

Continuous Hemodiafiltration with Polymyxin-B Immobilized Fiber Is Effective in Patients with Sepsis Syndrome and Acute Renal Failure

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Abstract: The aim of this study was first, to evaluate the effects of continuous hemodiafiltration (CHDF) alone or combined with CHDF and polymyxin-B immobilized fiber (PMX) on survival rates of patients with sepsis and acute renal failure, and second, to evaluate the changes in plasma levels of inflammatory cytokines before and after treatment with CHDF and PMX and CHDF alone in these patients. Forty-eight patients with septic shock and acute renal failure were enrolled in this study. The survival rate of all patients at 28 days was 25% for those with CHDF

and 75% for those with PMX and CHDF treatment. Combination treatment produced a significant reduction of plasma levels of endotoxin and interleukin-6 compared to the basal values and to the treatment with CHDF alone. From these data, it is suggested that the combined therapy with PMX and CHDF is effective in improvement of survival rate of patients with septic shock and acute renal failure. **Key Words:** Acute renal failure—APACHE II—Cytokines—Continuous hemodiafiltration—Polymyxin B—Septic syndrome.

In critical care practice, it is important to treat the patients with severe sepsis or septic shock for improvement of survival rate (1,2). However despite the vigorous advances in supportive therapy, such as newly developed antibiotics, innovation of respiratory machines, and so forth, there are no remarkable increases in survival rates of these patients around the world (3). The few agents that have reached large-scale clinical study have been disappointing and as yet no treatment has shown an unequivocal survival benefit (4). Continuous venovenous hemofiltration (CHF) has been used successfully to control renal failure in septic patients even when they are in shock (5–8). The primary advantage of CHF is to provide smooth and continuous control of the volume status while avoiding cardiovascular instability (9). A potential benefit of CHF is to remove a number of proinflammatory mediators that may be involved in the development of multiple organ failure

(MOF) (10,11). However, data from experimental and clinical studies have been inconsistent and sometimes contradictory (12).

During sepsis, release of various endotoxins from microorganisms activates immune cascade systems including release of anti- and proinflammatory cytokines. As described above, these cytokines might be removed with CHF; however, it remains uncertain whether endotoxins are removed effectively with CHF (13). Recently, immobilized polymyxin-B fibers (PMXs), which were developed to remove endotoxin selectively, have been available clinically in Japan (14,15). This newly developed technique provides some successful results (16,17).

These 2 techniques listed above seem to be effective for treatment of critically ill patients with sepsis and acute renal failure (ARF). However, whether and to what extent combined PMX treatment and CHF provides interaction effects for the prognosis of these patients remains unexplored.

In the present study, we undertook to compare the efficacy of CHF and its combination with PMX for prognosis of critically ill patients with sepsis and ARF in a prospective randomized open trial. To do

Received December 2001; revised January 2002.

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this, we compared and analyzed plasma from patients with sepsis who had received CHF alone or its combination with PMX. We focused on those mediators (endotoxin, interleukin (IL)-1B, and IL-6) that are believed to play a pivotal role in the pathophysiology of sepsis.

METHODS

Patients

Patients with a clinical diagnosis of septic shock as defined by the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference (18) and ARF as defined by a rise of the serum creatinine 0.5 mg/dl daily and a urine output less than 20 ml/h despite volume correction and intensive diuretic therapy were enrolled in the study. Informed consent was obtained from a close family member. Patients who were less than 18 years old, pregnant, and organ-transplant recipients were not enrolled. Patients were not eligible if informed consent from the patient's family was not granted, if they were experiencing acute organ transplant rejection, or if they were in a chronic vegetative state.

Decisions concerning supportive care and surgical intervention were made by the patients' attending physicians and were not dictated by the study protocol.

Evaluation of the patients

Patients were followed for 28 days or until death. An APACHE II score was calculated at entry. Patients were stratified for the severity of illness at baseline according to the overall APACHE II score (19).

The primary source of infection, causative pathogen, and adequacy of antimicrobial therapy were determined in a blinded fashion by a specialist in the microbiology section.

Gram-negative sepsis was considered to be present at study entry when a culture of blood or body fluid from a site of suspected infection obtained from 2 days before through 2 days after the day of study entry grew a gram-negative organism. In a small number of patients, no infection site had been found despite an aggressive search for bacterial infection. However, in those patients, gram-negative infection was suspected mostly on the basis of high fever and increased number of white blood cells count with a left shift of differential count.

Study design

All patients receiving a regular antibiotic therapy and other supportive treatment including continuous venovenous hemodiafiltration (CHDF) were as-

signed randomly to a group with or without PMX treatment. The primary end points were mortality from all causes; at Day 28 after the PMX procedure and if patients were discharged from the hospital or transferred to another hospital within 28 days. The outcomes were ascertained by the patients' attending physicians.

Continuous venovenous hemodiafiltration

Vascular access was obtained via a double-lumen hemodialysis catheter (Vas-cath; Salt Lake City, UT, U.S.A.) into either the femoral or jugular vein (6,13).

CHDF was performed using the PMMA dialyzer (Toray Co., Tokyo, Japan) that contains a highly permeable polymethylmethacrylate membrane. Blood was pumped by a peristaltic pump with pressure alarms and air trap (JUN-500; Ubejunnkenn Co., Tokyo, Japan), at a rate of 100 to 150 ml/min, from the *arterial* lumen of the catheter through the hemofilter and back into the circulation via the *venous* lumen of the catheter. Dialysate was delivered to the filter and ultrafiltrate collected as in CHDF. A balanced and isotonic electrolyte solution (HF solita; Shimizu Co., Shizuoka, Japan) was infused through the efferent limb of the extracorporeal system to maintain zero fluid balance. A steady state rate of 1 L of ultrafiltrate per hour (approximately 20 ml/kg/min) and isovolumetric fluid replacement were achieved. An electrolyte solution was warmed to 38°C and infused in a postdilution mode to maintain a zero fluid balance. The anticoagulant was nafamostat mesilate (Torii Co., Ltd., Tokyo, Japan), and the usual doses were between 30 and 50 mg/h. The activated clotting time (ACT) was measured every 3 h, and the doses of nafamostat mesilate were adjusted to maintain the ACT around 150 s. When the ACT was more than 180 s, the administration of nafamostat mesilate was withdrawn transiently until the ACT approached around 150 s. After 12 to 24 h, the hemofilter was regularly replaced during CHDF.

Direct hemoperfusion for polymyxin-B immobilized fiber treatment

Direct hemoperfusion was carried out for 4 h at a flow rate of 100 ml/min through a venovenous catheter used for CHDF as before (14,17). During PMX treatment, CHDF was discontinued. All entry criteria were met within a 12 h period, and the PMX or CHDF was started within the next 8 h. In combination therapy, after the end of 4 h PMX treatment, CHDF was started. CHDF was continued at least for the next 24 h or until death. In addition, CHDF was discontinued when ARF was recovered.

Collection of plasma

Blood samples were obtained before the initiation of PMX treatment or CHDF and after these treatments. Blood samples were obtained at 6 h and 24 h after the initiation of the treatment. Immediately after collection, all plasma tubes were centrifuged at 4°C for 15 min and stored at -80°C until measurements.

Measurement of endotoxin

Serum endotoxin was assayed for endotoxin by the Endospey method (14). The normal upper limit was 9.8 pg/ml (14).

Determinations of cytokines

Plasma IL-1 β was measured by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions. This assay detected only IL-1 β , and the lower limit of detection in our laboratory was 20 pg/ml.

Plasma IL-6 was measured using an ELISA according to the manufacturer's instructions. This assay detected only IL-6, and the lower limit of detection in our laboratory was 20 pg/ml.

Clinical and laboratory evaluation

Vital signs were recorded frequently during the first 72 h, then every 8 h through Day 14, and then every 24 h until Day 28. Mean blood pressure was recorded continuously from a catheter in the brachial artery using a modified Oxford Medilog device before, during, and 12 h after PMX or CHDF treatment. Dopamine was used for inotropic support in a dose of 3 to 30 μ g/ml/min as required by the patient's clinical condition.

Some patients were ventilated mechanically if they needed respiratory support. Physical examinations and the results of laboratory tests (serum albumin, alkaline phosphatase, total bilirubin, serum urea nitrogen, calcium, chloride, carbon dioxide, creatinine, glucose, potassium, total protein, aspartate transaminase, sodium, uric acid, complete blood cell count including differential and platelet counts, prothrombin time, partial thromboplastin time, fibrin split products, urinalysis with microscopic examination, and arterial blood gas) were recorded at entry and on Days 3, 7, and 14.

Statistical analysis

All data were expressed as mean \pm standard deviation except survival rate. Demographic and baseline characteristics were analyzed using the Wilcoxon test (20,21) to assess the comparability of the groups with respect to factors possibly related to the outcome. Statistical comparisons within groups were conducted by use of analysis of variance for repeated

measures, followed by the Newman-Keuls test. Student's unpaired *t*-test was used for comparisons between groups. The effects of PMX treatment on serum endotoxin were compared by paired *t*-test and the comparison between each group was carried out by unpaired *t*-test. To analyze the difference in mortality over the 28 day period after therapy, Kaplan-Meier survival curves (22) were constructed for these study groups and compared by the Cochran-Mantel-Haenszel test. A *p* value of less than 0.05 was considered significant.

RESULTS

Demographics and outcome

Of the 48 patients with the sepsis syndrome, 35 were men and 13 were women. The mean age was 65 \pm 1 years (range 46-87 years). Among the patients with the sepsis syndrome, 24 patients received PMX treatment and CHDF and 24 patients were treated only with CHDF. Two groups were well balanced with respect to demographic characteristics and underlying diseases (Table 1). The causative organisms are shown in Table 2. Antibiotic therapy was judged to be adequate when the patient received an antibiotic to which each isolated organism was sensitive.

The survival rate of all patients at 14 days was 25% for those with CHDF and 75% for those with PMX and CHDF treatment, and these were maintained at the end of the study (Fig. 1). At the end of the study, 3 patients in PMX and CHDF treatment and 4 patients in CHDF remained on hemodialysis

TABLE 1. Demographic character of the patients

	CHDF	CHDF and PMX
Number of patients	24	24
Age	64 \pm 2	65 \pm 2
Sex	17/7	18/6
APACHE II score	25 \pm 2.3	25 \pm 2.1
Number of patients with relevant previous disease		
Cardiovascular		
Postoperation of open surgery	6	6
Nonsurgical	4	4
Abdominal operation related		
Perforation	6	5
Gastric resection	1	0
Colon resection	1	2
Pancreatitis	2	3
Cholecystitis	2	3
Peritonitis	2	1
Number of patients		
On ventilation support	6	7
With multiple organ failure	24	24

CHDF: continuous hemodiafiltration, PMX: direct hemoperfusion using column containing polymyxin-B mobilized fiber.

TABLE 2. Causative bacteria

Variable	CHDF	CHDF and PMX
Number	24	24
<i>Escherichia coli</i>	5	7
<i>Pseudomonas aeruginosa</i>	6	5
MRSA	6	6
<i>Enterococcus</i>	3	5
<i>Clostridium difficile</i>	1	0
Unknown	3	1

MRSA: Methicillin resistant *Staphylococcus aureus*, CHDF: continuous hemodiafiltration; PMX: direct hemoperfusion using column containing polymyxin-B mobilized fiber.

therapy. Between these 2 groups, there was a significant difference in survival rate ($p < 0.01$).

Effects of continuous hemodiafiltration with polymyxin-B immobilized fiber treatment and continuous hemodiafiltration on blood pressure and biochemical findings

Treatment with PMX and CHDF in combination increased blood pressure from 78 ± 2 to 94 ± 4 mm Hg ($p < 0.001$) at 24 h after the start of treatment. However, treatment with CHDF alone did not change the mean blood pressure (75 ± 3 to 75 ± 4 mm Hg). There were no significant differences between the 2 groups (Table 3).

Dose of dopamine

Patients treated with CHDF only needed a relatively larger dose of dopamine at the end of the treatment. On the other hand, patients treated with PMX and CHDF did not receive the increases of the dose of dopamine (Fig. 2).

Adverse effects of polymyxin-B immobilized fiber treatment

There were no serious adverse effects of PMX treatment throughout the study. Detailed evaluation of laboratory values revealed no abnormality that was attributable to therapy.

TABLE 3. Biochemical findings in patients with the septic syndrome and acute renal failure before and after 48 h of treatment with CHDF or CHDF and PMX

	CHDF (n = 24)		CHDF + PMX (n = 24)	
	Before	48 h after	Before	48 h after
Blood urea nitrogen (mg/dl)	82.5 ± 12.1	56.3 ± 9.8	83.4 ± 11.3	58.3 ± 10.6
Serum creatinine (mg/dl)	3.8 ± 1.5	2.1 ± 0.9	3.6 ± 1.2	2.0 ± 1.1
Serum sodium (mEq/l)	140.5 ± 3.6	138.7 ± 4.1	141.0 ± 4.1	139.6 ± 5.0
Potassium (mEq/l)	5.2 ± 1.3	4.6 ± 1.2	5.1 ± 1.4	4.4 ± 1.6
Calcium (mg/dl)	7.2 ± 1.2	7.3 ± 1.6	7.0 ± 1.0	7.1 ± 1.5
Phosphate (mg/dl)	5.9 ± 1.8	5.3 ± 1.6	5.5 ± 1.6	5.1 ± 1.3
Hematocrit (%)	31.5 ± 1.6	32.4 ± 1.7	32.3 ± 1.8	32.5 ± 1.8
Hemoglobin (g/dl)	8.7 ± 1.6	9.2 ± 1.7	8.5 ± 1.7	9.0 ± 1.5
White blood cell ($\times 10^3/\text{mm}^3$)	1560 ± 350	1470 ± 280	1680 ± 240	1250 ± 310
Platelet ($\times 10^3/\text{mm}^3$)	53 ± 3.5	51 ± 3.6	55 ± 5.8	51 ± 4.3

CHDF: continuous hemodiafiltration, PMX: direct hemoperfusion using column containing polymyxin-B mobilized fiber.

Survival rate(%)

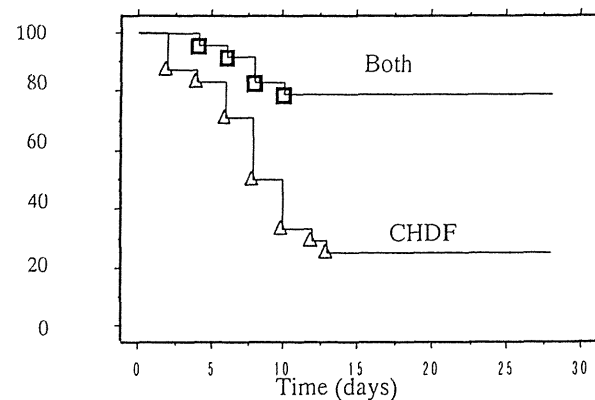


FIG. 1. Survival rate in patients with septic shock after treatment with PMX and CHDF and CHDF during 28 days is illustrated using Kaplan-Meier survival curves. Combination treatment with PMX and CHDF improved survival rate significantly compared to treatment with CHDF alone ($p < 0.01$). Comparison was made by the Cochran-Mantel-Haenszel test (CHDF: continuous hemodiafiltration).

Serum levels of endotoxin

At the start, there were no significant differences in endotoxin levels between these 2 groups. Treatment with PMX and CHDF in combination significantly reduced plasma levels of endotoxin; however, CHDF reduced it without significance (Fig. 3).

Changes in cytokines

Plasma levels of IL-6 were decreased significantly by CHDF and its combination with PMX (Fig. 4). Plasma levels of IL-1 β were significantly lowered by CHDF and its combination with PMX (Fig. 5).

DISCUSSION

In the present study, we compared the effects of the 2 kinds of treatments, CHDF and its combination with PMX, on outcome of patients with the sep-

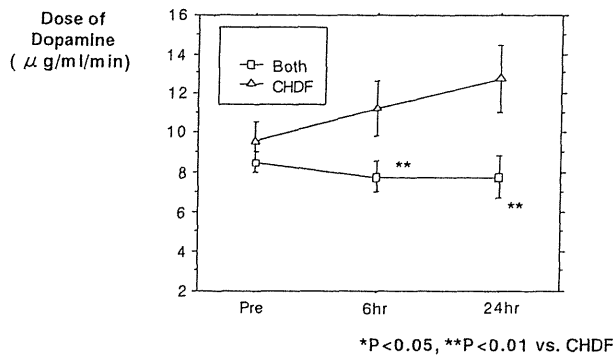


FIG. 2. Changes in the dose of dopamine for support of circulatory failure in patients with septic shock before, during, and after treatment with PMX and CHDF or CHDF alone is shown. Combination treatment produced a significant reduction of dose of dopamine at 24 h treatment compared to treatment with CHDF alone. **p < 0.01 compared to CHDF (PMX: polymyxin-B immobilized fiber, CHDF: continuous hemodiafiltration).

sis syndrome. In all patients enrolled in this study, the combination of CHDF and PMX had more beneficial effects on outcomes of patients with sepsis syndrome. Our previous study demonstrated that the treatment with PMX was effective in patients with APACHE II scores between 20 and 25 (23,24).

Meloni et al. (25) have demonstrated that CHDF is useful in septic patients to correct fluid overload and ARF, without affecting hemodynamic stability and oxygen balance. These results raise a possibility that this technique will improve the prognosis of septic patients complicated with ARF. In addition to these beneficial advantages, another potential benefit of hemofiltration is to remove a number of proinflammatory mediators that may be involved in the development of MOF (12,26).

During sepsis, proinflammatory cytokines, particularly, IL-1, IL-6, and IL-8 are released in excessive quantities from activated macrophages. This cytokine response is regulated by the intricate network

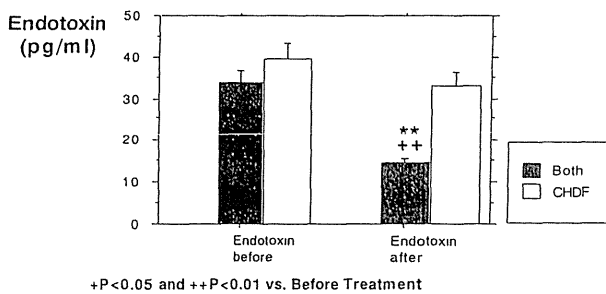


FIG. 3. The graph shows changes in plasma levels of endotoxin before and after treatment with PMX and CHDF or CHDF alone. Combination treatment produced a significant reduction of plasma levels of endotoxin (++p < 0.01 compared to the basal values and **p < 0.01 compared to CHDF alone) (PMX: polymyxin-B immobilized fiber, CHDF: continuous hemodiafiltration.)

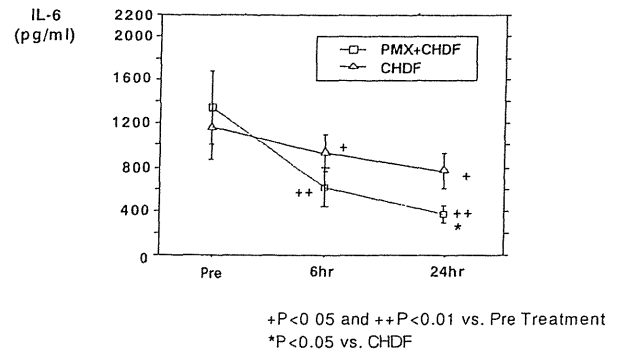


FIG. 4. The graph shows changes in plasma levels of interleukin (IL)-6 before and after treatment with PMX and CHDF or CHDF alone. Both combination treatment with PMX and CHDF and with CHDF alone reduced significantly plasma levels of IL-6, and at 24 h there was a significant difference between these 2 treatments (+p < 0.05 and ++p < 0.01 compared to the basal values and *p < 0.05 compared to CHDF alone) (PMX: polymyxin-B immobilized fiber, CHDF, continuous hemodiafiltration).

of mediators, including antiinflammatory cytokines and other endogenous antagonists (27). Combining this evidence, it is expected that CHDF will be useful for treatment of patients with the sepsis syndrome. However, data from experimental and clinical studies have been consistent and sometimes contradictory (12). This lack of consistency may come from clearance of a larger number of molecules in the circulation including mediators with more protective effects.

We measured several cytokines that have been considered to be indicators of severity of sepsis and organ failure. In the present study, 3 cytokines, IL-1 β , and IL-6 all were elevated when the therapeutic hemoperfusion/hemofiltration was started. After ap-

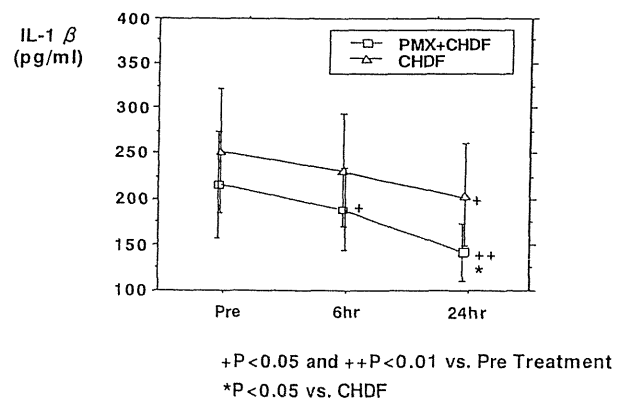


FIG. 5. The graph shows changes in plasma levels of interleukin (IL)-1 β before and after treatment with PMX and CHDF or CHDF alone. Both combination treatment with PMX and CHDF and with CHDF alone reduced significantly plasma levels of IL-1 β and at 24 h there was a significant difference between these 2 treatments (+p < 0.05 and ++p < 0.01 compared to the basal values and *p < 0.05 compared to CHDF alone) (PMX: polymyxin-B immobilized fiber, CHDF, continuous hemodiafiltration.)

plication of treatments, tumor necrosis factor- α did not show any significant changes. However, plasma levels of IL-1 β were decreased with both types of treatments. Similarly, plasma levels of IL-6 were significantly decreased with treatments. However, in the pathological process of the sepsis syndrome, a variety of pro- and antiinflammatory cytokines are released and affect each other. It is therefore unlikely that the removal of 1 of various cytokines is a determinant for improvement of septic shock.

In our studies, the plasma endotoxin, IL-6, and IL-1 β all were elevated and those levels are similar with the values reported previously. Moreover, Casey et al. (28) reported that the prognosis of patients with sepsis correlated with these plasma levels of the cytokines. Combined with these data it is indicated that the therapeutic procedures with CHDF and its combination with PMX might produce beneficial effects.

Despite these beneficial effects of removal of cytokines, CHDF did not improve the prognosis of patients. One of the other major factors except cytokines is possibly endotoxin.

Endotoxin is the most active and potent mediator stimulating various cellular responses resulting in septic shock. It should be kept in mind that the elimination of endotoxin effectively from the circulation in patients with sepsis-induced ARF and/or MOF is reasonable (12). PMX has been developed selectively to remove endotoxin and has been available clinically in Japan (29). There is strong evidence that the septic state is due to a combination of direct and indirect effects of endotoxin. Endotoxin releases a vast array of mediators including pro- and antiinflammatory cytokines. Based on this concept, it is reasonable to remove and/or to antagonize endotoxin for treating patients with sepsis and septic shock (30,31). In this study, the plasma levels of endotoxin were decreased significantly with CHDF and PMX but not with CHDF alone. In Japan, in the reports of Kodama et al. and Tani et al. in multicenter trials using PMX in patients with sepsis, this treatment decreased the plasma endotoxin concentration and reduced morbidity with improvement of parameters such as blood pressure and oxygen consumption index (32,33). Moreover, Ebihara et al. and Nakamura et al. provided supportive evidence that PMX treatment produced a reduction of erythropoietin, IL-6, and endothelin (16,34).

In the present study, treatment without PMX did not increase blood pressure as the result of no reduction of intravenous administered doses of dopamine. From these data, it would be expected that application of PMX might improve the prognosis in

patients with the sepsis syndrome when PMX was combined with CHDF. This indicates that in patients with the sepsis syndrome removal of only cytokines does not improve the pathophysiological process of septic shock. Indeed, combination therapy with PMX and CHDF produced a significant improvement of survival rate of patients with the sepsis syndrome. In our recent study (23,24), treatment with PMX alone improved the survival rate of patients with the sepsis syndrome. In that study, we evaluated the severity of the patients with the APACHE II score system and did not assess these patients as having sepsis, severe sepsis, and septic shock. The majority of patients with more than 30 points of APACHE II score should be classified as having septic shock instead of sepsis. It is therefore likely that in our recent report the prognoses of these patients presumably having septic shock were not improved with PMX treatment. Combining our recent and current data, it is suggested that patients with sepsis be treated with PMX and patients with septic shock be treated with PMX and CHDF in combination. This kind of notion is partially agreed to by Jaber and Pereira (35): that the early institution of PMX treatment may halt the sepsis syndrome at an early stage.

In conclusion, although in patients with septic shock the sole removal of either cytokines or endotoxin did not produce beneficial effects, the combined therapy with PMX and CHDF is effective in improvement of survival rate of these patients.

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