A pilot study of coupled plasma filtration with adsorption in septic shock*

Claudio Ronco, MD; Alessandra Brendolan, MD; Gerhard Lonnemann, MD; Rinaldo Bellomo, MD, FRCP; Pasquale Piccinni, MD; Antonio Digito, MD; Maurizio Dan, MD; Marco Irone, MD; Giuseppe La Greca, MD; Paola Inguggiato, MD; Umberto Maggiore, MD; Concetta De Nitti, BS; Mary Lou Wratten, PhD; Zaccaria Ricci, MD; Ciro Tetta, MD

Objective: To test the hypothesis that nonselective plasma adsorption by a hydrophobic resin (coupled plasmafiltration and adsorption) could improve hemodynamics and restore leukocyte responsiveness in patients with septic shock.

Design: Prospective, pilot, crossover clinical trial.

Setting: General intensive care unit in a teaching hospital.

Subjects: Ten patients with hyperdynamic septic shock.

Interventions: Patients were randomly allocated to 10 hrs of either coupled plasma filtration adsorption plus hemodialysis (treatment A) or continuous venovenous hemodiafiltration (treatment B) in random order. We measured the change in mean arterial pressure, norepinephrine requirements, and leukocyte tumor necrosis factor-α (TNF-α) production (both spontaneous and lipopolysaccharide-stimulated) after 10 hrs of each treatment. We also tested TNF-α production from normal human adherent monocytes incubated with patients' plasma obtained before and after the resin, both with or without incubation with an anti-interleukin-10 monoclonal antibody.

Results: Mean arterial pressure increased after 10 hr by 11.8 mm Hg with treatment A and by 5.5 mm Hg with treatment B (p = .001). There was an average decrease of norepinephrine requirement of 0.08 μg/kg/min with treatment A and 0.0049 μg/kg/min with treatment B (p = .003). All patients but one survived. Spontaneous and lipopolysaccharide-induced TNF-α production from patients' whole blood increased over time with treatment A. This increase was more marked in blood drawn after the device (plasmafiltrate-sorbent plus hemodialyzer) (p = .009). Preresin plasma suppressed lipopolysaccharide-stimulated production of TNF-α by 1 × 10⁶ cultured adherent monocytes from healthy donors. This suppressive effect was significantly reduced after passage of plasma through the resin (p = .019) and after incubation with anti-interleukin-10 monoclonal antibodies (p = .028).

Conclusions: In patients with septic shock, coupled plasmafiltration-adsorption combined with hemodialysis was associated with improved hemodynamics compared with continuous venovenous hemodiafiltration. This result might be related to its ability to restore leukocyte responsiveness to lipopolysaccharide. These findings suggest a potential role for blood purification in the treatment of septic shock. (Crit Care Med 2002; 30:1250–1255)

Key Words: sepsis; shock; cytokines; dialysis; hemodialysis; plasmafiltration; immunosuppression; lipopolysaccharide; tumor necrosis factor-α; hemodynamics; norepinephrine; interleukin-10

A cute renal failure is increasingly seen as part of the multiple organ dysfunction syndrome in critically ill patients (1, 2). Multiple organ dysfunction syndrome is the most frequent cause of death in patients admitted to intensive care units (3). Severe sepsis and septic shock are the primary causes of multiple organ dysfunction syndrome (4, 5) and develop as a result of the host response to infection (6) and to bacterial wall components, such as the lipid-A containing lipopolysaccharide (LPS). The host response involves both the cellular and humoral arms of the immune system and the generation of pro- and anti-inflammatory molecules (7–9). The anti-inflammatory response may lead to a state of “immunoparalysis” (10, 11). Continuous renal replacement therapies (CRRT) remove several soluble pro- and anti-inflammatory mediators simultaneously (12–14), albeit at low rates (13, 15, 16). However, large pore membranes, such as those used for plasmafiltration, may enhance cytokine removal and clearance (14). We recently observed that the use of coupled plasma filtration adsorption (CPFA) improved survival in a rabbit model of septic shock (17). The outcome was not correlated with single cytokines or mediators (such as tumor necrosis factor-α [TNF-α], platelet-activating factor, or endotoxin), but rather with a global sepsis severity score. The results of this study suggested that a nonselective removal of various septic mediators was beneficial. Thus, we performed a pilot,
prospective, crossover study to test the hypothesis that CPFA combined with hemodialysis might exert similar beneficial effects on hemodynamics and leukocyte responsiveness in humans with established septic shock and that it might prove superior to continuous venovenous hemodiafiltration (CVVHDF) alone.

PATIENTS AND METHODS

Patients

Patients were included in the study if they satisfied each of the following criteria: age 18 to 80 yrs; acute renal failure with oliguria (<200 mL in the last 12 hrs), requiring renal replacement therapy according to published criteria (3), sepsis and multiple organ dysfunction syndrome (4), and hemodynamic instability requiring norepinephrine infusion despite previous fluid loading. Patients were excluded if they suffered from any condition that would contraindicate extracorporeal treatment such as clinical evidence of infectious disease, imminent death, or lack of consent.

Severity of illness and predicted mortality were assessed in all patients using the Acute Physiology and Chronic Health Evaluation. Patients with an Acute Physiology and Chronic Health Evaluation II score >20 were included. All patients were continuously monitored for cardiac output, heart rate, central venous pressure, pulmonary artery pressure, systemic vascular resistance, mean arterial pressure (MAP), and hourly pulmonary artery occlusion pressure.

Study Design

This was a pilot, prospective, crossover study comparing a session of 10 hrs of CPFA to a session of 10 hrs of CVVHDF (in random order) with an overnight washout period between the two techniques. We planned to enroll the first ten eligible patients admitted in the Intensive Care Unit of St. Bortolo Hospital, Vicenza, Italy, from May 1999 to January 2000. Randomization was carried out using tables of random numbers.

The study was approved by the institutional review board. Informed consent was signed by the legal proxy.

Dialysis Technique

CPFA and CVVHDF were performed with the circuits and functional parameters described in Figure 1. A double lumen venous catheter was used for vascular access. Heparin was administered prefiltter and standardized at 5 IU/kg/hr. Subsequent adjustments were made according to coagulation parameters. A three-pump CRRT machine (Multimat BIC, Bellco, Mirandola, Italy) was used for the study. CPFA was performed with a polyether-sulfone plasmafilter (0.7 m², MPS 07, Bellco) placed in series with a highly permeable polysulfone hemodialyzer (1.2 m², BLS627, Bellco). Plasmafiltration rate was maintained between 30 and 40 mL/min. CVVHDF was performed with a highly permeable polysulfone hemodialyzer (1.2 m², BLS627, Bellco). Dialysate flow rate was 30 mL/min. The diastolic outflow plus ultrafiltration rate was 32 to 38 mL/min in both treatments. Blood flow averaged 155 ± 20 mL/min for both treatments. Sterile bicarbonate buffer was used as the dialysate (for both techniques) and as the reinfusion fluid (for CVVHDF alone) with the following composition (in mmol/L): Na⁺, 140; K⁺, 1.5; Ca²⁺, 2; Mg²⁺, 0.75; Cl⁻, 108; bicarbonate, 35; glucose, 5.5 (BC 35, Gambro, Lund, Sweden). Adsorption cartridges (4.3 and 7.5, and 1.7 and 5.9 pg/mL at 23, 75, and 230 pg/mL, respectively.

In Vitro Assessment of Cell Responsiveness

Spontaneous and LPS-Stimulated TNF-α Production. Spontaneous and LPS-stimulated whole blood production of TNF-α were determined by withdrawing 5 mL of blood at sites 1 to 3 at T0, T5, and T10 (Fig. 1). Whole blood was added to either pyrogen-free polypropylene tubes containing 0.5 mL RPMI 1640 culture medium for spontaneous production or to tubes containing 0.5 mL RPMI 1640 culture medium with LPS (Escherichia coli, serotype 055:B5, Sigma Chemical, St. Louis, MO; final concentration 20 ng/mL, 2.5 endotoxin units/ng) for LPS-stimulated production. LPS was initially dissolved in physiologic saline and sonicated for 10 mins to avoid aggregation or adhesion to the tube. After incubation at 37°C for 3 hrs, the blood was centrifuged at 900 × g for 20 mins (4°C). The plasma samples were stored at −80°C until they were assayed for TNF-α as described in the preceding section.

In Vitro Assessment of the Effect of Septic Plasma on TNF-α Production by Normal Adherent Cells. The effect of septic plasma IL-10 on TNF-α production by normal inflammatory cells was determined by withdrawing 5 mL of
plasma filtrate before and after the resin cartridge (sites 4 and 5, Fig. 1). These samples were incubated with $1 \times 10^6$ adherent monocytes from healthy subjects (1 mL plasma per 1 mL RPMI, prepared as described in Ref. 18) in the presence or absence of LPS (20 ng/mL, final concentration), with or without monoclonal antibodies to IL-10 (MAb-217, R&D Systems). TNF-α concentrations were corrected for hematocrit. The following controls were performed: normal monocytes with or without LPS (20 ng/mL, in the presence or absence of normal donor plasma) and normal monocytes incubated with patients’ plasma filtrate with a nonrelevant antibody (Cod B8895, Sigma).

**Clinical Parameters and Blood Chemistry**

Plasma creatinine, urea nitrogen, sodium, potassium, calcium, and bicarbonate were monitored using arterial blood at the beginning and at the end of each session. Total plasma protein and red blood cell, white blood cell, and platelet counts were measured at the beginning and at the end of the session. The same samples were collected during CVVHDF from the arterial blood line.

Weight balance was maintained throughout the two sessions of the study. Norepinephrine requirement was adjusted on the basis of the hemodynamic response to maintain a desired blood pressure. All patients were in a vasodilated hyperdynamic state for which norepinephrine was appropriate.

**Statistical Analysis**

The outcome measure of the treatment was the change in MAP and norepinephrine requirement at T10 with respect to T0. The analysis was based on a multivariate analysis of variance model examining MAP and norepinephrine requirement at T0 and T10, with the interaction term “treatment by time” being the outcome measure.

To verify whether the advantage of CPFA was related to the ability of the device (plasmafiltrate-sorbent plus hemodialyzer) to affect patients’ leukocyte responsiveness, we examined whether TNF-α production changed after the passage of blood through the device. We estimated the change in TNF-α production in the course of the treatment and tested whether the change differed with the sampling sites within the extracorporeal circuit, either in the presence or in the absence of LPS stimulation. This analysis was performed using an analysis of variance model for repeated measures with site (three levels), time (four levels), and LPS (two levels) as within-subjects factors. In this model, the interaction “time by site” was the measure of interest. After estimating the model, we compared the differences between the interaction term time by site at sites 1 (i.e., predevice plasma sample), 2 (i.e., prehemodialyzer), and 3 (i.e., posthemodialyzer plasma sample) (Fig. 1). Orthogonal polynomials were used for the comparisons.

To study the presence and the nature of inhibitors of monocyte activity, we examined TNF-α production from normal monocytes that were incubated with plasma from patients on CPFA. The aim of this analysis was to verify whether the plasma inhibitory activity changed after passage through the resin and was influenced by incubation with anti-IL-10 antibodies. We also used an analysis of variance model for repeated measures with anti-IL-10 antibodies (two levels), PRE/POST alone (two levels), and time (four levels) as within-subjects factors.

All the analyses described above were carried out using the GLM procedure of SPSS statistical software (SPSS 10.0 statistical software package; SPSS, Chicago, IL).

Finally, we verified whether TNF-α expression from normal monocytes differed after incubation with plasma from septic patients or from healthy donors. Comparisons were made with plasma obtained at T0 and T10, in the presence or absence of LPS, before and after the cartridge, using a two-sample Student’s t-test.

**RESULTS**

**Treatment Feasibility and Technical Aspects**

Despite the high mean Acute Physiology II score (27.6 ± 1.8), the high requirement for norepinephrine (0.25 ± 0.3 μg/kg/min), and severity of illness (Table 1), all patients completed the treatment sequence. One patient died within 48 hr of enrollment after completing the treatment sequence. All other patients survived.

 Patients were randomly allocated to treatment sequence AB (A = CPFA; B = CVVHDF) (six patients) or to BA (four patients). No clotting episodes or cessation of therapy for technical reasons were recorded during treatments. Total protein, albumin, and platelet counts were unchanged after each treatment.

The pressure profile in the circuit was modified by the presence of the plasmafiltration-adsorption device. In particular, the pressure in the “arterial” (inflow) line before the plasmafilter in CPFA displayed an average increase of 30% compared with the value recorded during standard CVVHDF. None of these problems had a negative impact on the treatment performance. In both CPFA and CVVHDF, MAP was stabilized, and the treatments were well tolerated. Fluid balance was maintained at zero due to a pump-controlled fluid balance.

**Mean Arterial Pressure**

In the entire group of patients, MAP increased during all the treatments, being on average 74.0 mm Hg (range, 67–82 mm Hg) at T0 and 82.8 mm Hg (range, 76–95 mm Hg) ($p < .0001$) at T10 (Fig. 2). From T0 to T10, mean arterial pressure increased by 11.8 mm Hg with treatment A and 5.5 mm Hg with treatment B ($p = .001$).

**Norepinephrine Requirement**

The requirement of norepinephrine was reduced at the end of both treatments for all patients (from 0.13 to 0.08 μg/kg/min, $p = .001$) (Fig. 3). CPFA in-

<table>
<thead>
<tr>
<th>Patient</th>
<th>APACHE II Score</th>
<th>Failing Organs</th>
<th>Tx Sequence</th>
<th>NE (μg/kg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>27</td>
<td>3</td>
<td>AB</td>
<td>0.19</td>
</tr>
<tr>
<td>2</td>
<td>28</td>
<td>4</td>
<td>AB</td>
<td>0.11</td>
</tr>
<tr>
<td>3</td>
<td>27</td>
<td>4</td>
<td>BA</td>
<td>0.15</td>
</tr>
<tr>
<td>4</td>
<td>28</td>
<td>3</td>
<td>AB</td>
<td>0.08</td>
</tr>
<tr>
<td>5</td>
<td>30</td>
<td>4</td>
<td>BA</td>
<td>0.16</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>4</td>
<td>BA</td>
<td>0.18</td>
</tr>
<tr>
<td>7</td>
<td>24</td>
<td>4</td>
<td>BA</td>
<td>0.18</td>
</tr>
<tr>
<td>8</td>
<td>26</td>
<td>5</td>
<td>AB</td>
<td>0.22</td>
</tr>
<tr>
<td>9</td>
<td>27</td>
<td>4</td>
<td>AB</td>
<td>0.19</td>
</tr>
<tr>
<td>10</td>
<td>29</td>
<td>4</td>
<td>AB</td>
<td>0.15</td>
</tr>
</tbody>
</table>

APACHE, Acute Physiology and Chronic Health Evaluation; Tx, treatment; NE, norepinephrine; AB, treatment sequence of coupled plasma filtration adsorption plus hemodialysis, then continuous venovenous hemodiafiltration; BA, treatment sequence of continuous venovenous hemodiafiltration, then coupled plasma filtration adsorption plus hemodialysis.
duced a significant reduction of norepinephrine requirement at T10 (mean reduction = 0.08 μg/kg/min) compared with CVVHDF (mean reduction = 0.0049 μg/kg/min) (p = .003).

**Cytokine Plasma Levels**

During CPFA, TNF-α and IL-10 were almost undetectable in the plasmalitract effluent from the sorbent cartridge at all time points (TNF-α at T10: pre cartridge values = 25.3 ± 12.2 pg/mL, post cartridges values = 0.1 ± 1.3 pg/mL; IL-10: pre cartridge values = 120.2 ± 12.2 pg/mL, post cartridges values = 0.2 ± 0.3 pg/mL). Despite such adsorption, the circulating plasma levels of these two cytokines were not changed (TNF-α: 34.1 ± 13.2 pg/mL at T1, 28.9 ± 7.8 pg/mL at T5, and 30.4 ± 11.1 pg/mL at T10; IL-10: 120.4 ± 21.2 pg/mL at T1, 139 ± 13.2 pg/mL at T5, and 123.4 ± 11.2 pg/mL at T10). This lack of change was also seen with CVVHDF.

**In Vitro Assessment of Cell Responsiveness**

**Spontaneous and LPS-Stimulated Production of TNF-α**

Both spontaneous and LPS-stimulated production of TNF-α increased during the course of CPFA (p < .001) (Fig. 4). This increase depended on the site of sampling (p < .001), being lower at site 1 than at sites 2 and 3 (p = .047 and p = .006, respectively; p = .009 for the comparison of site 1 with site 2 plus site 3).

**DISCUSSION**

Sepsis remains a very complicated and dynamically regulated syndrome with...
The spontaneous in vitro production of TNF-α by patients’ whole blood was normal in all patients at the start, but it was increased at the end of treatment with CPFA. The spontaneous in vitro production of TNF-α was also further increased by passage through the hemofilter (site 3). These changes in in vitro cell responsiveness could be due to a less biocompatible circuit, exposure to cytokine-inducing substances, removal of a dialysable suppressive “uremic toxi,” or removal of other inhibitors of cell responsiveness.

In vitro assessment of LPS-stimulated TNF-α production showed a marked inhibition at the start of treatment in all patients. An eight- to ten-fold increase in LPS-stimulated TNF-α production was observed after 10 hrs of CPFA at both sites 2 and 3, suggesting that this effect was affected both by diffusion/convection. Only a five-fold increase could be seen in LPS-stimulated TNF-α production after CVVHDF.

Finally, the in vitro assessment of the effect of septic plasma on TNF-α production by normal inflammatory cells showed that at the start of treatment, LPS-stimulated TNF-α production was suppressed by plasma obtained before and after the resin. Although such suppression was decreased by passage through the resin, it was not fully eliminated by it. We speculate that the resin was not able to completely adsorb suppressive soluble factors in a biologically significant amount in a single pass. However, after 10 hrs of CPFA, the inhibitory effect of septic plasma was markedly attenuated. The ability of CPFA to restore immune cell responsiveness may be clinically beneficial. Several studies reported the suppression of cytokine production in leukocytes from septic patients (7, 27–29). Postoperative sepsis has been shown to be associated with immediate monocyte defects that may affect both pro- and anti-inflammatory cytokine secretion, which suggests that immunosuppression is a primary rather than a compensatory response to sepsis (29). Survival has been shown to be correlated with the recovery of the proinflammatory but not the anti-inflammatory response (7, 29).

The resin effects shown in Figure 6 may have been due to the removal of IL-10, the principal down-regulator of the innate immune response, because anti-IL-10 monoclonal antibodies blocked
the *in vitro* inhibitory effect of septic plasma on normal inflammatory cell responsiveness. Figure 6 shows that in particular, there was a marked difference between the “pre” cartridge plasma at T0 vs. the plasma at T10 when the plasma was incubated with monoclonal antibody against IL-10. This gives support to the idea that IL-10 may be playing an important role. However, it does not exclude the possibility that other cofactors may be simultaneously adsorbed and also play a role in the increased responsiveness.

Thus, our findings suggest that CPFA was more effective than CVVHDF at restoring immune cell homeostasis, probably because it combined nonselective removal of sepsis-associated mediators and uremic toxins. CPFA also improved hemodynamics better than CVVHDF. This study also suggests that IL-10 may be, at least in part, responsible for sepsis-associated immunoparalysis and that immune mechanisms participate in vascular dysfunction because the restoration of *in vitro* cell responsiveness coincided with hemodynamic improvement.

ACKNOWLEDGMENTS

We thank the Nephrology and Intensive Care staff (Flaviano Ghiotto, Vincenzo Tessore, and Ettore Pagani) who contributed by setting up the extracorporeal real system and Luisa Sereni for performing the laboratory determinations of cytokines.

REFERENCES

19. Deleted in proof
20. Deleted in proof
21. Deleted in proof