in the intensive care unit (ICU) setting experience a high rate of mortality, and this risk is compounded when their course is complicated by acute renal failure (ARF). Published studies estimate that 5% to 20% of ICU patients experience an episode of ARF during the course of their illness, often accompanied by multiorgan dysfunction [1–3]. Despite advances in supportive care and innovations in renal replacement therapies over the past three decades, the mortality rate for these patients remains high, with most studies citing death rates in excess of 50% [4, 5]. The association between ARF and death in ICU patients appears to be independent of other complications, suggesting that patients die in part from, and not only with, ARF [6–8].

Severe illness of almost any etiology is accompanied by a generalized host inflammatory response. This has been referred to as the systemic inflammatory response syndrome (SIRS) [9, 10]. Central to this process is the release of a cascade of potent inflammatory mediators into the systemic circulation, including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and IL-6. It is now recognized that this intense proinflammatory reaction is often followed temporally by a compensatory anti-inflammatory response syndrome (CARS), during which time anti-inflammatory cytokines, including IL-10, are liberated into the bloodstream [10]. In some clinical settings, including uremia and sepsis, dysregulation of the inflammatory process is characterized by simultaneous release of both proinflammatory and anti-inflammatory mediators [11]. The dysregulation of the inflammatory response in septic and critically ill patients has been implicated as an important mechanism underlying the

development of multiple organ system dysfunction, septic shock, and death.

Given the clear association between persistent activation of the inflammatory response and multisystem organ failure, several studies have examined the prognostic role of plasma levels of the various inflammatory mediators in predicting outcomes in critically ill patients in general [12–14]. However, few studies have focused on severely ill patients with ARF. We hypothesized that extensive immune dysregulation would be associated with all-cause mortality in critically ill patients with ARF, based on the exceedingly high mortality rates observed in this patient population, and the complex metabolic derangements induced by reduction in glomerular filtration rate, hypercatabolism, and the effects of dialysis.

METHODS

Study cohort

This study was performed as a part of the Program to Improve Care in Acute Renal Disease (PICARD) network. The PICARD study is a prospective observational cohort examining the natural history, practice patterns, and outcomes of treatment in critically ill patients with ARF, conducted at five academic medical centers in the United States (Cleveland Clinic Foundation, Cleveland, Ohio; Maine Medical Center, Portland, Maine; Vanderbilt University, Nashville, Tennessee; University of California, San Diego, California; University of California, San Francisco, California). The study period was from February 1, 1999 to August 31, 2001. All adult (age ≥18 years) ICU patients with ARF in whom a nephrology service consultation was received were considered for the study. ARF was defined using standard laboratory parameters. For patients with no prior history of kidney disease, or baseline creatinine values of <1.5 mg/dL, ARF was defined by an increase in serum creatinine of at least 0.5 mg/dL occurring over a 48-hour period. For those patients with pre-existing chronic renal insufficiency (baseline creatinine values ≥1.5 mg/dL), ARF was defined by a rise in serum creatinine of ≥1 mg/dL from baseline occurring over 48 hours. Exclusion criteria included previous dialysis, kidney transplantation, ARF from urinary tract obstruction or from hypovolemia responsive to fluids, as well as prisoners and pregnant patients. The study was approved by the Institutional Review Board of each participating hospital, and informed consent was obtained from all study participants or their next-of-kin.

As a substudy of PICARD, patients enrolled at Vanderbilt University Medical Center and Maine Medical Center were asked to undergo serial measurements of biomarkers of inflammation. A total of 414 ARF cases from these two centers were initially evaluated, with 201 (49%) meeting entry criteria. This particular study required an additional consent requesting blood sampling for cytokine determination, and the 98 patients who agreed to sign the additional consent form comprised the analytic sample. Of these participants, 25 (26%) were at Maine Medical Center and 73 (74%) were at Vanderbilt University Medical Center. Patients were followed prospectively from the time of initial nephrology service consultation until death or hospital discharge. We compared results obtained in patients with ARF with healthy control patients ($N = 48$) and patients with end-stage renal disease (ESRD) on maintenance hemodialysis ($N = 42$).

Clinical and laboratory data

The study consisted of baseline and serial measurements of biomarkers of inflammation beginning at the time of nephrology consultation and each week thereafter over the course of hospitalization. Renal function was assessed daily from records of urine output, blood urea nitrogen (BUN) level, and serum creatinine level. Patients were considered to be oliguric when urine output fell below 400 mL per 24 hours. The origin of ARF was classified as ischemic, nephrotoxic, or multifactorial acute tubular necrosis, multisystem disease (i.e., systemic lupus erythematosus, rapidly progressive glomerulonephritis), or uncertain. Complete recovery of renal function was defined as the return of serum creatinine to <2.0 mg/dL, or return to within 20% of baseline creatinine concentration for patients with underlying chronic renal disease. Recovery was deemed to be partial when the above conditions were not met, but the patient was no longer dialysis-dependent. Vital signs, hemodynamic data (where available), and general laboratory data were recorded for the first ICU day and each day from the time of nephrology consultation, and generic severity-of-illness scores were computed from these variables. At the time of initial nephrology assessment, patients were evaluated for the presence of SIRS, sepsis, or septic shock as defined by American College of Chest Physicians/Society of Critical Care Medicine guidelines [15]. Specifically, SIRS was defined as the systemic inflammatory response to an unspecified stimulus manifested by the presence of two or more of the following: (*1*) a body temperature greater than 38°C or less than 36°C; (*2*) a heart rate greater than 90 beats per minute; (*3*) on a ventilator, or tachypnea, manifested by a respiratory rate greater than 20 breaths per minute, or hyperventilation, as indicated by a $PaCO_2$ of less than 32 mm Hg; and (*4*) a white blood cell count greater than 12,000/mm^3 or less than 4,000/mm^3, or the presence of more than 10% immature neutrophils. Sepsis included the above criteria when an infectious source was documented or strongly suspected. For our study purposes, sepsis and severe sepsis (sepsis associated with organ dysfunction, hypoperfusion, or hypotension) were considered in the same category, as all

patients included in the study had evidence of renal organ dysfunction. Septic shock was defined as sepsis with hypotension, despite adequate fluid resuscitation, along with the presence of perfusion abnormalities.

Biomarkers of inflammation

Inflammatory markers followed during the study included the proinflammatory cytokines IL-1β, IL-6, IL-8, and TNF-α; the acute-phase reactant C-reactive protein (CRP); and the anti-inflammatory cytokine IL-10. Baseline venous blood samples for cytokine determination were collected within 48 hours of nephrology consultation for ARF study subjects and predialysis for ESRD patients on maintenance hemodialysis. Blood samples were taken into EDTA tubes and clot activator tubes for plasma and serum separation, respectively. Samples were immediately centrifuged, and the plasma and serum stored at −70°C until analysis. Cytokine concentrations were measured in duplicate by enzyme-linked immunosorbent assay (ELISA) with kits from BioSource International (Camarillo, CA, USA). IL-1β, IL-6, and TNF-α were measured in plasma, and IL-8 and IL-10 in serum. The detectable limits and interassay coefficients of variation for the cytokines were 2.0 pg/mL and 5% for IL-1β, 2.0 pg/mL and 6% for IL-6, 0.7 pg/mL and 5% for IL-8, 1.0 pg/mL and 3% for IL-10, and 3.0 pg/mL and 10% for TNF-α, respectively. Serum CRP was measured using high-sensitivity nephelometry (Dade Behring, Inc., Newark, DE, USA).

Analysis plan

We focused on determining the associations among biomarkers of inflammation with mortality. The primary outcome measure was all-cause in-hospital mortality, with ICU mortality analyzed as a secondary outcome. Demographic data such as age, sex, and race were also tabulated. IL-1β and IL-10 levels below the reference laboratory's limit of detection (<2 pg/mL for IL-1β and <1 pg/mL for IL-10) were assigned values of 1.1 and 0.5 pg/mL, respectively, for statistical analysis. Additionally, the distributions of IL-1β, IL-6, IL-8, IL-10, and TNF-α were highly skewed, and values for these inflammatory markers were natural log-transformed before inference testing.

For univariate analysis of single time point data, comparisons among ARF patients and control patients, as well as among survivors and nonsurvivors, were assessed using the Student t test or Mann-Whitney U test for continuous variables; discrete data were analyzed by Pearson χ^2 test or Fisher exact test. Logistic regression was employed to evaluate independent predictors of mortality. Odds ratios (OR) and 95% CI were derived from model parameter coefficients and standard errors, respectively. In multivariable analyses, we adjusted for confounding by age, sex, and race. We also evaluated whether other nondemographic predictors of mortality confounded the cytokine-mortality relation, including signs of sepsis and generic severity of illness scores [e.g., Acute Physiology and Chronic Health Evaluation (APACHE) III]. The trends of the inflammatory markers over the time were estimated using the restricted/residual maximum likelihood (REML)-based mixed effect model to adjust for the intra-correlation effect for the patients who had multiple measurements. The model reported herein was selected based on Akaike's Information Criterion (AIC) and Schwarz's Bayesian Criterion (BIC) [16]. Results are expressed as mean ± SD or median and range, where appropriate. All tests of significance were two-sided, and differences were considered statistically significant with P value < 0.05. SAS version 8.2 (Statistical Analysis System for Windows; SAS Institute, Inc., Cary, NC, USA) was used for data analyses.

RESULTS

Patient characteristics

The demographic and clinical characteristics for the 98 patients at the time of nephrology consultation are summarized in Table 1. The mean age (±SD) of the total study cohort was 60.3 (±15.7) years, with 59% male patients and 96% white patients. The predominant etiology of ARF based on clinical presumption was ischemic (47%) or multifactorial acute tubular necrosis (40%). The mean (±SD) BUN and creatinine at the time of nephrology consultation were 60.6 (±30.1) mg/dL and 3.2 (±1.3) mg/dL, respectively, and median urine output was 927.5 [interdecile range 102 to 3178] mL per 24 hours. A number of the participants were classified as having SIRS (5%), sepsis (25%), or septic shock (3%) at the time of nephrology consultation. The average number of nonrenal organ systems in failure at the time of initial assessment by the nephrology consultant was 2.0 (±1.0), and these were primarily respiratory failure (74%) and cardiovascular failure (63%). Notably, only three patients in the cohort had isolated renal failure, without additional organ systems in failure, at the time of renal consultation. Fifty-five patients received renal replacement therapy, predominantly intermittent hemodialysis (43 patients), during the course of their illness, and 50% of the total cohort recovered renal function (19% with partial recovery and 31% with complete recovery) before hospital discharge or death (Table 2).

Healthy control patients consisted of 48 nonpregnant, generally well individuals with no known cardiovascular disease or diabetes mellitus. This subset was composed of 30 women (62%) and 18 men (38%), the majority of whom were white (92%). The mean age (±SD) for healthy control patients was 49.1 (±16.1) years ($P < 0.001$ vs. ARF patients).

Table 1. Patient demographics and clinical features as assessed on the day of nephrology consultation according to survival status

Demographics and history	Total $N = 98$	Survivors $N = 54$	Nonsurvivors $N = 44$	P value
Age *years*	60.3 (15.7)	58.5 (16.9)	62.6 (14.0)	NS
Male %	59.2	55.6	63.6	NS
White %	95.9	96.3	95.5	NS
Diabetes mellitus%	35.7	35.2	36.4	NS
ARF on CRI %	29.9	28.3	31.8	NS
History of cardiac disease %	49.0	40.7	59.1	0.07
History of liver disease %	16.3	14.8	18.2	NS
Surgical %	57.8	64.0	50.0	NS
Etiology of ARF %				
Ischemic	46.9	50.0	43.2	NS
Nephrotoxic	8.2	9.3	6.8	NS
Multisystem	1.0	1.9	0.0	NS
Multifactorial	39.8	35.2	45.5	NS
Unknown	4.1	3.7	4.5	NS
Renal function indicators				
Blood urea nitrogen *mg/dL*	60.6 (30.1)	54.7 (27.7)	67.1 (31.6)	0.06
Creatinine *mg/dL*	3.2 (1.3)	3.2 (1.4)	3.3 (1.1)	NS
Median urine output (interdecile range) *mL*	927.5 (102–3178)	1285.0 (255–4130)	425.0 (41–2352)	0.001
Oliguria %	27.6	15.7	44.2	0.002
SIRS %	5.1	8.0	2.6	NS
Sepsis or septic shock %	28.6	34.0	28.9	NS
Organ system failure %				
Respiratory	73.6	68.8	79.5	NS
Cardiac	62.5	62.5	62.5	NS
Hepatic	23.0	18.8	28.2	NS
Hematologic	23.0	25.0	20.5	NS
Central nervous system	23.0	22.9	23.1	NS
Severity-of-illness scores				
APACHE II	23.0 (6.4)	22.7 (5.9)	23.4 (7.0)	NS
APACHE III	77.7 (18.5)	72.1 (16.5)	84.3 (18.8)	0.002
SAPS II	53.8 (15.7)	49.7 (12.4)	58.7 (17.9)	0.04
SOFA	5.6 (2.7)	5.2 (2.5)	6.2 (2.9)	0.08

Abbreviations are: ARF, acute renal failure; CRI, chronic renal insufficiency; SIRS, systemic inflammatory response syndrome; APACHE, Acute Physiology and Chronic Health Evaluation; SAPS, simplified acute physiology score; SOFA, sequential organ failure assessment. Data are presented as mean (SD) unless otherwise indicated. P values are for survivors vs. nonsurvivors.

Table 2. Patient outcomes

	Survivors	Nonsurvivors	P value
Hemodialysis %	40.7	75.0	0.001
Renal function recovery %			
Partial	24.5	13.6	NS
Complete	54.7	4.5	<0.001
Mortality %			
Hospital mortality		44.9	
Mortality day, median (interdecile range)		10.5 (4–36)	
ICU mortality		37.8	
30-day mortality		36.7	

ICU, intensive care unit.

Forty-two patients on maintenance hemodialysis (≥12 months) served as additional controls. They were of similar age (mean ± SD, 58.4 ± 17.3 vs. 60.3 ± 15.7 for ARF patients, $P = 0.52$) and sex distributions (62% male vs. 59% male, $P = 0.76$) to the study participants with ARF. The percentage of black subjects with ESRD was much higher than in the cohort with ARF (62% vs. 4%, $P < 0.001$). Twenty-nine percent of the ESRD group had diabetes mellitus ($P = 0.42$ vs. ARF patients). The mean (±SD) monthly dose of dialysis delivered for the ESRD group at the time of cytokine determination was 1.6 (±0.3) for Kt/V and 75.3 (±8.0) for urea reduction ratio.

As noted in the **Methods** section, there was a group of patients from both centers who refused consent for cytokine analysis ("refusers," $N = 103$), but who were included in the overall PICARD study. A comparison of the demographic characteristics showed that refusers were slightly older than study participants (mean age ± SD, 64.8 ± 14.1 years vs. 60.3 ± 15.7 years, $P = 0.04$). However, there were no significant differences in sex, race/ethnicity, history of diabetes mellitus, or renal parameters, including serum creatinine, BUN, and presence of oliguria, at the time of consultation. Both groups had, on average, two organ systems in failure in addition to ARF upon initial nephrology evaluation ($P = 0.64$). A similar fraction of subjects in both groups (56% of participants and 46% of refusers, $P = 0.15$) underwent renal replacement therapy over the course of the study. In-hospital mortality was statistically higher in the study participants than the refusers (45% vs. 30%, $P = 0.03$), although recovery of renal function was comparable in the two groups.

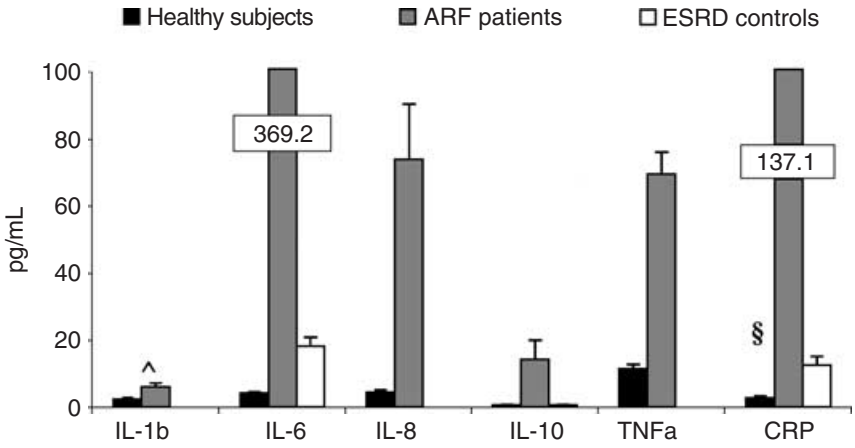

Fig. 1. Cytokine values for study participants at the time of nephrology consultation versus healthy subjects and end-stage renal disease (ESRD) control subjects (only IL-6, IL-10, and CRP available). Bars and error bars represent mean ± SEM for each time point. $^{\wedge}P = 0.031$ for interleukin (IL)-1β (ARF study patients vs. healthy subjects); $P < 0.001$ for all other comparisons (ARF study patients vs. healthy subjects and ARF study patients vs. ESRD control patients). §CRP in mg/L.

Overall survival

The in-hospital mortality rate for the study cohort was 45%. The median survival time for those patients who died was 10.5 days (interdecile range 4 to 36 days). Likewise, the ICU mortality rate for the study cohort was 38%, indicating that the majority of deaths occurred in the ICU setting. Table 1 displays the demographic and clinical features according to survival status. Neither demographic characteristics, presumed etiology of ARF, nor serum indicators of renal function were associated with mortality. Not surprisingly, mortality rates were higher among those patients who were oliguric (70% vs. nonoliguric mortality of 36%, $P = 0.002$) and for those who required dialysis (60% vs. 26%, $P = 0.001$). Mean APACHE III and Simplified Acute Physiology Score (SAPS) II scores as assessed at the time of nephrology consultation were significantly higher among nonsurvivors compared with patients who survived to hospital discharge (84.3 vs. 72.1, $P = 0.004$ and 58.7 vs. 49.7, $P = 0.04$ for APACHE III and SAPS II scores, respectively).

Plasma cytokine levels are elevated at the time of nephrology consultation and predict mortality

Baseline cytokine and CRP values determined from blood obtained within 48 hours of study entry are depicted in Figure 1. As illustrated, critically ill patients with ARF had marked simultaneous increases in plasma levels of all cytokines ($P < 0.05$ for IL-1β; $P < 0.001$ for IL-6, IL-8, IL-10, and TNF-α) and CRP ($P < 0.001$) when compared with healthy control subjects. Likewise, the values of IL-6, IL-10, and CRP were also 10- to 20-fold higher in the critically ill ARF patients compared with ESRD patients on maintenance hemodialysis ($P < 0.001$ for all). The relationships among baseline cytokine and CRP concentrations of study participants and various physiologic variables as assessed at the time of nephrology consultation are presented in Table 3.

Table 3. Correlations among baseline inflammatory biomarkers and physiologic variables measured on the day of nephrology consultation

	IL-1β	Il-6	IL-8	IL-10	TNF-α	CRP
Age	−.089	.014	−.067	.028	.037	.219
	.398	.892	.533	.797	.723	.063
BUN	.316	.004	.234	.306	.275	−.220
	.004[a]	.972	.038[a]	.006[a]	.011[a]	.086
Creatinine	−.001	−.012	.132	.023	.147	.015
	.996	.918	.243	.840	.176	.909
UOP	−.136	−.221	−.295	−.258	−.224	−.058
	.204	.038[a]	.006[a]	.016[a]	.032[a]	.636
APACHE III	.016	.142	.458	.186	.289	−.006
	.889	.208	<.001[a]	.104	.008[a]	.963
SAPS II	.127	.021	.310	.405	.203	.029
	.390	.888	.028[a]	.004[a]	.157	.863

Abbreviations are: IL, interleukin; TNF, tumor necrosis factor; CRP, C-reactive protein; BUN, blood urea nitrogen; UOP, urine output; APACHE, Acute Physiology and Chronic Health Evaluation; SAPS, simplified acute physiology score. Values presented are Pearson correlation coefficients (upper) and *P* values (lower).

[a]Denotes statistically significant correlations. Data that did not fit a normal distribution were log transformed before analysis (IL-1β, IL-6, IL-8, IL-10, TNF-α, creatinine, and urine output).

Several cytokines, reflecting both the proinflammatory and anti-inflammatory cascades, were elevated at baseline in nonsurvivors compared with those patients who survived to hospital discharge. Specifically, the proinflammatory cytokines IL-6 and IL-8 were significantly higher in nonsurvivors versus survivors [median 234.7 (interdecile range 64.8 to 1775.9) pg/mL vs. 113.5 (46.1 to 419.3) pg/mL, $P = 0.02$ for IL-6; 35.5 (14.1 to 237.9) pg/mL vs. 21.2 (8.5 to 87.1) pg/mL, $P = 0.03$ for IL-8]. Similarly, the median value for the anti-inflammatory cytokine IL-10 was 3.1 (0.5 to 41.9) pg/mL for nonsurvivors compared with 2.4 (0.5 to 16.9) pg/mL for survivors ($P = 0.04$). Each natural log unit increase of IL-6, IL-8, and IL-10 was associated with an OR (95% CI) of fatal outcome of 1.65 (1.09 to 2.49), 1.54 (1.03 to 2.30), and 1.34 (1.01 to 1.80), respectively. Figure 2 illustrates the risk profiles for the inflammatory biomarkers according to quartiles. Notably, for both IL-6 and IL-10, baseline cytokine values above the 25th percentile were associated with a progressively

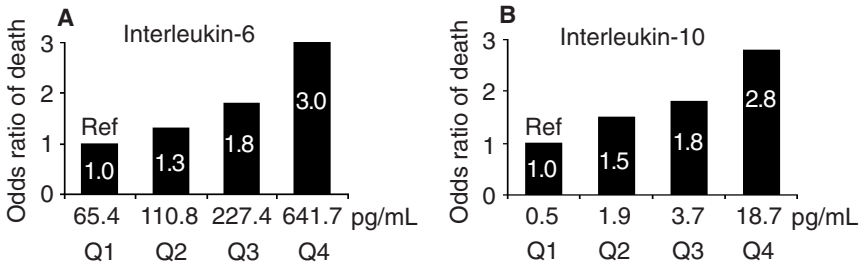

Fig. 2. Risk profiles for interleukin-6 (IL-6) and interleukin-10 (IL-10) according to quartiles. Values along the x-axis represent the median baseline cytokine concentrations of the respective quartiles. $P = 0.02$ for IL-6 trend (*A*); $P = 0.05$ for IL-10 trend (*B*). Ref, reference.

Table 4. Baseline plasma cytokine levels according to presence of SIRS, sepsis, or septic shock at the time of nephrology consultation

	No SIRS/no sepsis $N = 63$	SIRS/sepsis/septic shock $N = 34$	*P* value
IL-1β	2.0 (1.1–20.4)	1.1 (1.1–9.2)	0.24
IL-6	158.2 (30.9–932.0)	156.9 (67.8–575.4)	0.99
IL-8	24.1 (8.0–245.3)	34.8 (13.8–120.6)	0.40
IL-10	2.4 (0.5–26.5)	3.4 (0.5–28.2)	0.23
TNF-α	47.4 (24.3–99.1)	67.8 (36.5–143.1)	0.001
CRP	102.6 (28.8–275.8)	148.3 (67.8–271.1)	0.08

Abbreviations are: IL, interleukin; TNF, tumor necrosis factor; CRP, C-reactive protein; SIRS, systemic inflammatory response syndrome. Values are median (interdecile range) for each variable. Cytokines are expressed as pg/mL; CRP is expressed as mg/L.

higher risk of mortality ($P = 0.02$ and $P = 0.05$ for IL-6 and Il-10 trends, respectively; $P = 0.04$ for IL-8 trend, data not shown). Although numerically higher, the difference in median values for IL-1β, TNF-α, and CRP between the two groups did not reach statistical significance. The associations among inflammatory markers and ICU mortality were similar to those with in-hospital mortality (data not shown).

The presence or absence of SIRS or sepsis was not a major determinant of plasma cytokine concentration in this subgroup of critically ill ARF patients. Table 4 depicts the baseline plasma concentrations of the proinflammatory and anti-inflammatory markers examined in this study by SIRS/sepsis status as assessed on the day of nephrology consultation. TNF-α was consistently higher in patients with SIRS, sepsis, or septic shock compared with patients without these conditions ($P = 0.001$). No significant differences were observed in the remaining plasma cytokine values among the groups. Likewise, the presence of SIRS, sepsis, or septic shock was not independently associated mortality ($P = 0.24$).

In multivariable analysis of cytokines as predictors of in-hospital mortality, adjustment for demographic factors (age, race, sex) and sepsis status did not extinguish the significant associations among baseline IL-6, IL-8, and IL-10 values and mortality ($P = 0.02$ for IL-6, $P = 0.03$ for IL-8, and $P = 0.05$ for IL-10). Likewise, when adjusted for severity of illness (APACHE III), IL-6 remained an independent predictor of in-hospital mortality ($P = 0.04$), while IL-8 and IL-10 sustained marginal significance ($P = 0.06$ and $P = 0.07$ for IL-8 and IL-10, respectively). Interestingly, after the predictive value of cytokines were adjusted for urine output on the day of nephrology consultation, which itself was significantly associated with in-hospital death, only IL-6 maintained a trend toward significance ($P = 0.08$), while IL-8 and IL-10 were no longer independently associated with mortality ($P = 0.23$ and $P = 0.28$, respectively).

Levels of inflammatory markers decrease over time

To assess the relationship between changes in inflammatory markers over time and in-hospital mortality, we measured cytokine and CRP values at weekly intervals from study entry in patients who remained in the ICU. The inflammatory biomarkers were generally lower in survivors compared with nonsurvivors over serial measurements, with all but IL-1β and CRP attaining statistical significance (Table 5). The proinflammatory cytokine IL-6 and the anti-inflammatory cytokine IL-10 showed significant decreases over the study period ($P = 0.01$ and $P = 0.04$, respectively), and the differences in these cytokine levels between survivors and nonsurvivors remained statistically significant ($P = 0.001$ and $P = 0.04$, respectively, for IL-6 and IL-10). This phenomenon is represented graphically for IL-6 in Figure 3. There were also significant declines in overall IL-1β and CRP levels, while IL-8 and TNF-α levels appeared to change little over time. These trends were not affected by whether or not patients received renal replacement therapy.

DISCUSSION

The current study was designed to evaluate whether biomarkers of inflammation were associated with mortality in a cohort of 98 critically ill patients with ARF. At the time of nephrology consultation, the proinflammatory cytokines IL-6 and IL-8, as well as the anti-inflammatory cytokine IL-10 were significantly higher in nonsurvivors than in those patients who survived to hospital discharge. For each natural log unit increase in levels of IL-6, IL-8, and IL-10, the odds of death increased by point estimates of 65%, 54%, and 34%, respectively, corresponding to increases in relative risk of approximately 30%, 25%, and 15%, respectively [17]. The association between cytokine concentrations and in-hospital mortality was maintained

Table 5. Relationship of changes in inflammatory markers over time to in-hospital survival

	N	Survivors	Nonsurvivors	*P* value
IL-1β *pg/mL*				0.80[a]
Baseline	93	1.1 (1.1–11.1)	3.0 (1.1–19.0)	<0.001[b]
Week 1	29	9.9 (1.1–49.6)	17.8 (1.1–28.9)	
Week 2	14	27.6 (1.1–61.8)	30.7 (5.0–33.2)	
IL-6 *pg/mL*				0.001[a]
Baseline	91	113.5 (46.1–419.3)	234.7 (64.8–1775.9)	0.01[b]
Week 1	34	90.7 (22.4–735.6)	173.1 (59.9–1349.0)	
Week 2	15	63.0 (29.9–454.5)	252.5 (34.2–564.0)	
IL-8 *pg/mL*				0.006[a]
Baseline	90	21.2 (8.5–87.1)	35.5 (14.1–237.9)	0.70[b]
Week 1	33	28.3 (7.7–85.4)	45.7 (18.3–182.7)	
Week 2	17	21.3 (7.2–51.3)	40.4 (20.5–114.6)	
IL-10 *pg/mL*				0.04[a]
Baseline	90	2.4 (0.5–16.9)	3.1 (0.5–41.9)	0.04[b]
Week 1	33	1.8 (0.5–13.9)	2.9 (1.0–13.9)	
Week 2	16	2.2 (0.5–65.6)	2.2 (1.2–7.2)	
TNF-α *pg/mL*				0.03[a]
Baseline	96	50.7 (26.6–120.3)	58.7 (29.6–105.6)	0.66[b]
Week 1	33	45.9 (29.0–73.0)	67.9 (37.7–123.7)	
Week 2	16	58.3 (30.6–101.7)	77.8 (57.7–203.8)	
CRP *mg/L*				0.36[a]
Baseline	73	109.8 (38.2–233.1)	122.4 (30.8–328.4)	<0.001[b]
Week 1	31	66.0 (22.6–270.9)	65.7 (9.4–253.9)	
Week 2	15	53.2 (6.9–184.2)	124.4 (57.0–153.8)	

Abbreviations are: IL, interleukin; TNF, tumor necrosis factor; CRP, C-reactive protein. Values are presented as median (interdecile range). Data were analyzed using the mixed effect model; values for IL-6, IL-8, IL-10, and TNF-α were log transformed before analysis due to the non-normal distribution of these variables. *N* denotes the number of study patients at each time point.

[a]Denotes *P* values between survivors and nonsurvivors over the course of multiple measurements.

[b]Denotes *P* values for changes in cytokine levels over time.

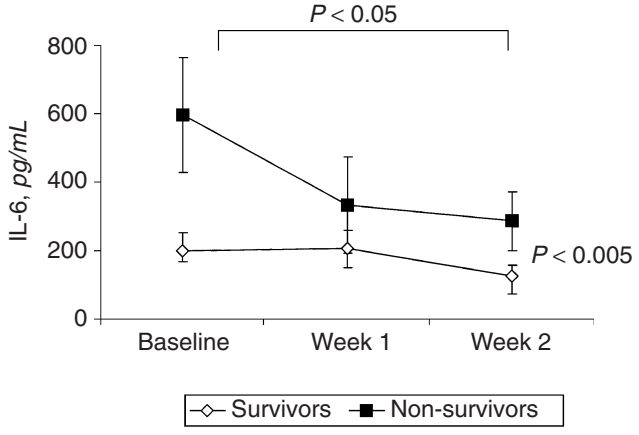

Fig. 3. Changes in plasma interleukin (IL)-6 concentrations over three weeks among survivors and nonsurvivors. Points and error bars represent mean ± SEM for each time point.

even after adjusting for demographics (age, race, sex) and sepsis status.

Cytokines are proteins with potent pleiotrophic biologic effects at picomolar concentrations [18]. Proinflammatory cytokines, including TNF-α, IL-1β, IL-6, and IL-8 are required for the initiation of an effective inflammatory response to infection and/or tissue injury. Proinflammatory cytokines are able to induce each other in a series of cascade events, thereby resulting in synergistic potentiation of pathobiologic effects. High concentrations of proinflammatory cytokines have been reported to correlate with the prognosis of sepsis and the development of multiorgan dysfunction syndrome. In animal models, infusion of high concentrations of proinflammatory cytokines can lead directly to the development of multiorgan system failure [19]. Several, but not all, studies have suggested that levels of circulating proinflammatory cytokines can be used as a measure of severity of illness and/or as a prognostic marker for patients with sepsis and multiorgan system failure.

There are, however, important distinctions between the present study and the body of literature reporting plasma cytokines in critically ill patients, especially patients with sepsis. In many sepsis studies, the proinflammatory cytokines IL-1 and TNF-α predict mortality, while in the present study we did not observe such a relationship. Instead, the significant predictors of survival were IL-6 and IL-8, often considered secondary inflammatory cytokines that are more distal in inflammatory cascades than IL-1 or TNF-α. It is noteworthy that an association between IL-6 and mortality has also been demonstrated in patients with chronic kidney disease [20, 21]. These findings suggest that renal failure may in and of itself confer an altered cytokine profile, even in the context of critical illness. Another potential explanation for this discrepancy between our study and previous studies in patients with sepsis may be the timing of cytokine determination in the overall course of illness. The fact that earlier markers of inflammation, namely IL-1 and TNF-α, were not associated with mortality in our cohort, while those later in the inflammatory cascade (i.e., Il-6 and IL-8) were may indicate that at the time of nephrology consultation and blood sampling, the patients had been inflamed for an extended period. Finally, the alterations in cytokine metabolism caused by underlying disease(s) might account for our observed results [22].

In this study we also measured plasma levels of the prototypical anti-inflammatory cytokine IL-10. Interleukin-10 is known to counter-regulate the cascade of proinflammatory cytokines that develop as a result of tissue injury and as part of the acute-phase reaction, including TNF-α, IL-1, and IL-6. Several previously reported studies demonstrate that in the sepsis syndrome, elevated plasma IL-10 levels are associated with poor survival, a finding similar to the present study of critically ill patients with ARF [23, 24]. Interleukin-10 can directly inhibit the monocyte inflammatory response to endotoxin and other stimuli, and a number of studies have demonstrated that monocyte hyporesponsiveness in sepsis and multiorgan dysfunction syndromes in critically ill patients is also strongly associated with mortality [25]. The increase in IL-10 and other anti-inflammatory

cytokines after an acute-phase inflammatory response has been termed the "counter anti-inflammatory response syndrome," or CARS. Data from the present study would suggest that in critically ill patients with ARF, dysregulation of proinflammatory and anti-inflammatory cytokine networks may proceed in parallel and the overall degree of cytokine network disruption may be an important prognostic indicator.

A surprising finding in this study is that plasma cytokine levels did not differ substantially between patients with or without the diagnosis of sepsis. ARF frequently develops as a complication of sepsis, and the prevalence of ARF in patients who develop sepsis ranges from 9% to 40% in published studies [26]. Patients with ARF and sepsis are also reputed to have a higher mortality rate than nonseptic patients with ARF. Although the conventional wisdom is that sepsis precedes ARF, data from the larger PICARD cohort suggest that the relationship between sepsis and ARF may be more complex than previously appreciated [abstract; Mehta RL et al: *J Am Soc Nephrol* 13:246A, 2002] [27].

Another notable finding in this study is the inverse association of baseline circulating cytokine levels with 24-hour urine output on the day of renal consultation. While urine output may reflect the glomerular filtration rate (GFR), it is unlikely that GFR, per se, has a significant impact on cytokine levels in critically ill ARF patients. Indeed, the levels of cytokines we observed in ARF patients were several-fold higher than those obtained from ESRD patients who had minimal to no residual GFR. Alternatively, the resulting volume overload associated with a diminished urine output may itself be a significant mediator of plasma cytokine elevation in ARF similar to that observed in patients with congestive heart failure, and may account for the loss of significance between cytokine concentrations and mortality when adjusted for the impact of urine output [28].

This study has several important limitations. The sample size was small. While the data were collected prospectively, the timing of consultation and subsequent measurement of cytokines were nonuniform. Therefore, events occurring early in the course of ARF may not have been captured. Although levels of several cytokines were associated with mortality, there was a large degree of interpatient variability in cytokine levels. Thus, we were unable to establish with precision clinically relevant plasma concentrations of cytokines that could predict outcome or response to therapy in individual patients. Moreover, circulating cytokines have short half-lives, and there may be considerable intrapatient variability over time. Incorporating these facts, one could infer that our ability to find a "signal" between cytokines and survival is even more noteworthy. Finally, several investigators have suggested that plasma cytokine levels may not reflect monocyte responsiveness to proinflammatory stimuli and thus, may not accurately reflect the physiologic state of the immune mechanisms at the time that they are assayed [29–31].

CONCLUSION

In critically ill patients with ARF, proinflammatory and anti-inflammatory cytokines are markedly elevated in the presence or absence of sepsis, and associated with significant and clinically meaningful increases in the risk of death. The variable timing of assessment and the heterogeneous population make it impossible to reach precise conclusions regarding the specific utility of cytokine measurements in patients with ARF. However, the demonstrated association between cytokines and mortality in this important subset of the critically ill supports the extension of ongoing research efforts exploring the inflammatory state in ARF.

ACKNOWLEDGMENTS

This work is supported in part by NIH grants R01 DK53411, DK53412, DK53413, and 1K24 DK62849. Dr. Edith Simmons is supported by NIH grant 2T32 DK07569 and K12 RR17697. Dr. M. Tugrul Sezer is supported by International Society of Nephrology Fellowship Training Grant. This work was presented in abstract form at the 35th Annual Meeting and Scientific Exposition of the American Society of Nephrology, Philadelphia, PA, November, 2002.

Reprint requests to T. Alp Ikizler, M.D., Division of Nephrology, Vanderbilt University Medical Center, S-3223 MCN, 1161 21st Ave. South & Garland, Nashville, TN 37232–2372.
E-mail: alp.ikizler@vanderbilt.edu

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