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Original Articles

Plasma inflammatory and apoptosis markers are associated with dialysis dependence and death among critically ill patients receiving renal replacement therapy

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ABSTRACT

Background. Survivors of critical illness complicated by acute kidney injury requiring renal replacement therapy (RRT) are at an increased risk of dialysis dependence and death but the mechanisms are unknown.

Methods. In a multicenter, prospective, cohort study of 817 critically ill patients receiving RRT, we examined association between Day 1 plasma inflammatory [interleukin (IL)-1β, IL-6, IL-8, IL-10 and IL-18; macrophage migration inhibitory factor (MIF) and tumor necrosis factor]; apoptosis [tumor necrosis factor receptor (TNFR)-I and TNFR-II and death receptor (DR)-5]; and growth factor (granulocyte macrophage colony stimulating factor) biomarkers and renal recovery and mortality at Day 60. Renal recovery was defined as alive and RRT independent.

Results. Of 817 participants, 36.5% were RRT independent and 50.8% died. After adjusting for differences in demographics, comorbid conditions; premorbid creatinine; nephrotoxins; sepsis; oliguria; mechanical ventilation; RRT dosing; and severity of illness, increased concentrations of plasma IL-8 and IL-18 and TNFR-I were independently associated with slower renal recovery [adjusted hazard ratio (AHR) range for all markers, 0.70–0.87]. Higher concentrations of IL-6, IL-8, IL-10 and IL-18; MIF; TNFR-I and DR-5 were associated with mortality (AHR range, 1.16–1.47). In an analysis of multiple markers simultaneously, increased IL-8 [AHR, 0.80, 95% confidence interval (95% CI) 0.70–0.91, $P < 0.001$] and TNFR-I (AHR, 0.63, 95% CI 0.50–0.79, $P < 0.001$) were associated with slower recovery, and increased IL-8 (AHR, 1.26, 95% CI 1.14–1.39, $P < 0.001$); MIF (AHR, 1.18, 95% CI 1.08–1.28, $P < 0.001$) and TNFR-I (AHR, 1.26, 95% CI 1.02–1.56, $P < 0.03$) were associated with mortality.

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Conclusions. Elevated plasma concentrations of inflammatory and apoptosis biomarkers are associated with RRT dependence and death. Our data suggest that future interventions should investigate broad-spectrum immune-modulation to improve outcomes.

Keywords: acute kidney injury, biomarkers, mortality, renal recovery, renal replacement therapy

INTRODUCTION

Individuals surviving critical illness with acute kidney injury (AKI) requiring renal replacement therapy (RRT) are at an increased risk for RRT dependence and death in the subsequent months [1–3]. More than one-half of patients die, and of survivors, 25% of patients are dependent on RRT at 2 months following acute illness [1, 4]. This increased risk persists long after resolution of acute illness [5–8] and is not fully explained by differences in underlying demographic characteristics; premorbid renal function; comorbid disease burden or severity of illness [5, 9–11]. If acute illness plays a causal role in nonrecovery of renal function, the mechanisms are unclear.

Critically ill patients with AKI have higher circulating plasma concentrations of inflammatory [12, 13] and apoptosis [14–16] biomarkers compared with patients without AKI. For instance, there is a 1.5-fold increase in circulating proinflammatory marker interleukin (IL)-6 [13, 16] and apoptosis markers soluble tumor necrosis factor receptor (TNFR)-I and TNFR-II [16] in patients with AKI compared with those without AKI. These concentrations are 5-fold higher than in patients with end-stage renal disease and 10-fold higher than in healthy controls [12]. While these markers have been implicated in susceptibility to AKI [13, 16, 17], and subsequent risk of death in patients with AKI [12], the relationship of these biologic processes to renal recovery following acute illness has not been examined.

We conducted a cohort study entitled Biological Markers of Recovery for the Kidney (BioMaRK) as an ancillary study to the Veterans Affairs (VA)/National Institute of Health (NIH) acute renal failure trial network (ATN) [1] study of patients receiving RRT and examined associations between a set of candidate molecules defined *a priori*, and renal recovery and mortality. Molecules were selected in three implicated pathways: inflammatory [IL-6, IL-8, IL-10, IL-18, IL-1β, macrophage migration inhibitory factor (MIF), tumor necrosis factor (TNF)]; [12, 17–19] apoptosis [TNFR-I, TNFR-II and death receptor (DR)-5]; [15, 20] and growth factors (granulocyte-macrophage colony-stimulating factor [GM-CSF]) [21].

We examined the hypothesis that lower baseline concentrations of plasma inflammatory and apoptosis markers, and higher concentrations of growth factors are associated with renal recovery and survival. Our goal was not to examine biomarker prediction of outcomes, but rather, the relative contribution of each marker to the overall association with the outcomes of interest. In order to examine whether failure to recover kidney function is not confounded by death, we performed sensitivity analyses using five mutually exclusive categories of renal recovery.

MATERIALS AND METHODS

Study design and selection of participants

The BioMaRK study was a multicenter, prospective, nested, observational cohort study conducted as an ancillary study to the VA/NIH ATN clinical trial. The ATN study was a multicenter, randomized clinical trial ($n = 1124$) comparing intensive and less-intensive RRT strategies in critically ill patients and is described in detail elsewhere [1, 22]. As per the primary ATN trial, patients with chronic kidney disease (defined as premorbid serum creatinine >2 mg/dL in men and >1.5 mg/dL in women) or prior kidney transplantation were excluded. The ATN trial found no difference in 60-day mortality and renal recovery between the two RRT strategies. The BioMaRK study included all participants in the ATN study who gave additional written consent to blood collections for sample banking. We obtained approval from the institutional review boards of the University of Pittsburgh and all other participating sites.

Blood sample collection

Blood samples were collected on Day 1 of enrollment in ATN and BioMaRK studies. Since the ATN study protocol allowed for participants to be enrolled in the trial if they had received no more than one session of intermittent hemodialysis or sustained low-efficiency dialysis or received continuous renal-replacement therapy less than 24 h before randomization, 68.5% of participants ($n = 560$) had received RRT at the time of enrollment in BioMaRK. Of participants who were receiving RRT, blood samples were drawn prior to RRT initiation in case of intermittent hemodialysis. Of participants receiving continuous RRT, samples were collected prior to protocolized RRT dosing. Of participants who did not receive any RRT, blood samples were collected before first protocolized RRT initiation. Details of biomarker assays are provided in Supplementary material, Item S1 and intra-assay and inter-assay coefficients of variation for each marker are shown in Supplementary material, Table S1.

Data collection

We prospectively ascertained baseline characteristics including demographic; cause of AKI; other clinical, physiologic and laboratory data at the time of entry into the ATN study. We collected individual comorbid illnesses and assessed comorbidity using the Charlson comorbidity score [23]. Severity of illness was ascertained at enrollment using the acute physiology and chronic health evaluation (APACHE)-II [24], and the Cleveland Clinic intensive care unit acute renal failure score [25]. We defined acute organ dysfunction as a new sequential organ failure assessment score of ≥3 in any of six organ systems [26]. All participants were followed daily until hospital discharge, death or Day 28 after randomization, whichever occurred first.

Outcome ascertainment

Our primary outcomes were renal recovery and mortality at Day 60, and corresponding time to event outcomes. Renal recovery specified *a priori* was defined as being alive and independent from RRT by Day 60 irrespective of the participant's discharge location. Outcomes were ascertained daily during hospitalization, and at Days 28 and 60 using telephone and/or mail follow up. Time to recovery was defined as time to dialysis independence as in ATN study. Survival data on patients who could not be contacted was ascertained using the VA beneficiary identification and records locator system, the National Center for Health Statistics National Death Index database or the Social Security Administration's Death Master File [1].

Statistical analysis

We first performed an outcome-stratified analysis comparing baseline characteristics by renal recovery and mortality. Continuous data were compared using Student's *t*-test or Wilcoxon rank-sum test and categorical data using the χ^2 test or Fisher's exact test. Left-censored biomarker data were imputed using the lower limit of detection. Plasma biomarker data were log-transformed and analyzed in natural logarithm scale. For participants with no record of comorbid condition ($n = 94$), we assumed that the condition was absent. For participants with missing data elements for calculating the APACHE II score ($n = 44$), SOFA score ($n = 201$) and Cleveland CLINIC ICU acute renal failure score ($n = 146$), we imputed the score using regression-based maximum likelihood method as previously published using the same dataset [27].

As our goal was to examine association (and not risk prediction), we built models with individual biomarker concentration and outcome in a cohort of 682 subjects without missing data. We fitted logistic regression models and computed risk-adjusted odds ratios (ORs). We examined the time to renal recovery and mortality using Cox proportional hazards regression and computed risk-adjusted hazard ratios (HRs). For time to renal recovery analyses, time to independence from RRT was modeled only among survivors at Day 60. Participants who became independent of RRT but died before Day 60 were treated as nonrecovery throughout the study period. For all analyses, ORs and HRs were calculated for each natural log-unit increase in biomarker concentration. We plotted Kaplan–Meier failure curves stratified by quartiles of biomarker concentrations and compared differences across quartiles using the log-rank test.

In order to examine the relative contribution of multiple biomarkers, we included markers simultaneously in the regression analysis, while adjusting for all baseline covariates and other biomarkers. For sensitivity analyses, we fitted multinomial logistic regression using all five categories of renal recovery. Statistical analyses were performed using SAS software version 9.3 (SAS Institute, Cary, NC), with statistical significance set at $P < 0.05$.

Sensitivity analysis

To ascertain that the association between individual biomarkers and renal recovery is not confounded by death, we performed sensitivity analyses by varying the definitions of renal recovery and using five mutually exclusive categories that were specified *a priori*. We defined renal recovery as complete; partial; dependent on RRT; death on RRT and death without RRT. Complete recovery was defined as independence from RRT and return of serum creatinine to no more than 150% of baseline level before death or hospital discharge, as proposed by the Acute Dialysis Quality Initiative [28]. Partial renal recovery was defined as independence from RRT with final serum creatinine higher than 150% of baseline level before death or hospital discharge [28].

RESULTS

Characteristics of study participants

Participants in the BioMaRK and ATN cohorts were similar (Supplementary material, Table S2). Of 817 participants, 298 (36.5%) were alive and free of RRT and 415 (50.8%) died by Day 60. Participants who recovered renal function and who survived were younger and had lower comorbid disease burden (Table 1). Among those who recovered renal function, there was a lower prevalence of chronic liver disease; and, among survivors, there was a higher prevalence of immunocompromised state and lower prevalence of chronic hypoxemia compared with nonsurvivors. Participants who had renal recovery and who were survivors were generally less severely ill.

Despite similar premorbid renal function, those who recovered renal function and survivors had higher serum creatinine prior to initiation of RRT. Patients receiving RRT at lower creatinine also tended to be more oliguric (mean serum creatinine in those with and without oliguria, 3.79 ± 1.63 versus 5.01 ± 1.87 mg/dL, $P < 0.001$). Use of mechanical ventilation was higher among those who did not recover and in nonsurvivors. Nephrotoxin exposure was more frequently noted as the cause of AKI in those who had renal recovery. The hospital length of stay was longer among survivors [median(interquartile range, IQR), 33(19–54) versus 9(3–20), $P < 0.001$] and among those who recovered kidney function [30(17–48) versus 13(4–29), $P < 0.001$], the latter primarily due to higher mortality among those who did not recover. Premorbid serum creatinine data were available only in 683 subjects (83.6%). There was no difference in missing premorbid creatinine values between those who did and did not recover (18.8 versus 15%, $P = 0.16$) and survivors and nonsurvivors (16.1 versus 16.6%, $P = 0.84$).

Association of biomarker concentration with renal recovery

Figure 1 shows median biomarker concentrations, stratified by renal recovery and Table 2 shows raw biomarker concentration. Of 11 biomarkers, 3 had moderate to heavy proportion of measurements censored below detection thresholds: IL-1β (62.7%), TNF (63.7%) and GM-CSF (25.3%). At baseline, concentrations of plasma inflammatory (IL-6, IL-8, IL-10, IL-18 and MIF) and apoptosis (TNFR-I, TNFR-II and DR-5) biomarkers were modestly elevated in those who did not recover renal function compared with those who

Table 1. Baseline characteristics of participants stratified by renal recovery and mortality

Characteristic	No (%)					
	Recovery (n = 298)	Nonrecovery (n = 519)	P-value	Survivors (n = 402)	Nonsurvivors (n = 415)	P-value
Age, years, mean (SD)	56 (16.3)	62.9 (14.3)	<0.001	57.3 (15.6)	63.3 (14.7)	<0.001
Male gender	207 (69.5)	360 (69.4)	0.98	274 (68.2)	293 (70.6)	0.45
Race						
White	225 (75.5)	401 (77.3)	0.87	300 (74.6)	326 (78.6)	0.60
Black	46 (15.4)	78 (15)		67 (16.7)	57 (13.7)	
Hispanic	19 (6.4)	30 (5.8)		26 (6.5)	23 (5.5)	
Other	8 (2.7)	10 (1.9)		9 (2.2)	9 (2.2)	
Comorbid condition						
Charlson comorbidity index, mean (SD)[a]	2.2 (2.4)	2.6 (2.4)	0.02	2.3 (2.4)	2.7 (2.5)	0.03
Cardiovascular disease	112 (37.6)	197 (38)	0.92	144 (35.8)	165 (39.8)	0.25
Chronic hypoxia	22 (7.4)	58 (11.2)	0.08	31 (7.7)	49 (11.8)	0.049
Liver disease	23 (7.7)	71 (13.7)	0.01	38 (9.4)	56 (13.5)	0.07
Diabetes	89 (29.9)	149 (28.7)	0.73	124 (30.8)	114 (27.5)	0.29
Malignancy	55 (18.5)	102 (19.6)	0.68	69 (17.2)	88 (21.2)	0.14
Immunocompromised	53 (17.8)	70 (13.5)	0.10	71 (17.7)	52 (12.5)	0.04
Severity of illness						
APACHE-II score, mean (SD)[b]	23.7 (6.8)	27.7 (6.8)	<0.001	23.8 (6.6)	28.5 (6.8)	<0.001
Cleveland Clinic ICU Acute Renal Failure score, mean (SD)[c]	10.8 (3.4)	12.3 (3.1)	<0.001	10.9 (3.3)	12.6 (2.9)	<0.001
SOFA score, mean (SD)[d]	12.3 (3.7)	14.6 (3.7)	<0.001	12.4 (3.7)	15.1 (3.6)	<0.001
Renal function prior to onset of AKI						
Premorbid serum creatinine[e]	1.1 (0.4)	1.1 (0.3)	0.20	1.1 (0.4)	1.1 (0.3)	0.67
Estimated GFR, mL/min/1.73 m^2[e,f]						
≥60	182 (67.9)	312 (62.5)	0.33	238 (64.8)	256 (59.9)	0.56
45–59	55 (20.5)	121 (24.2)		79 (21.5)	97 (24.2)	
30–44	31 (11.6)	66 (13.2)		50 (13.6)	47 (11.8)	
Serum creatinine before RRT initiation	4.5 (2)	3.7 (1.5)	<0.001	4.4 (2)	3.7 (1.5)	<0.001
Presence of oliguria	208 (69.8)	446 (85.9)	<0.001	300 (74.6)	354 (85.3)	<0.001
Use of mechanical ventilation	209 (70.1)	451 (86.9)	<0.001	292 (72.6)	368 (88.7)	<0.001
Diagnosis of sepsis	189 (63.4)	340 (65.5)	0.55	254 (63.2)	275 (66.3)	0.36
Cause of AKI						
Ischemia	235 (78.9)	414 (79.9)	0.72	319 (79.4)	330 (79.7)	0.90
Nephrotoxins	90 (30.2)	108 (20.8)	0.003	116 (28.9)	82 (19.8)	0.003
Sepsis	153 (51.3)	271 (52.3)	0.79	206 (51.2)	218 (52.7)	0.69
Multifactorial causes	159 (53.4)	281 (54.2)	0.81	221 (55)	219 (52.9)	0.55

[a]According to Charlson *et al.* [23] without the age.
[b]Acute physiology and chronic health evaluation includes initial values of 12 routine physiologic measurements, age and previous health status ranging from 1 to 71; An increasing score is closely correlated with the subsequent risk of hospital death [24].
[c]The Cleveland clinic intensive care unit acute renal failure score can range from 1 to 20, with higher scores predictive of increased risk of death [25].
[d]Sequential Organ Failure Assessment score includes six organ systems with scores ranging from 0 to 4 for each organ system (with the renal system score included in the calculation of the total SOFA score); higher scores indicate more severe organ dysfunction [26].
[e]Premorbid serum creatinine was available only in 683 of the 817 (83.6%) subjects.
[f]Estimated GFR was calculated using four-variable Modification of Diet in Renal Disease estimation equation [29].
SD, standard deviation; ICU, intensive care unit; APACHE, Acute Physiology and Chronic Health Evaluation; SOFA, Sequential Organ Failure Assessment; RRT, renal replacement therapy; GFR, glomerular filtration rate; AKI, acute kidney injury.

recovered. We found no difference in biomarker concentration between those who did and did not receive RRT within the previous 24 h prior to enrollment in BioMaRK (Supplementary material, Table S3).

Supplementary material, Table S4 shows the unadjusted and Table 3 shows the adjusted ORs (AORs) and adjusted hazard ratios (AHRs) for association between individual biomarker concentration and renal recovery, and time to renal recovery. When adjusted for differences in age; sex; race; Charlson comorbidity score without age; premorbid serum creatinine; history of chronic hypoxemia, liver disease and immunocompromised state; nephrotoxin exposure; diagnosis of sepsis; presence of oliguria prior to RRT; use of mechanical ventilation, APACHE-II score and intensity of RRT, each natural log unit increase in plasma IL-8 [AOR, 0.74, 95% confidence interval (95% CI) 0.65–0.85], IL-18 (AOR, 0.82, 95% CI 0.70–0.96), MIF (AOR, 0.86, 95%CI 0.76–0.97) and TNFR-I (AOR, 0.62, 95% CI 0.46–0.84) concentrations were associated with lower odds of renal recovery (Table 3).

Figure 2 shows Kaplan–Meier failure plots for time to renal recovery stratified by quartiles of biomarker concentration. Higher biomarker concentrations were associated with lower probability of renal recovery for plasma inflammatory (IL-8 and IL-18) and apoptosis (TNFR-I) markers. From Cox models, we found that higher concentrations of plasma IL-8 (AHR, 0.81, 95% CI 0.73–0.89), IL-18 (AHR, 0.87, 95% CI 0.78–0.97) and TNFR-I (AHR, 0.70, 95% CI 0.60–0.82) were associated with increased time to renal recovery (Table 3).

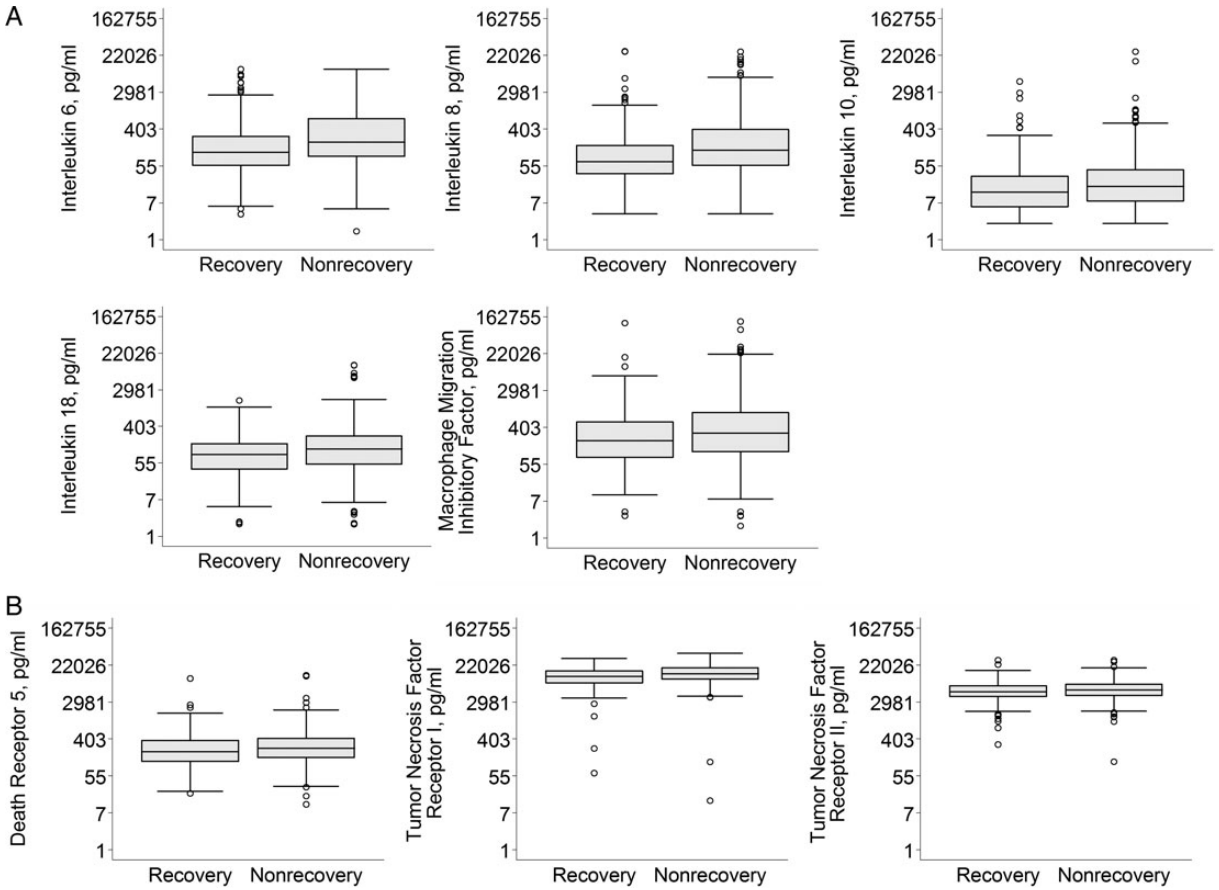

FIGURE 1: Plasma inflammatory and apoptosis biomarker concentrations in subjects receiving RRT, stratified by renal recovery. Boxplot summaries of plasma inflammatory (**A**) and apoptosis (**B**) biomarker concentrations are displayed in natural logarithm scale and labeled with their corresponding biomarker concentration in picograms/milliliter. The vertical box represents the 25th percentile (bottom line), median (middle line) and 75th percentile (top line) values. The lowest datum (lower whisker) represents 1.5 times the interquartile range of the lower quartile and the highest datum (upper whisker) represent 1.5 times the interquartile range of the upper quartile. The open circles represent the outliers. *P < 0.05.

Table 2. Biomarker concentration stratified by renal recovery and mortality

Biomarker	Median (IQR)			
	Recovery	Nonrecovery	Survivors	Nonsurvivors
Inflammation				
IL-6	114 (56.6–272)	197 (91.6–710)**	124 (56.6–280)	230 (103–911)**
IL-8	69.5 (36–166)	128 (57.3–393)**	71.5 (35.8–164)	146 (63–505)**
IL-10	13.3 (6–31.4)	17.9 (8.2–44.3)**	13.4 (6.2–29.5)	19.2 (8.5–56)**
IL-18	87.9 (39.1–157)	118 (50.7–238)**	86.6 (39.3–155)	127 (52.7–254)**
IL-1β[a]	22.7 (22.7–34.3)	22.7 (22.7–45.7)	22.7 (22.7–32.6)	22.7 (22.7–47.9)**
MIF	196 (78.8–540)	298 (106–891)**	184 (70.7–531)	337 (139–1003)**
TNF[a]	2.4 (2.4–3.7)	2.4 (2.4–3.4)	2.4 (2.4–3.6)	2.4 (2.4–3.5)
Apoptosis				
TNFR-I	11 919 (8294–15 958)	13 698 (10 167–18 805)**	12 173 (8630–16 247)	13 779 (10 312–19 147)**
TNFR-II	5179 (3939–7194)	5700 (4254–7775)*	5285 (4005–7217)	5729 (4300–8084)*
DR-5	201 (120–370)	240 (147–417)**	200 (120–357)	261 (154–448)**
Growth factor				
GM-CSF[a]	8.5 (3.1–20.6)	8.8 (3.1–20)	7.9 (3.1–19)	9.4 (3.9–21.6)

[a]25.3% of GM-CSF values, 62.7% of IL-1β and 63.7% of TNF values were below the detection thresholds and were censored.
*P < 0.05; **P < 0.01.
IQR, interquartile range. Median biomarker concentrations are expressed in picograms/milliliter. All biomarkers were measured in 817 subjects except DR-5, which was measured in 816 subjects.

Table 3. Association between individual biomarker concentration and renal recovery and mortality

Biomarker	(95% CI)			
	Renal recovery		Mortality	
	OR for renal recovery	HR for time to renal recovery	OR for death	HR for time to death
Inflammation				
IL-6	0.91 (0.81–1.01)	0.93 (0.86–1.01)	1.19 (1.07–1.32)*	1.19 (1.12–1.27)*
IL-8	0.74 (0.65–0.85)*	0.81 (0.73–0.89)*	1.50 (1.32–1.71)*	1.36 (1.27–1.46)*
IL-10	0.88 (0.77–1.01)	0.92 (0.83–1.03)	1.30 (1.14–1.49)*	1.24 (1.16–1.33)*
IL-18	0.82 (0.70–0.96)***	0.87 (0.78–0.97)***	1.35 (1.16–1.58)*	1.27 (1.15–1.40)*
MIF	0.86 (0.76–0.97)***	0.92 (0.85–1.01)	1.37 (1.22–1.54)*	1.26 (1.17–1.35)*
Apoptosis				
TNFR-I	0.62 (0.46–0.84)**	0.70 (0.60–0.82)*	1.61 (1.16–2.24)**	1.47 (1.16–1.86)**
TNFR-II	0.88 (0.64–1.23)	0.90 (0.70–1.16)	1.17 (0.85–1.60)	1.16 (0.95–1.43)
DR-5	0.91 (0.74–1.12)	0.91 (0.78–1.06)	1.36 (1.10–1.67)**	1.24 (1.09–1.41)$

Multivariable logistic regression models with corresponding OR were constructed to examine association between each individual biomarker concentration (per natural log unit) and renal recovery and mortality. All models were adjusted for differences in age; race; sex; Charlson comorbidity score without age; history of chronic hypoxemia, liver disease and immunocompromised state; premorbid serum creatinine; nephrotoxic cause of AKI; diagnosis of sepsis; presence of oliguria at initiation of RRT; use of mechanical ventilation; APACHE-II score, and intensity of RRT. Models were not adjusted for other biomarkers.

For the recovery model, an OR of >1 indicates that higher biomarker concentration is associated with increased renal recovery, and an OR <1 indicates nonrecovery. For the mortality model, OR >1 indicates that higher biomarker concentration is associated with increased mortality and OR <1 indicates lower mortality.

Multivariable Cox models with corresponding HR were constructed to examine association between individual biomarker concentration and time to event outcomes. Models were adjusted for baseline covariates as above. For time to renal recovery model, a HR >1 indicates that higher marker concentration is associated with faster recovery and <1 indicates slower recovery. For time to death, a HR of >1 indicates that higher marker concentration is associated with shorter time to death and <1 indicates longer time to death. The models included 682 subjects due to missing premorbid creatinine data in 134 subjects and DR-5 marker levels in 1 subject. Models were not constructed for IL-1 β, TNF, and GM-CSF due to high censoring of biomarker data.

*P < 0.001; **P < 0.01; ***P < 0.05; $P = 0.001.

IL, interleukin; MIF, macrophage migration inhibitory factor; TNFR, tumor necrosis factor receptor; DR, death receptor.

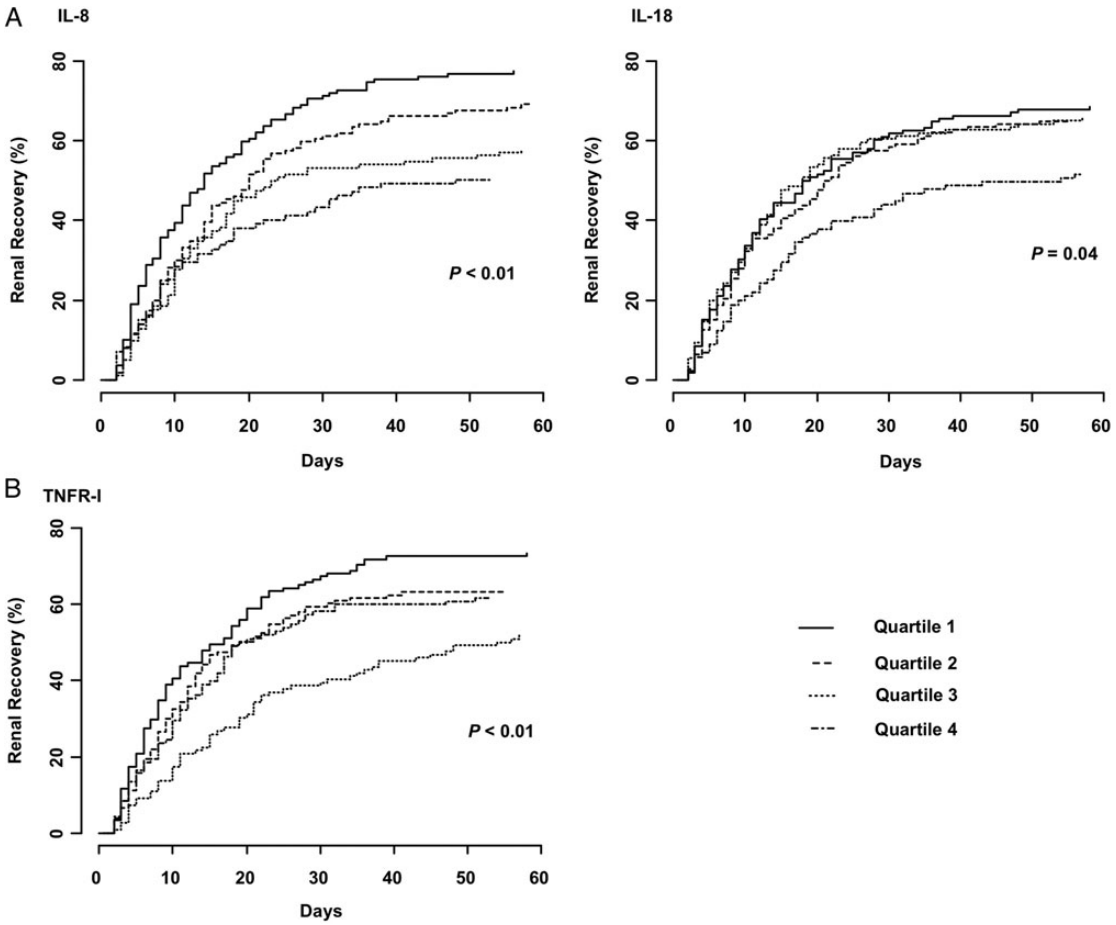

FIGURE 2: Kaplan–Meier failure plots showing time to renal recovery stratified by quartiles of plasma inflammatory (**A**) and apoptosis (**B**) biomarker concentrations. Markers were compared across quartiles for trend using a log-rank test for ordered survival curves. Higher plasma IL-8, IL-18 and TNFR-I concentrations are associated with slower renal recovery.

ORIGINAL ARTICLE

Association of biomarker concentration with mortality

Plasma inflammatory (IL-6, IL-8, IL-10, IL-18 and MIF) and apoptosis (TNFR-I, TNFR-II and DR-5) biomarkers were modestly elevated in nonsurvivors compared with survivors (Figure 3). When adjusted for differences in age; sex; race; Charlson comorbidity score; premorbid creatinine; history of chronic hypoxemia, liver disease and immunocompromised state; nephrotoxin exposure; diagnosis of sepsis; oliguria prior to RRT; use of mechanical ventilation, APACHE-II score, and intensity of RRT, per natural log increase in plasma inflammatory (IL-6, IL-8, IL-10, IL-18 and MIF: AOR range for all markers, 1.19–1.50) and apoptosis marker (DR-5: AOR, 1.36, 95% CI 1.10–1.67; and TNFR-I: AOR, 1.61, 95% CI 1.16–2.24) concentrations were associated with increased mortality (Table 3).

Per quartile increase in plasma inflammatory (IL-6, IL-8, IL-18 and MIF) and apoptosis (TNFR-I) marker concentrations were associated with decreased time to death (Figure 4). Using a Cox model after adjustment for baseline covariates, higher plasma inflammatory marker (IL-6, IL-8, IL-10, IL-18 and MIF: AHR range, 1.19–1.36) and apoptosis marker concentrations (TNFR-I: AHR, 1.47, 95% CI 1.16–1.86; and DR-5: AHR, 1.24, 95% CI 1.09–1.41) were associated with an increased hazard of death (Table 3).

Multimarker models of renal recovery and mortality

Table 4 shows the multivariable analyses of multiple makers to examine whether specific markers are independently associated with clinical outcomes. When adjusted for differences in baseline covariates, higher plasma concentrations of IL-8 (AHR, 0.80, 95% CI 0.70–0.91, $P < 0.001$) and TNFR-I (AHR, 0.63, 95% CI 0.50–0.79, $P < 0.001$) were associated with lower probability of recovery of kidney function. Higher plasma concentrations of IL-8, MIF and TNFR-I (AHR, 1.26, 1.18 and 1.26, respectively) were associated with increased risk of death.

Sensitivity analyses

Of the 817 participants, 217 (26.5%) had complete and 81 (9.9%) had partial renal recovery; 107 (13.1%) were RRT dependent; 327 (39.9%) died on RRT and 87 (10.6%) died without RRT. Compared with complete recovery (as the reference category), higher plasma IL-6 was associated with lower odds of partial renal recovery (OR, 0.79, 95% CI 0.66–0.95) (Supplementary material, Table S5). We also found that higher plasma inflammatory (IL-6, IL-8, IL-10, IL-18 and MIF: OR range, 1.26–1.45) and apoptosis markers (TNFR- I, TNFR-II and DR-5: OR range, 1.26–2.02) were associated

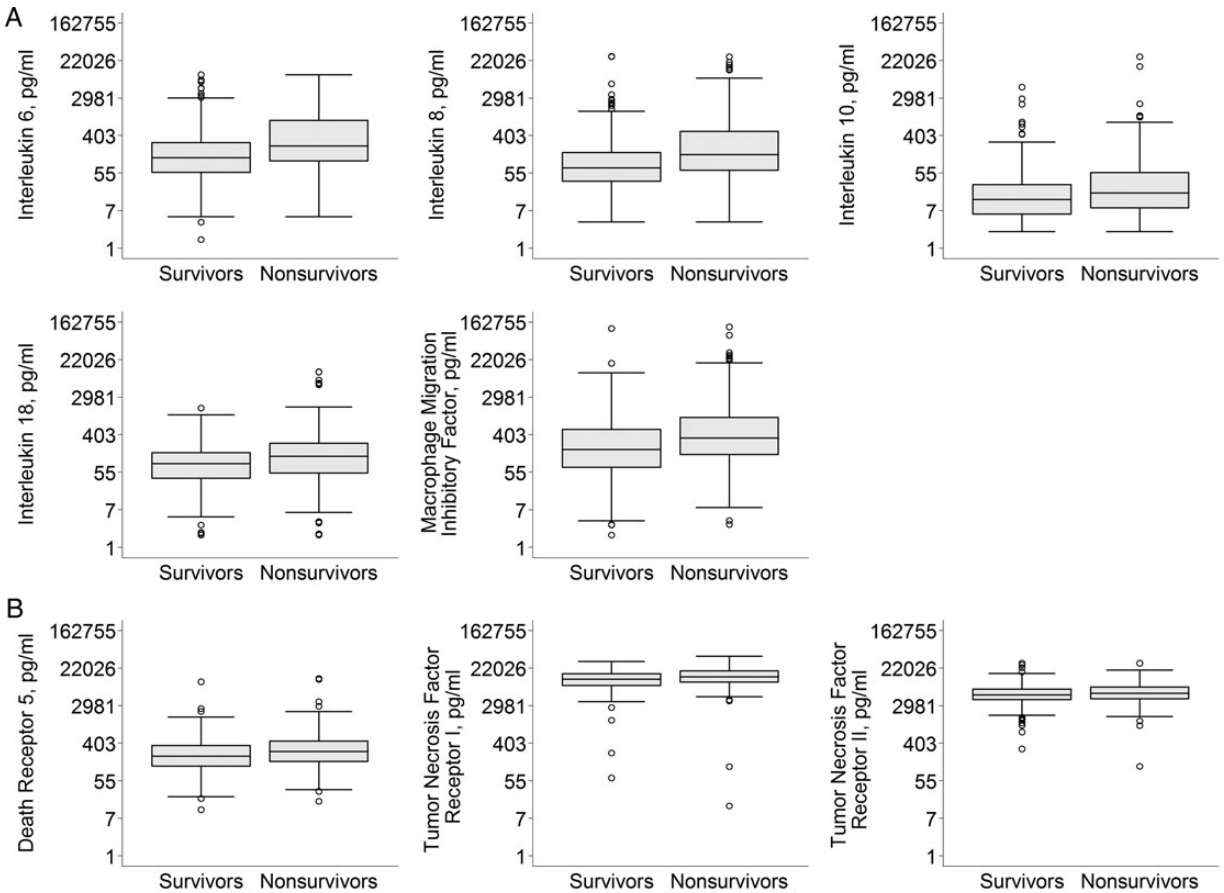

FIGURE 3: Plasma inflammatory and apoptosis biomarker concentrations stratified by 60-day mortality. Boxplot summaries of plasma inflammatory (**A**) and apoptosis (**B**), biomarker concentrations are displayed in natural logarithm scale and labeled with their corresponding biomarker concentration in picograms/milliliter. The vertical box represents the 25th percentile (bottom line), median (middle line) and 75th percentile (top line) values. The lowest datum (lower whisker) represents 1.5 times the interquartile range of the lower quartile and the highest datum (upper whisker) represents 1.5 times the interquartile range of the upper quartile. The open circles represent the outliers. *$P < 0.05$.

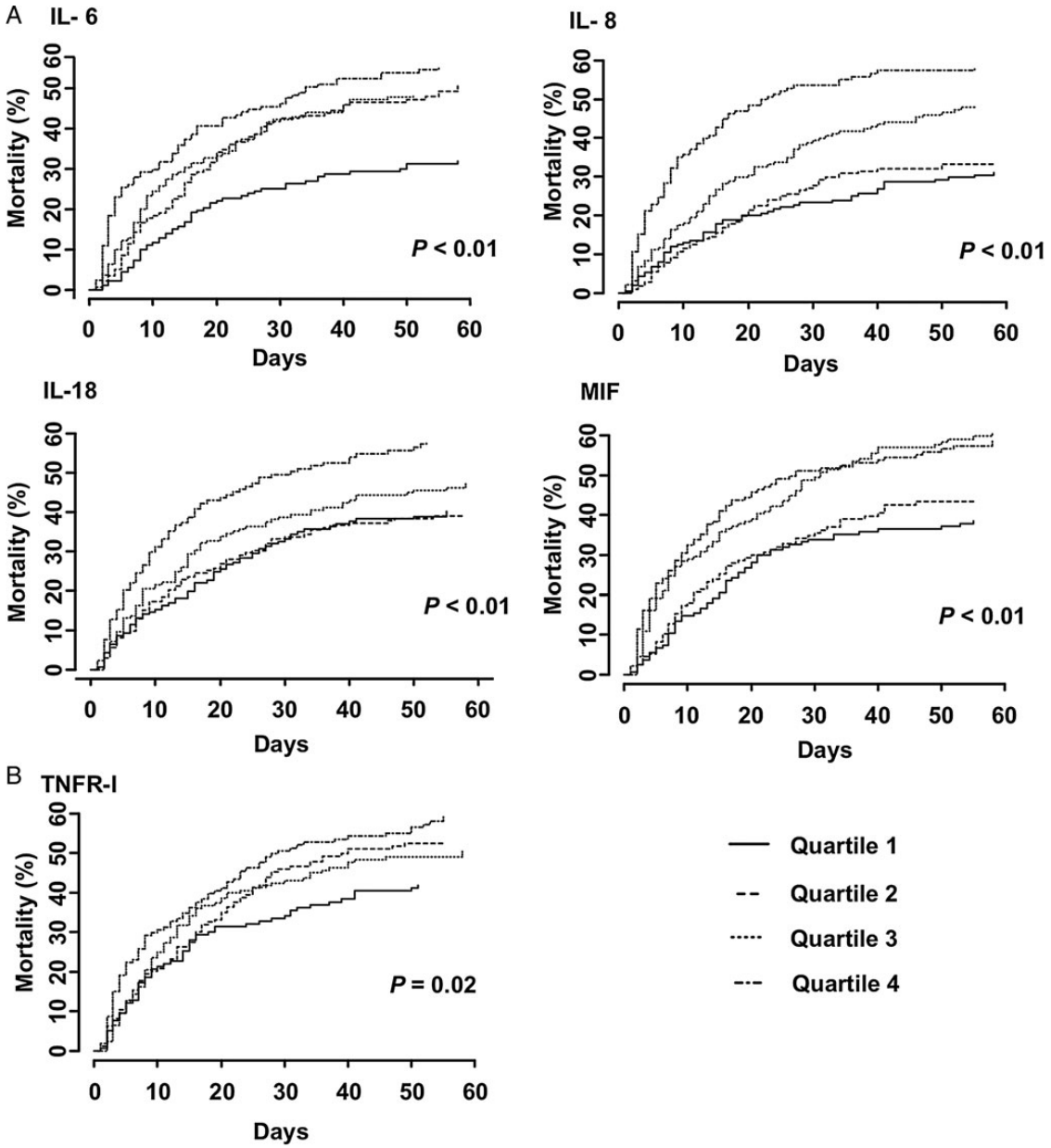

FIGURE 4: Kaplan–Meier failure plots showing time to death stratified by quartiles of plasma inflammatory (**A**) and apoptosis (**B**) biomarker concentrations. Markers were compared across quartiles for trend using a log-rank test for ordered survival curves. Higher plasma IL-6, IL-8, IL-18, MIF and TNFR-I concentrations are associated with faster time to death.

with increased risk of RRT dependence. While higher concentrations of plasma IL-6 (OR, 1.17, 95% CI 10.1–1.36) and IL-8 (OR, 1.34, 95% CI 1.12–1.59) were associated with increased risk of death on RRT, increased concentrations of MIF were associated with lower risk of death without RRT (OR, 0.81, 95% CI 0.69–0.95). Subgroup analyses of biomarker concentrations by etiology of AKI did not change our results (data not shown).

DISCUSSION

Among critically ill individuals with AKI requiring RRT, we found increased circulating concentrations of plasma inflammatory and apoptotic biomarkers at initiation of renal support in those who subsequently did not recover kidney function and among those who died. When adjusted for differences in participant characteristics and severity of illness, higher plasma concentrations of IL-8, IL-18 and TNFR-I were associated with subsequent RRT dependence, while plasma IL-6, IL-8, IL-10, IL-18, MIF, TNFR-I and DR-5 were associated with increased risk of death. When adjusted for baseline characteristics and 'other markers', plasma IL-8 and TNFR-I were independent predictors of RRT dependence and plasma IL-8, MIF and TNFR-I were predictors of mortality. This study, to the best of our knowledge, is the first large-scale investigation of the association between immune and apoptosis biomarkers and the patient-centered outcomes of

Table 4. Multivariable analyses of independent effects of multiple biomarkers on clinical outcomes

Biomarker	Adjusted HR (95% CI)	P-value
Renal recovery		
IL-8	0.80 (0.70–0.91)	<0.001
TNFR-I	0.63 (0.50–0.79)	<0.001
Mortality		
IL-8	1.26 (1.14–1.39)	<0.001
MIF	1.18 (1.08–1.28)	<0.001
TNFR-I	1.26 (1.02–1.56)	0.03

Only significant results for time to renal recovery and mortality from Cox models are shown. All models were adjusted for differences in age; race; sex; Charlson comorbidity score without age; history of chronic hypoxemia, liver disease and immunocompromised state; premorbid serum creatinine; nephrotoxic cause of AKI; diagnosis of sepsis; presence of oliguria at initiation of RRT; use of mechanical ventilation, intensity of RRT and other biomarkers. Models were not adjusted for APACHE II score due to multicolinearity between APACHE II score and biomarkers.
For time to renal recovery model, a HR >1 indicates that per natural log increase in biomarker concentration is associated with faster recovery and <1 indicates slower recovery. For time to death, a HR of >1 indicates that per natural log increase in biomarker concentration is associated with shorter time to death and <1 indicates longer time to death.
The models included 682 subjects due to missing premorbid creatinine data in 134 subjects and DR-5 marker levels in 1 subject. Models were not constructed for IL-1β, TNF and GM-CSF due to high censoring of biomarker data.
IL, interleukin; MIF, macrophage migration inhibitory factor; TNFR-I, tumor necrosis factor receptor-I.

independence from RRT and mortality in patients with AKI. The results of our study have important implications for designing interventions to enhance renal recovery and to lower mortality in patients with AKI.

Higher cytokine concentrations observed at baseline in patients ultimately dependent on RRT and nonsurvivors could be due to elevations before occurrence of acute illness due to chronic health conditions, or due to an interaction between poor chronic health status and acute illness. We speculate that the latter is likely because circulating cytokine concentrations were lower than we observed in prior studies, even in individuals with end-stage renal disease [12, 30]. Our findings were not confounded by severity of illness because the association between increased biomarker concentration and RRT dependence persisted after adjusting for APACHE score. The significant association between various biomarkers and RRT dependence in the sensitivity analysis also suggests that our findings were not confounded by death. Furthermore, the magnitude of association and the dose–response relationship (Figures 2 and 4) between individual biomarkers and outcomes suggest that these markers are unlikely to be just surrogates or an epiphenomenon in severe illness.

In our study, the risk of RRT dependence and death appeared to be greatest for increased IL-8 concentrations. IL-8, a chemokine, is an important mediator of innate and adaptive immunity and has been implicated in the pathogenesis of AKI [31–33]. Higher IL-8 concentrations may reflect a persistent proinflammatory milieu among renal tubular cells impairing renal recovery. IL-18 is produced by macrophages and other cell types present in the kidney during ischemia–reperfusion injury [34]. Higher serum IL-18 concentrations have been associated with hospital mortality in patients receiving RRT [35].

IL-6 is a pleiotropic cytokine and higher concentrations have been associated with increased susceptibility [13] and mortality in patients with AKI [12]. The cytokine, MIF, exerts a variety of biologic responses including macrophage activation, enhancement of adherence and phagocytosis, and higher MIF concentrations have been associated with severe AKI [36]. Stimulation of Fas/TNFR receptor family by ligands triggers cell apoptosis. Higher concentrations of TNFR have been associated with susceptibility to AKI and mortality [15].

Our data extend the findings of other studies in which pro- and anti-inflammatory cytokines are associated with poor outcomes [12, 16, 37]. As several molecules were associated with adverse outcomes in our study, immunomodulation strategies that include inhibition of single molecules are unlikely to be successful and that broad-spectrum modulation of multiple molecules is essential to improve outcomes. Furthermore, since associations persisted after adjusting for baseline clinical variables, patient selection for immune modulation therapies will require monitoring of biomarker concentrations.

Our study has several important limitations. Firstly, being an observational study, our findings are hypotheses generating and cannot prove cause and effect between biomarkers and adverse outcomes. Secondly, we did not examine biomarker concentrations prior to onset of severe illness for practical reasons. Larger differences in immune response may have occurred during this period, which might have increased susceptibility to critical illness, which may represent a surrogate for unknown underlying illness. Thirdly, we did not examine local (i.e. renal tissue or urinary) concentrations of these markers, which could have been different from serum levels. Fourthly, three of the biomarkers studied were below the detection thresholds and were thus censored confounding interpretation. Finally, we made a conscious decision not to adjust any of our analyses for multiple comparisons—as recommended in studies of natural phenomena like ours [38]. We recognize that this approach increases risk of type I error for null associations but at the same time decreases the risk of type II error for those associations that are not null.

Our study has several major strengths. Firstly, being a large multicenter prospective cohort study, our finding of association between high concentrations of immune and apoptosis markers and adverse outcomes in critically ill patients receiving RRT is highly generalizable. Secondly, we measured biomarkers that have been implicated in renal injury and recovery and thus able to gain insight on mechanisms and long-term outcomes. Thirdly, we were able to compare the marker concentrations in patients who are at risk for nonrecovery unlike other case–control studies that compared markers in critically ill patients with AKI to that of healthy controls or end-stage renal disease [12]. Finally, by varying definitions of renal recovery, we were able to examine the influence of biomarkers on various degrees of clinically meaningful outcomes.

In summary, our results show that in critically ill patients receiving RRT, elevated concentrations of inflammatory and apoptosis biomarkers are associated with nonrecovery of kidney function and continued dependence on RRT and risk of death. Future studies should examine whether broad-spectrum immune modulation of inflammatory and apoptosis markers improves outcomes. The importance of the chemokine IL-8, as a marker of nonrecovery and death, is also notable.

SUPPLEMENTARY DATA

Supplementary data are available online at http://ndt.oxfordjournals.org.

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CONFLICT OF INTEREST STATEMENT

None declared. The content of this paper is solely the responsibility of the authors and does not necessarily represent the official views of NIDDK or NIH.

(See related article by Ronco and Bonventre. Stigmata of death: for kidneys and patients. *Nephrol Dial Transplant* 2014; 29: 1797–1798.)

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ORIGINAL ARTICLE

Inflammatory stress reduces the effectiveness of statins in the kidney by disrupting HMGCoA reductase feedback regulation

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ABSTRACT

Background. Patients with chronic kidney disease (CKD) are unlikely to gain the same benefit from conventional doses of statins as do patients with cardiovascular disease alone. This study investigated whether inflammation accompanying CKD causes statin resistance.

Methods. Inflammatory stress was induced by adding cytokines and lipopolysaccharide (LPS) to human mesangial cells (HMCs) *in vitro*, and *in vivo* by subcutaneous casein injection in apolipoprotein E, scavenger receptors class A and CD36 triple knockout mice.

Results. Inflammatory stress exacerbated cholesterol accumulation and was accompanied *in vitro* and *in vivo* by increased HMGCoA reductase (HMGCoA-R) mRNA and protein expression mediated via activation of the sterol regulatory element-binding protein cleavage-activating protein (SCAP)/sterol regulatory element-binding protein 2 pathway. Atorvastatin reduced HMGCoA-R enzymatic activity and intracellular cholesterol synthesis *in vitro*; however, inflammatory stress weakened these suppressive effects. Atorvastatin at concentrations of 15 μM inhibited HMGCoA-R activity by 50% (IC_{50}) in HMCs, but the same concentration in the presence of interleukin (IL)-1β resulted in only 30% inhibition of HMGCoA-R

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