Oxidative Stress Is Increased in Critically Ill Patients with Acute Renal Failure

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Abstract. Patients with acute renal failure (ARF) experience a high mortality rate. Dysregulated inflammation and altered metabolism may increase oxidative stress in ARF patients. Thirty-eight patients who met the Program to Improve Care in Acute Renal Disease (PICARD) Study inclusion criteria underwent plasma protein oxidation and plasma cytokine measurements. For comparison, similar measurements were also performed in 21 critically ill patients without ARF, 28 patients with ESRD, and 49 healthy subjects. Plasma protein thiol oxidation was measured by spectrophotometry. Plasma protein carbonyl content and cytokine concentrations were measured by ELISA. Plasma protein thiol oxidation and carbonyl content were markedly different in ARF patients compared with healthy subjects, ESRD patients, and critically ill patients (P < 0.001 in all cases). There were significant but less marked differences in plasma protein oxidation between ESRD patients and critically ill patients compared with healthy subjects. Plasma protein thiol oxidation in ARF patients improved with dialysis (P < 0.001); however, there was significant plasma oxidant reaccumulation during the interdialytic period (P < 0.001) not due to rebound equilibration of compartmentalized solutes. Plasma proinflammatory cytokine levels were significantly higher (P < 0.05) in ARF patients and critically ill patients than in healthy subjects. Plasma protein oxidation is markedly increased in ARF patients compared with healthy subjects, ESRD patients, and critically ill patients. Increased oxidative stress may be an important target for nutritional and pharmacologic therapy in ARF patients.

Critically ill patients who develop acute renal failure (ARF) experience a high mortality rate that is not entirely explained by sepsis, advanced age, or underlying comorbid conditions (1–5). Recent studies emphasize that the development of ARF makes an important independent contribution to risk-adjusted mortality, whether the cause is radiocontrast toxicity, antibiotic toxicity, or after cardiac surgery (6–8). Metabolic derangements related to hypercatabolism, dysregulated inflammation, and multiple organ system failure are highly prevalent in this patient population, and decreased renal clearance of byproducts of inflammation and hypercatabolism may exacerbate these systemic disorders. At present, no effective pharmacologic or metabolic strategies have been demonstrated to reduce the mortality in this group of patients (4).

A likely consequence of the dysregulated inflammatory response in patients with ARF is an increase in oxidative stress. In inflammatory disorders, stimulated phagocytic cells, in addition to producing excess cytokines, are major producers of reactive oxygen species. Renal failure itself is now recognized as an additional stimulus for increased oxidative stress (9,10). Furthermore, an increase in oxidative stress is considered an important pathogenic mechanism in the development of ischemic and toxic renal tubular injury (11–15). However, few clinical studies have examined the prevalence of oxidative stress or the effects of dialysis on oxidative stress parameters in patients with ARF, and none has included control groups to address comparisons between effects of critical illness and renal failure (16). We hypothesized that critically ill ARF patients have excess oxidation of plasma protein, an excellent in vivo biomarker of oxidative stress status (17), compared with healthy subjects, ESRD patients, and critically ill patients with normal renal function. We further hypothesized that hemodialysis therapy would improve the redox status of critically ill patients with ARF. To test these hypotheses, we compared measurements of plasma protein oxidation in patients with ARF, healthy subjects, critically ill patients, and ESRD patients.

Materials and Methods

Patients

Critically Ill Patients with ARF. The Program to Improve Care in Acute Renal Disease (PICARD) Study is a five-center prospective cohort study investigating potential treatable causes of excess morbidity and mortality in critically ill patients with ARF. The characteristics of the PICARD population as well as the inclusion and exclusion criteria have been published elsewhere (18,19). For the present subset study, 38 patients who met PICARD inclusion criteria...
at the Maine Medical Center underwent measurement of plasma protein oxidation as well as plasma cytokine measurements. In 14 (37%) of 38 cases, plasma samples were obtained within 24 h of consultation, and in 28 (74%) of the cases, plasma samples were obtained within 72 h of consultation. At the time of study enrollment, duration of ARF had been 7.5 ± 2.0 d, as calculated by the number of days elapsed between the day when patients met PICARD inclusion criteria and the day when blood was drawn. On the day when plasma samples were obtained, all patients were evaluated with the Simplified Acute Physiology Score (SAPS) II and Sequential Organ System Failure (SOFA) as described previously (20,21). Sepsis was defined by ACCP/SCCM guidelines (22).

Demographic and biochemical characteristics are shown in Table 1. Twenty-seven of these patients were kept on mechanical ventilation during the study period. Lung Injury Scores (LIS) were calculated by the formula (arterial oxygen pressure + fraction of inspired oxygen + positive end expiratory pressure/3) and are included in Table 1. None of the patients were receiving drugs that are known to interfere with the oxidative stress bioassays used in this study during their intensive care unit (ICU) stay, including mesna and N-acetyl cysteine.

Twenty-seven of these patients received dialysis during their ICU stay. The timing of initiation of dialysis and the dialysis prescription were at the discretion of the attending nephrologist. All hemodialysis treatments were performed with a dual lumen polyurethane dialysis catheter placed percutaneously in a central vein and with a high-flux biocompatible membrane using bicarbonate-based dialyse.

**Critically Ill Patients without ARF.** For evaluating the effects of uremia on the study outcomes, 21 critically ill patients without ARF served as control subjects. Identification of the absence of ARF was done by serum creatinine and urine output. With aims to study independent groups and maintain a 1:2 ratio of controls to experiments, we attempted to recruit a minimum of 19 patients. Indeed, a total of 21 patients met the inclusion criteria during the enrollment period and were included for comparison. Demographic and biochemical characteristics are shown in Table 1. Fifteen of these patients were kept on mechanical ventilation during the study period. LIS are included in Table 1.

**ESRD.** For further clarifying the influence of uremia in the study outcomes, 28 patients with ESRD were included in the statistical analyses for comparisons. Inclusion criteria consisted of patients who were aged 18 to 80 and receiving maintenance hemodialysis therapy. Exclusion criteria consisted of inability to give informed consent and presence of overt infection or known metastatic cancer, and demographics are shown in Table 1. In general, they were adequately dialyzed (urea reduction ratio, 75.9 ± 1.3%) and nourished (serum albumin, 3.7 ± 0.1 g/dl), with high levels of acute-phase inflammation (C-reactive protein, 29.2 ± 11.3 mg/L).

**Healthy Subjects.** A group of 49 healthy subjects were used for comparison of biomarkers of oxidative stress status and inflammation. Healthy subjects were obtained from health care facility employees and a local geriatric primary care practice. The mean age of healthy subjects was 52.7 ± 2.6 yr (range, 22–93)._41_% were male, and 92% were white. Demographic and biochemical characteristics are shown in Table 1. The study was approved by the Institutional Review Board, and informed consent was obtained from all study participants or the next-of-kin.

**Blood Sampling**

Blood sampling occurred 9.1 ± 2.3 and 7.7 ± 1.9 d from admission in the ICU, in ARF and critically ill patients, respectively. Blood was drawn into Vacutainer serum separator tubes that contained clot

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**Table 1.** Patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>ARF</th>
<th>Critically Ill</th>
<th>ESRD</th>
<th>Healthy</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>38</td>
<td>21</td>
<td>28</td>
<td>49</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>69.3 ± 2.1</td>
<td>60.5 ± 0.8</td>
<td>61.4 ± 3.0</td>
<td>52.7 ± 2.6</td>
</tr>
<tr>
<td>Gender (% male)</td>
<td>55</td>
<td>57</td>
<td>48</td>
<td>41</td>
</tr>
<tr>
<td>Race (% white)</td>
<td>97</td>
<td>100</td>
<td>83</td>
<td>92</td>
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<tr>
<td>Sepsis (%)</td>
<td>18</td>
<td>29</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>SOFA</td>
<td>8.6 ± 0.7 (2–16)</td>
<td>5.7 ± 0.6 (1–12)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>SAPS II</td>
<td>44.9 ± 2.3 (22–85)</td>
<td>43.0 ± 2.3 (22–63)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>SAPS IIb</td>
<td>31.9 ± 2.2 (6–69)</td>
<td>32.2 ± 2.1 (6–51)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>LIS</td>
<td>33.9 ± 2.1</td>
<td>31.7 ± 2.5</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>55</td>
<td>24</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>70 ± 6.4a</td>
<td>20 ± 1.9</td>
<td>59 ± 3.7</td>
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<tr>
<td>Creatinine (mg/dl)</td>
<td>3.6 ± 0.2c</td>
<td>0.7 ± 0.1</td>
<td>8.2 ± 0.7</td>
<td>N/A</td>
</tr>
<tr>
<td>Phosphorus (mg/dl)</td>
<td>5.8 ± 0.4c</td>
<td>3.1 ± 0.3</td>
<td>6.3 ± 0.4</td>
<td>N/A</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>2.6 ± 0.1</td>
<td>2.7 ± 0.1</td>
<td>3.78 ± 0.6</td>
<td>N/A</td>
</tr>
<tr>
<td>Prealbumin (mg/dl)</td>
<td>14.3 ± 2.0</td>
<td>N/A</td>
<td>32.2 ± 2.0</td>
<td>N/A</td>
</tr>
<tr>
<td>Transferrin (mg/dl)</td>
<td>148 ± 11</td>
<td>N/A</td>
<td>179 ± 7</td>
<td>N/A</td>
</tr>
<tr>
<td>Total bilirubin (mg/dl)</td>
<td>2.0 ± 0.5</td>
<td>1.4 ± 0.4</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>10.5 ± 0.2</td>
<td>10.4 ± 0.3</td>
<td>11.8 ± 0.2</td>
<td>N/A</td>
</tr>
<tr>
<td>WBC (thou/μl)</td>
<td>13.6 ± 1.0</td>
<td>14.0 ± 1.5</td>
<td>7.6 ± 0.5</td>
<td>N/A</td>
</tr>
</tbody>
</table>

* BUN, blood urea nitrogen; NA, not applicable; N/A, not available; SOFA, sequential organ failure assessment; SAPS, simplified acute physiologic score; LIS, lung injury score; WBC, white blood cells.
* Excluding points related to age. Data are expressed as means ± SEM, with ranges in parenthesis.
* Significant difference compared with critically ill.
activator. Tubes were kept at room temperature and centrifuged within 1 h of blood draw. For plasma measurements, blood was drawn into Vacutainer tubes that contained EDTA. Tubes were placed on ice and centrifuged within 1 h. Plasma and serum samples were stored at −70°C until analysis.

**Plasma Protein Thiol Oxidation**

Thiol groups were assayed according to the method of Ellman (23) as modified by Hu et al. (24) as we have described. Briefly, 1 ml of buffer that contained 0.1 M Tris and 10 mM EDTA (pH 8.2) was added to cuvettes, followed by 50 μl of sample plasma and 50 μl of DTNB reagent (10 mM 5′5′dithio-bis[2-nitrobenzoic acid] in methanol). Blanks were run for each sample, prepared as above, with the exception that there was no DTNB in the methanol. Samples, blanks, and reagent blanks were run in duplicate. After incubation for 15 min at room temperature, sample absorbance was read at 412 nm on a Lambda 2 spectrophotometer (Perkin Elmer, Norwalk, CT). After the absorbances of the sample blank and reagent blank were subtracted from the absorbance for the sample, the concentration of thiol groups was calculated using the TNB molar extinction coefficient of 14,100 M/cm, and results are reported as micromoles per liter. The interassay and intra-assay coefficients of variation for this assay were 4 and 2%, respectively.

**ELISA for Protein-Associated Carbonyl Groups**

Carbonyl groups were measured using the Zentech PC Test (Protein Carbonyl Enzyme Immunoassay Kit) from Zenith Technology (Dunedin, New Zealand). This kit follows the method outlined by Buss et al. (25) [as amended by Winterbourn and Buss (26)], which uses derivatization of protein carbonyls in samples and oxidized protein standards with dinitrophenylhydrazine, followed by ELISA with an anti-DNP antibody and standard ELISA techniques for labeling and visualizing labeled molecules. Absorbance was read at 450 nm on the MRX Revelation microplate reader (Dynex Technologies, Chantilly, VA). A standard curve was plotted, and the carbonyl concentration of samples was read off the curve, using the MRX Revelation software. The interassay and intra-assay coefficients of variation were 13 and 10%, respectively.

**Plasma Cytokine Determination**

Plasma cytokine concentrations (IL-1β, TNF-α, IL-8, and IL-6) were determined by ELISA with kits from BioSource International (Camarillo, CA). 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 8% for IL-6, 0.7 pg/ml and 5% for IL-8, and 3.0 pg/ml and 10% for TNF-α.

**Statistical Analyses**

The main outcomes followed in this study were the continuous variables plasma protein carbonyl content, plasma protein reduced thiol content, and plasma cytokine levels. Comparisons of one-time data among the four different study groups were completed by using a one-way ANOVA for normally distributed variables and the Kruskal-Wallis H test for nonnormally distributed variables. Once it was determined that differences existed among the means, the Bonferroni post hoc range test was used to determine which means were different. Separate additional comparisons between each study group were completed by the t test or the Mann-Whitney U test, using the Bonferroni test to adjust the observed significance level for multiple comparisons. Analyses of changes in study variables comparing pre-dialysis; immediately after dialysis; and 30 min, 60 min, and 24 h after dialysis were completed with a general linear model repeated measures ANOVA. In this case, the Bonferroni post hoc range test was used to determine which specific means were different. Analyses of possible predictors of study variables were completed by linear regression model. Correlations among continuous data were performed by the Pearson correlation coefficients. Comparisons between categorical data were done by the χ² test. All tests were two-tailed, and a P = 0.05 was accepted to indicate statistical significance. All data are expressed as means ± SEM, unless otherwise noted. The SPSS statistical software program (version 11.5, SPSS, Chicago, IL) was used for all analyses.

**Results**

**Plasma Protein Thiol Content**

The assay for plasma protein thiol content measures the major source of reducing equivalents (or antioxidant capacity) available in the plasma (27,28). An increase in oxidative stress results in depletion of plasma thiol content. We found significantly lower thiol content in patients with ARF (191 ± 7 μM) compared with critically ill patients without ARF (243 ± 14 μM; P < 0.001), with ESRD patients (280 ± 11 μM; P < 0.001), and with healthy subjects (416 ± 6 μM; P < 0.001). Differences in median values are illustrated in Figure 1. Plasma protein thiol content was also significantly lower when ESRD and critically ill patients were compared with healthy subjects (P < 0.001).

**Plasma Protein Carbonyl Content**

Plasma protein carbonyl content measures reactive aldehyde content as an index of oxidative injury (17,29). Significantly higher levels of protein carbonyl content were detected in critically ill patients with ARF (0.408 ± 0.074 nmol/mg protein) compared with healthy subjects (0.032 ± 0.004 nmol/mg protein) compared with healthy subjects.

![Figure 1](https://via.placeholder.com/150)

**Figure 1.** Plasma protein thiol content in the four study groups. Boxplots show the median, interquartile range, and outliers. *P < 0.001 versus healthy; § P < 0.001 versus ARF.
protein; $P < 0.001$), ESRD patients ($0.076 \pm 0.009$ nmol/mg protein; $P < 0.001$), and critically ill patients without ARF ($0.097 \pm 0.019$ nmol/mg protein; $P < 0.001$). The levels of plasma protein carbonyl content were also significantly different when critically ill and ESRD patients were compared separately with healthy patients. Differences in median values are illustrated in Figure 2.

**Effects of Hemodialysis on Plasma Protein Oxidation**

Changes in plasma protein thiol content before and immediately after dialysis were examined in patients who received dialysis during the ICU stay. For these patients, dialysis treatment length was 195 ± 10 min, with mean blood flow of 247 ml/min and dialysis flow of 500 ml/min for all patients. Urea reduction ratio was 45 ± 4%. As shown in Figure 3, there was a significant increase in plasma protein thiol content after the hemodialysis procedure compared with the predialysis period ($213 \pm 17$ µmol/L postdialysis versus $169 \pm 12$ µmol/L predialysis; $P < 0.001$). However, this increase was considerably less than what has been previously observed in ESRD patients (28,30) and did not result in normalization of plasma protein thiol content. Hemodialysis had no significant effect on plasma protein carbonyl content in critically ill patients with ARF ($P = 0.13$ post versus predialysis; Figure 4), similar to previous observations in ESRD patients (28).

It is widely known that during the hemodialysis procedure, solute compartmentalization can result in postdialysis rebound once equilibration has taken place (31,32). To determine whether there may be significant intracellular stores of oxidants that could equilibrate with plasma proteins after the dialysis procedure (postdialysis rebound), we additionally examined plasma protein thiol and carbonyl content 30 and 60 min after the dialysis procedure in eight hemodialysis sessions.

![Figure 3](image3.png)

*Figure 3. Effects of hemodialysis on plasma protein thiol oxidation. $*P < 0.001$ for post- versus predialysis period.*

Our results showed no significant changes in levels of plasma protein thiol content or plasma protein carbonyl content either at 30 or at 60 min after dialysis compared with immediately after dialysis (data not shown), demonstrating a lack of “rebound” equilibration of oxidized solute.

**Examination of Interdialytic Changes in Plasma Protein Oxidation**

Because plasma protein thiol oxidation is biochemically reversible and likely occurs as low molecular weight oxidants accumulate, we also measured plasma protein thiol content 24 h after 12 hemodialysis sessions. By 24 h after dialysis, plasma protein thiol content had decreased to levels seen before the dialysis procedure (158 ± 20 µmol/L predialysis versus $157 \pm 23$ µmol/L at 24 h after dialysis) and had decreased significantly from the immediate postdialysis values ($211 \pm 25$ µmol/L immediately after versus $157 \pm 23$ µmol/L 24 h after dialysis; $P < 0.001$). There were no significant differences in plasma carbonyl concentration as a result of the dialysis procedure or 24 h after the dialysis procedure (data not shown). These data demonstrate that there is significant plasma oxidant reaccumulation during the interdialytic period.

**Plasma Cytokine Levels**

Critically ill patients who have ARF and manifest increased oxidative stress will likely also have an increase in cytokine...
production (33). We therefore measured plasma levels of the cytokines IL-1β, TNF-α, IL-6, and IL-8 in these patients simultaneously with oxidative stress biomarker measurements. Table 2 demonstrates that plasma levels of IL-6, IL-8, and TNF-α were markedly elevated in patients with ARF compared with healthy subjects. In critically ill patients, plasma levels of IL-6, IL-8, and TNF-α were also elevated compared with healthy subjects. Plasma TNF-α levels were significantly higher in ARF patients compared with critically ill patients without ARF. Plasma IL-1β levels did not differ between patient groups. We also examined the correlation among plasma cytokines and plasma protein thiol and carbonyl content. As shown in Table 3, plasma protein thiol content was significantly and inversely associated with plasma cytokines and plasma protein thiol and carbonyl content. Furthermore, the observed inverse association between plasma protein thiol content and proinflammatory cytokines IL-6, TNF-α, and IL-8, as well as with plasma protein carbonyl content.

Predictors of Biomarkers of Oxidation in ARF Patients

We examined whether comorbidities and demographic factors were predictive of levels of plasma protein thiol and carbonyl content in patients with ARF. In univariate analysis, we examined the predictive power of age, gender, oliguria, severity of illness scores, sepsis, and whether patients received hemodialysis during the ICU stay. We found that none of these factors was a significant predictor of plasma protein thiol or carbonyl content (data not shown).

Discussion

To date, few studies have examined oxidative stress in critically ill patients with ARF. In this study, we demonstrated that there is excess plasma protein oxidation in critically ill patients with ARF, as evidenced by diminished thiol content and increased carbonyl content. These significant changes are above and beyond that observed in critical illness without renal failure or in ESRD patients, suggesting that the combination of these two conditions, namely loss of renal function and acute critical illness, has additive effects on oxidative stress status. Given the high mortality rate observed in this population, these data suggest that increased oxidative stress may be a potential target for future pharmacologic and therapeutic applications.

Oxidative stress is known to modify plasma proteins, and these modifications can serve as excellent in vivo biomarkers of oxidative stress status. The ready accessibility of plasma proteins for sampling, the relatively long plasma half-lives of many proteins, and the well-characterized biochemical pathways of protein and amino acid oxidation make plasma protein oxidation an attractive in vivo biomarker of oxidative reactions (17,34–37). Thiols are organic sulfur derivatives that are characterized by the presence of sulphydryl residues at the active site. Halliwell and others (24,27,38) have demonstrated that protein-associated thiols, particularly in the albumin molecule, constitute a major defense against oxidative stress in plasma. Plasma thiol groups are also effective in scavenging free radicals and myeloperoxidase-generated oxidants. Of particular note, the level of plasma protein thiol depletion is substantially greater in ARF patients than in ESRD patients or critically ill patients without ARF. These data suggest that production of reactive oxygen species by dysregulated phagocytic cells is greater than that seen in the “microinflammation” that frequently accompanies uremia (39,40). Support for this concept is also demonstrated by the high plasma levels of proinflammatory cytokines in this study. Furthermore, the observed inverse association between plasma protein thiol content and plasma levels of proinflammatory cytokines IL-6, IL-8, and TNF-α suggest that inflammation and oxidative stress are closely linked in this patient population.

Excess carbonyl formation in plasma proteins has previously been observed in studies of oxidative stress after cardiopulmonary bypass surgery and in critically ill patients (41,42). In this study, we demonstrated that plasma protein carbonyl formation is markedly increased in critically ill patients with ARF. As with plasma protein thiol oxidation, levels of plasma protein carbonyl formation are higher in critically ill patients with ARF than in ESRD patients or in critically ill patients without ARF. Previous work by our group and other investigators has demonstrated that myeloperoxidase-catalyzed reactive oxygen species (derived from activated monocytes and neutrophils) potently stimulate plasma protein thiol oxidation and plasma protein carbonyl formation in uremia (28,43,44). The magnitude of the increased plasma protein oxidation observed in ARF patients suggests that increased production and decreased clearance of oxidants have additive effects on plasma carbonyl levels.

An important observation in this study is that hemodialysis has only a limited and transient beneficial effect on the redox status of plasma protein thiol groups with no discernible effect on protein carbonyl content. We have previously shown that in ESRD patients, the dialysis procedure results in a marked improvement in plasma protein and amino acid thiol oxidation while having no significant effect on plasma protein carbonyl

### Table 2. Proinflammatory cytokine concentrations in study groups

<table>
<thead>
<tr>
<th></th>
<th>IL-1β (pg/ml)</th>
<th>IL-6 (pg/ml)</th>
<th>IL-8 (pg/ml)</th>
<th>TNF-α (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td>4.7 ± 1.7</td>
<td>2.9 ± 0.6</td>
<td>4.6 ± 0.7</td>
<td>10.6 ± 1.0</td>
</tr>
<tr>
<td>Critically ill</td>
<td>2.5 ± 0.4</td>
<td>290.2 ± 70.3b</td>
<td>32.4 ± 12.2b</td>
<td>23.5 ± 3.0b</td>
</tr>
<tr>
<td>ARF</td>
<td>2.8 ± 0.3</td>
<td>190.6 ± 32.4b</td>
<td>44.3 ± 13.1b</td>
<td>56.2 ± 5.3b,c</td>
</tr>
</tbody>
</table>

* ARF, acute renal failure.

b $P < 0.05$ versus healthy subjects.

* $P < 0.005$ versus critically ill patients.
levels, as a result of dialytic removal of thiol binding oxidants (28,45). The results in the present study suggest that intermittent renal replacement therapy is less effective in ameliorating the consequences of oxidative stress in ARF patients (postdiagnosis 26% increase in thiol content in ARF patients in this study versus 55% increase in thiol content in previous ESRD studies) (28). Although this observation may be related to less efficient clearance during hemodialysis in ARF patients (46,47), it is also likely that excess oxidant production is substantially higher in this patient population than maintenance hemodialysis patients. The lack of substantial disequilibrium or intracellular compartmentalization in the pool of oxidants in critically ill patients with ARF, as shown in this study, suggests that the beneficial influence of the hemodialysis procedure is indeed limited. It is also possible that the specific pathways of excess reactive oxygen species production are different and potentially less reversible in critically ill patients with ARF than in ESRD patients.

Evidence of a nearly universal increase in plasma protein oxidation in critically ill patients with ARF suggests that increased oxidative stress may be a target for therapeutic intervention in this patient population (48). Recent studies suggest that administration of N-acetylcysteine, a source of reduced thiols, may be beneficial in restoring or preserving renal function in patients who are at risk for ARF (49–51); and N-acetylcysteine may also be beneficial in reducing inflammation during critical illness (52). Mesna, another thiol-containing antioxidant, has recently been demonstrated to have protective effects on renal function in a model of ischemic renal failure (53). Whether and which type of antioxidant administration would be helpful in the setting of critically ill patients with ARF will likely be the source of subsequent investigation.

There are several limitations to the present study. Most of the data were obtained at a single time point, whereas the clinical course of development and manifestations of ARF in critically ill patients is heterogeneous and varies over time. Similarly, for critically ill patients without ARF, the time course of oxidative stress may vary over the course of the illness. In addition, in this study, we relied exclusively on assays of plasma protein oxidation to detect an increase in oxidative stress. Although plasma protein oxidative stress bioassays are increasingly being used for in vivo detection of oxidative stress, assays of lipid oxidation, DNA oxidation, and lipoprotein oxidation could also be used to give a more holistic view of oxidative stress status in this patient population. Similarly, additional measurements of specific pathways of inflammation, including measurement of myeloperoxidase activity and measures of the acute-phase inflammatory response such as C-reactive protein, may have provided additional information regarding the cause of oxidative stress in this patient population. Measuring antioxidant capacity, including antioxidant enzymes and total plasma antioxidant capacity, could also contribute to enhanced understanding of oxidative stress in patients with ARF. Further research is likely to provide an enhanced understanding of the causes and consequences of oxidative stress in this patient population.

In summary, critically ill patients with ARF manifest a marked increase in plasma protein oxidation, including plasma

| Table 3. Correlations within study variables |
|-------------------------------|----------------|----------------|----------------|----------------|----------------|
|                               | IL-1β          | IL-6           | IL-8           | TNF-α          | Thiols         |
| **IL-1β**                     |                |                |                |                |                |
| Pearson correlation           |                |                |                |                |                |
| **IL-6**                      |                |                |                |                |                |
| Pearson correlation           |                |                |                |                |                |
| **IL-8**                      |                |                |                |                |                |
| Pearson correlation           |                |                |                |                |                |
| **TNF-α**                     |                |                |                |                |                |
| Pearson correlation           |                |                |                |                |                |
| **Thiols**                    |                |                |                |                |                |
| Pearson correlation           |                |                |                |                |                |

*a Correlation is significant at the 0.05 level (two-tailed).

*b Correlation is significant at the 0.01 level (two-tailed).
protein thiol group oxidation and carbonyl formation. Increased plasma protein oxidation in critically ill patients with ARF occurs in the setting of a generalized increase in circulating inflammatory cytokines. In contrast to patients who have ESRD and receive chronic hemodialysis therapy, plasma protein thiol oxidation is not as substantially improved with hemodialysis in ARF patients. Further studies using antioxidant therapy in an attempt to decrease the high morbidity and mortality for this patient population are indicated.

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References


