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ORIGINAL ARTICLE

Effects of hemoperfusion with an immobilized polymyxin-B fiber column on cytokine plasma levels in patients with abdominal sepsis

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ABSTRACT

Aim. The beneficial role of hemofiltration with immobilized polymyxin-B fiber (PMX) columns in sepsis, especially sepsis due to gram-negative bacteria, has previously been emphasized. Although the efficacy of PMX-B fiber-mediated hemofiltration in reducing plasma levels of cytokines has been reported, other studies did not confirm this observation. Here we report the effects of PMX-B fiber-mediated hemofiltration on outcome and cytokine plasma levels in patients with abdominal sepsis.

Methods. Twelve consecutive patients admitted to the Intensive Care Unit (October 2006-December 2007) for severe sepsis/septic shock from abdominal infection were treated with standard therapy and 2 cycles of hemofiltration with PMX cartridges. Clinical data and plasma levels of IL-6, IL-10 and TNF-α were measured 24 hours before and after PMX treatment.

Results. Plasma concentrations (pg/mL) of IL-6, IL-10 and TNF-α were significantly lower after hemofiltration with a PMX fiber column (279.9±69.2 *vs.* 130.9±18.4, 166.4±36.7 *vs.* 45.5±12.2, 83.1±13.5 *vs.* 23.9±5.1 pg/mL, respectively; P<0.05). After treatment, patients required lower doses of norepinephrine (0.3±0.1 *vs.* 0.8±0.1 μg/kg/min) and reduced lactate levels, recovery of respiratory function and improved Simplified Organ Failure Assessment (SOFA) scores. After 28 days, 6 patients (50%) had survived. Subgroup analysis demonstrated that survivors had higher IL-6 and lower IL-10 and TNF-α pre-treatment plasma levels (pg/mL) compared with deceased patients (324.4±41.1 *vs.*235.3±38.4; 98.5±16.1 *vs.* 234.3±48.6, 44.5±9.0 *vs.*121.6±52.3 pg/mL, respectively; P<0.05). No adverse events imputable to the treatment were recorded.

Conclusion. Hemofiltration with a PMX fiber column was able to reduce plasma levels of IL-6, IL-10 and TNF-α, especially in patients surviving at 28 days. Use of the technique was associated with lower norepinephrine support and an increased PaO_2/FiO_2 ratio. *(Minerva Anestesiol 2010;76:405-12)*

Key words: Sepsis - Polymyxin-B fiber column - Cytokines - Hemoperfusion.

Sepsis and septic shock are common in the Intensive Care Unit. Sepsis is defined as a systemic inflammatory response (SIRS) [1] to a proven or suspected infection,[1, 2] which can evolve to severe sepsis (acute organ dysfunction secondary to infection) and septic shock (severe sepsis plus hypotension not reversed with fluid resuscitation).[3] Severe sepsis is characterized by clinical and laboratory signs of multi-organ dysfunction syndrome (MODS) and may evolve to the final and most

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life-threatening clinical form of sepsis, septic shock with systemic hypoperfusion and multi-organ failure (MOF). Mortality rates reach 30% for severe sepsis and 70% for septic shock.[4, 5]

Sepsis may be defined as a maladaptive response of the host to a microbiological infection (bacterial/fungal/viral). The host response initially implicates the innate immune system, with activation of toll-like receptors (TLRs), nucleotide-binding domains, leucine-rich repeat-containing proteins (or Nod-like receptors, NLRs), and retinoic-acid-inducible gene I (RIG-I)-like helicases.[2] Gram-positive bacterial peptidoglycans bind to TLR-2, whereas Gram-negative lipopolysaccharide (LPS) interacts with TLR-4 and the CD14 protein complex on lymphocytes. TL receptors are linked to cytosolic activation of nuclear factor-kappa B (NF-kB).[6, 7] The interaction between receptors and microorganisms stimulates synthesis and release of pro-inflammatory molecules, such as tumor necrosis factor (TNF)-α and interleukin (IL)-6. Pro-inflammatory cytokines promote the expression of adhesion molecules on leucocytes and endothelial cells, chemotaxis and activation of neutrophils, monocytes/macrophages and lymphocytes, and injury to the endothelium, resulting in increased endothelial permeability and interstitial edema. Moreover, activation of endothelial cells leads to the production and release of nitric oxide (NO), one of the most potent vasodilators and a central player in the pathogenesis of septic shock.[8] Despite the hyperinflammation status at the beginning of the disease, the immune system usually passes through down-regulation phases and apoptosis, in which interleukin (IL)-10 is known to play a pivotal role.[9]

Polymyxins, obtained from the Gram-positive bacterium *Bacillus polymyxa*, are antibiotics known for decades for their ability to bind to LPS in the outer membrane of the Gram-negative bacterial cell wall. Although effective, nephrotoxic and neurotoxic adverse events [10] have limited the clinical application of these tools. Hemofiltration with polymyxin B (PMX) bound and immobilized to a polystyrene fiber column (Toraymyxin®, Toray Industries, Tokyo, Japan) has been proposed for patients with sepsis due to Gram-negative bacteria, to reduce circulating levels of LPS.[11, 12] Such an approach would overcome the adverse systemic effects of the drug. Beneficial effects of hemofiltration with PMX [13, 14] on morbidity and mortality have also been demonstrated in patients with acute lung injury (ALI), acute respiratory distress syndrome (ARDS) [15] and Gram-positive bacterial sepsis.[16] Although a recent systematic review of the literature demonstrated favorable effects of direct hemoperfusion with PMX on mean arterial pressure (MAP), vasoactive agent use (dopamine, dobutamine, norepinephrine), PaO_2/FiO_2 ratio, and mortality,[14] the only multicenter study on PMX cartridge hemofiltration in septic patients failed to demonstrate a decrease in LPS and cytokine plasma levels [17] after therapy.

Here we present the results of a pilot study designed to evaluate the potential benefits of hemofiltration with PMX cartridges in patients diagnosed with severe sepsis/septic shock due to proven or suspected abdominal infection.

Materials and methods

Twelve consecutive patients admitted to our Intensive Care Unit from October 2006 to December 2007 with severe sepsis/septic shock were treated with PMX cartridge. The inclusion criteria were: 1) proven or suspected abdominal source of infection; 2) the presence of positive blood culture upon admission; 3) the need for continuous veno-venous hemofiltration (CVVH) for renal failure, based on urinary flow <0.5 mL/h (lasting more than 6 hours), serum creatinine level (>2 mg/dL) and blood urea nitrogen level (>60 mg/dL) despite hemodynamic support optimization and full diuretic drug therapy. All patients were treated according to the conventional protocol for the management of critically ill patients.[18, 19] During the first 48 hours of admission, each patient underwent two cycles (2 hours each) of hemofiltration with a PMX cartridge (Toraymy-xin®, Toray Industries, Tokyo, Japan). Each cycle of PMX treatment lasted 2 hours, separated from the next by 24 hours of CVVH with a standard filter. Vascular access was obtained with 12 French two-lumen catheters (Arrow International Inc., US) via the femoral vein, after an ultrasound assessment of the vascular bed. Anticoagulation was afforded by heparin infusion. The ultrafiltration rate was set at 35 mL/kg/h, and bicarbonate or lactate substitution fluid was used.

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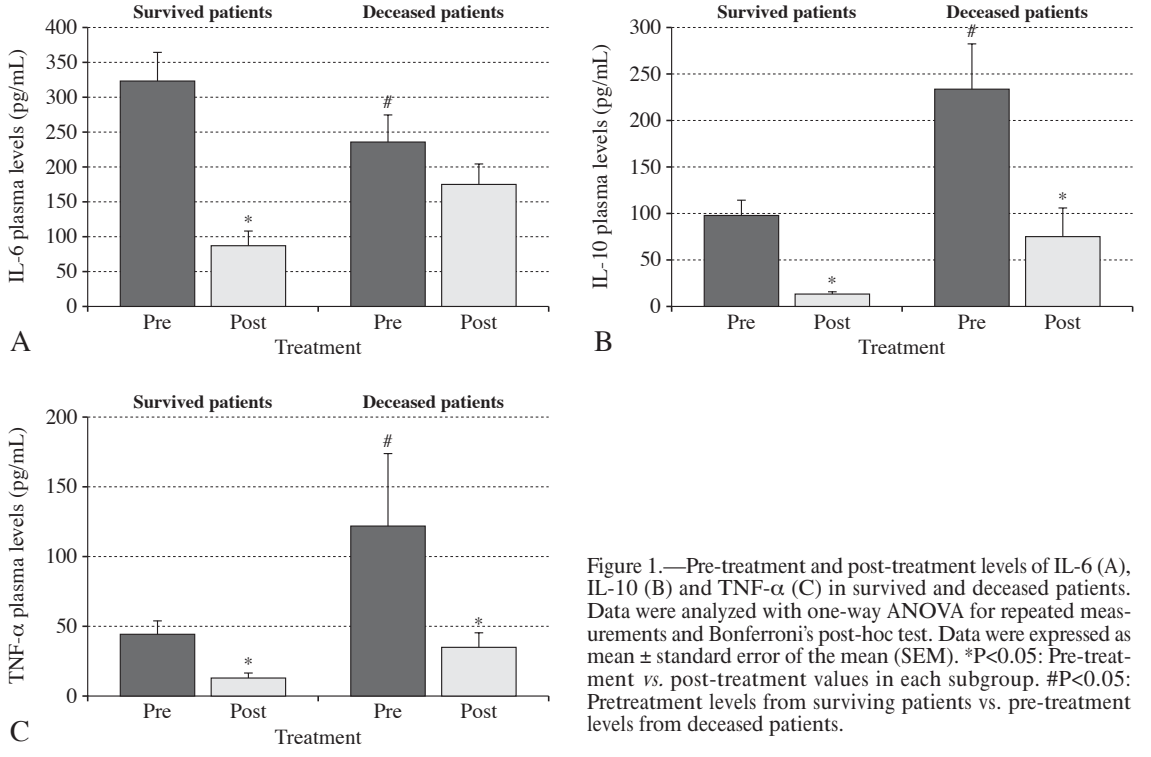

Figure 1.—Pre-treatment and post-treatment levels of IL-6 (A), IL-10 (B) and TNF-α (C) in survived and deceased patients. Data were analyzed with one-way ANOVA for repeated measurements and Bonferroni's post-hoc test. Data were expressed as mean ± standard error of the mean (SEM). *P<0.05: Pre-treatment *vs.* post-treatment values in each subgroup. #P<0.05: Pretreatment levels from surviving patients vs. pre-treatment levels from deceased patients.

Clinical and laboratory data were recorded in an ICU-database (FileMaker Pro 5.5v2, FileMaker, Inc, USA) 24 hours before and 24 hours after PMX treatment. Laboratory data included determination of plasma levels of IL-6, IL-10 (ELISA, Bender MedSystem, Burlingame, CA) and TNF-α (ELISA, Biosource Europe, Nivelles, BE). For each patient, the following parameters where collected: demographic data, Simplified Acute Physiology Score II (SAPS II), Sequential Organ Failure Assessment (SOFA) score, norepinephrine infusion immediately before and after treatment (□g/kg/min), the Horowitz ratio (PaO_2/FiO_2), arterial lactate (mmol/L), and plasma levels of IL-6 (pg/mL), IL-10 (pg/mL), TNF-α (pg/mL), and procalcitonin (ng/mL).

Patients were followed for 28 days. Primary outcomes of the current study were the mean differences between pre- and post-treatment levels of cytokines, and mean changes in respiratory and hemodynamic parameters. Differences in the above parameters were also evaluated in survivors and non-survivors.

Statistical analysis

Statistical analysis was performed with GraphPad Prism 5 (GraphPad Software Inc., San Diego, CA); continuous variables were analyzed with two-tailed Student's t-test and expressed as mean ± standard error of the mean (SEM). P value was considered significant at <0.05.

The study was performed in accordance with the principles of the Declaration of Helsinki and was approved by the Institutional Ethics Committee. Since this study did not require any deviation from the Institutional Protocol for Sepsis Treatment, the Institutional Ethics Committee waived the need for written informed consent.

Results

The twelve patients enrolled (7 males, 5 females) were relatively young (average, 53.7 years), with a SAPS II score of 61.5±6.6 (mean±SEM). No adverse events imputable to the treatment were registered. Baseline characteristics of studied patients were reported in Table I. Three patients were admitted

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TABLE I.—*Baseline characteristics of studied patients.*

Pt.	Gender	Age (y)	Diagnosis at admission	Severe sepsis/ septic shock	Time before sepsis (days)	SAPS II
1	Male	79	Peritonitis after gut perforation	Septic shock	5	73
2	Male	79	Peritonitis after gut perforation	Severe sepsis	4	24
3	Male	68	Major trauma	Septic shock	7	47
4	Male	45	Post-surgical peritonitis	Septic shock	9	90
5	Male	42	Major trauma	Septic shock	8	79
6	Male	43	Major trauma	Severe sepsis	7	64
7	Female	36	Post-surgical peritonitis	Septic shock	11	44
8	Female	58	Post-surgical peritonitis	Septic shock	16	73
9	Female	39	Post-surgical peritonitis	Septic shock	12	38
10	Male	58	Peritonitis after gut perforation	Septic shock	2	83
11	Female	42	Post-surgical peritonitis	Septic shock	11	73
12	Male	55	Post-surgical peritonitis	Severe sepsis	13	24

Pt: patient; SAPS: Simplified Acute Physiology Score.

with signs of severe sepsis but required vasoactive support within the first 24 hours after admission for the progression of disease, whereas all others had been in septic shock since admission to the ICU. The average time between hospital admission and organs' dysfunction was 8.75 days. Six patients (50%) were admitted for complications after abdominal surgery, three (25%) for complications after major trauma, and three (25%) for peritonitis (Table I). Microorganisms isolated from blood cultures were *Pseudomonas aeruginosa* (2 patients), *Serratia marcescent* (2 patients), *Enterobacter cloacae* (3 patients), *Escherichia coli* (3 patients), *Enterecoccus faecium* (2 patients). Three patients had also *Candida albicans* infections. All patients received full intensive care management, including antimicrobial therapy, mechanical ventilatory support, fluid resuscitation, amines. Two of them also received corticosteroids, whereas three received infusions of recombinant activated protein C (Table I).

Table II summarized clinical and laboratory data before and after 2 cycles of Toraymyxin® treatment of enrolled patients. The pre-treatment Simplified Organ Failure Assessment (SOFA) score decreased significantly after 2 cycles of PMX hemofiltration (from 17.5±1.3 to 13.2±1.7; p=0.0007), as did IL-6 plasma levels (from 279.9±69.2 to 130.9±18.4 pg/mL; p=0.0007), IL-10 (from 166.4±36.7 to

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TABLE I.—*Baseline characteristics of studied patients.*

Pt	Microorganism	N. of organ dysfunction	Antibiotic used	Other sepsis-spicific treatments	Outcome
1	Pseudomonas aeruginosa	3	Piperacillin/tazobactam Vancomycin Metronidazole	None	Survived
2	Serratia marcescens Candida albicans	2	Meropenem, Fluconazole Vancomycin Metronidazole	None	Survived
3	Enterobacter cloacae	3	Oxacillin, Imipenem, Vancomycin	Hydrocortisone	Survived
4	Pseudomonas aeruginosa Candida albicans	4	Piperacillin/tazobactam Vancomycin Fluconazole	None	Deceased
5	Enterecoccus faecium	3	Linezolid, Meropenem	Recombinant activated protein C	Deceased
6	Serratia marcescens	2	Oxacillin, Gentamicin, Imipenem Vancomycin	None	Deceased
7	Escherichia coli Candida albicans	3	Imipenem, Vancomycin Fluconazole Metronidazole	Recombinant activated protein C	Survived
8	Enterobacter cloacae	3	Piperacillin/tazobactam Vancomycin Fluconazole	Hydrocortisone	Deceased
9	Enterobacter cloacae	2	Piperacillin/tazobactam Vancomycin Fluconazole	Recombinant activated protein C	Survived
10	Enterecoccus faecium	4	Linezolid Piperacillin/tazobactam Fluconazole Metronidazole	None	Deceased
11	Escherichia coli	3	Meropenem, Linezolid, Fluconazole	None	Deceased
12	Escherichia coli	2	Piperacillin/tazobactam Vancomycin	None	Survived

Pt: patient; SAPS: Simplified Acute Physiology Score.

45.5±12.2 pg/mL; P<0.0001), TNF-α (from 83.1±13.5 to 23.9±5.1 pg/mL; P=0.0064), and lactates (from 10.9±2.1 to 3.2 ±0.5 mmol/L; P=0.0146). Procalcitonin plasma levels were reduced but not significantly (from 44.2±17.5 to 20.4±12.2 ng/mL; P=0.3837). In accordance with reduced levels of cytokines and lactate, norepinephrine infusion decreases significantly after PMX treatment (from 0.8±0.1 to 0.3±0.1 □g/kg/min; P=0.0403); the PaO_2/FiO_2 ratio also improved (from 116±14.8 to 229±24.5; P=0.0021) (Table II).

A subgroup analysis comparing survival (n=6) *vs.* non-survival (n=6) patients confirmed that the significant decrease in plasma cytokines after treatment observed in the overall population was associated with improved final outcomes (Table III; Figure 1, panel A-C), as well as reductions in plasma lactate and norepinephrine infusion, as well as an improved PaO_2/FiO_2 ratio (Table III). On average, survival patients had a length of stay (LOS) in the ICU of 12 days; LOS in the hospital was 25 days (Table III). None of the patients discharged from the ICU died during their hospital stay.

Discussion

In the present study, we found that PMX-hemofiltration was effective in reducing all

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TABLE II.—*Comparison of pre- and post-treatment clinical, hemodynamic, respiratory data and cytokine plasma concentrations of studied population.*

	Pre-treatment	Post-treatment	P
SOFA score	17.5±1.3	13.2±1.7	0.0007
Serum creatinine (mg/dL)	2.7±0.7	1.4±0.6	0.0219
Azotemia (mg/dL)	88.3±18.1	44.2±12.2	0.0351
IL-6 (pg/mL)	279.9±69.2	130.9±18.4	0.0131
IL-10 (pg/mL)	166.4±36.7	45.5±12.2	<0.0001
TNF-α (pg/mL)	83.1±13.5	23.9±5.1	0.0064
Procalcitonin (ng/mL)	44.2±17.5	20.4±12.2	0.3837
Norepinephrine (□g/Kg/min)	0.8±0.1	0.3±0.1	0.0403
PaO_2/FiO_2 ratio	116±14.8	229±24.5	0.0021
Lactates (mmol/L)	10.9±2.1	3.2 ±0.5	0.0146

Data were collected before and after the two cycles of PMX cartridge treatment. Statistic: two-tail Mann-Whitney test. Results are expressed as mean ± standard error of mean (SEM). SOFA: Sequential Organ Failure Assessment.

cytokines dosed (IL-6, IL-10, TNF-α; Table II). More importantly, 2 cycles of PMX-hemofiltration permitted a significant decrease in SOFA score and the need for vasoactive support (Table II). The observation that patients who were still alive at day 28 showed significantly higher levels of IL-6 upon admission than patients who died (Table III, Figure 1 panel A) suggests that pre-treatment levels of IL-6 could be related to the risk of death. In contrast, high pre-treatment levels of IL-10 (Table III, Figure 1B) and TNF-α (Table III, Figure 1, C) appeared to be related with a worse outcome. The finding that post-treatment levels of procalcitonin were lower in deceased patients than in survived patients (Table III) might be related with the immunosuppression/apoptosis of the late/final phase of septic shock. All these observations need to be confirmed with clinical investigations designed to include a larger sample, since it is not possible to conclusively assess the correlation between cytokine levels and survival in this limited sample.

A recent meta-analysis confirmed a potential role of Toraymyxin in improving hemodynamic and respiratory parameters in septic patients, but highlighted the need for larger multicentric controlled trials.[14] The most recent multicentric, randomized controlled study involved 6 European intensive care units.[17] In this paper, Vincent *et al.* confirmed the improvement of hemodynamic and renal function afforded by PMX-cartridge hemofiltration, but found no difference in plasma cytokine concentrations before and after PMX-treatment.

TABLE III.—*Comparison of clinical, hemodynamic, respiratory data and cytokine plasma concentrations between survived and deceased patient at 28 days from ICU admission.*

	Survival (N.=6)			Non survival (N.=6)		
Age (years)	52.5±6.5			54.8±5.6		
SAPS II score	62.6±7.6			60.4±10.7		
ICU LOS (days)	12±4			13±7		
Total Hospital LOS (days)	25±6			—		
	Pre-treatment	Post-treatment	P	Pre-treatment	Post-treatment	P
SOFA score	17.4±0.8	12.2±1.1	0.0012	17.6±1.3	14.2±3.9	0.2697
IL-6 (pg/ml)	324.4±41.1a	86.7±21.4	0.0322	235.3±38.4	175.0±28.9	0.4360
IL-10 (pg/ml)	98.5 ±16.1 b	14.3±1.6	0.0258	234.3±48.6	76.7±29.5	0.015
TNF-α (pg/ml)	44.5±9.0 c	13.0±3.1	0.0226	121.6±52.3	34.7±10.2	0.0331
Procalcitonin (ng/ml)	41.8±26.8	28.0±19.8	0.0775	46.6±32.7	12.8±9.5	0.4007
Norepinephrine (□g/Kg/min)	0.9±0.4	0.2±0.1	0.0022	0.7±0.1	0.4±0.2	0.8391
PaO_2/FiO_2 ratio	140.0±22.1	280.0±15.1	0.0215	92.0±19.65	178.0±20.5	0.0445
Lactates (mmol/l)	11.4±2.1	1.5±0.9	0.0171	10.3±3.7	4.9±0.9	0.1778

Data were collected before and after the second cycle of PMX cartridge treatment. Data were analyzed with one-way ANOVA for repeated measurements and Bonferroni's post-hoc. Data are expressed as mean ± standard error of mean (SEM). aP=0.0215: pre-treatment levels of surviving patients vs pre-treatment levels of deceased patients. bP=0.0294: pre-treatment levels of surviving patients vs pre-treatment levels of deceased patients. cP=0.0187: pre-treatment levels of surviving patients vs pre-treatment levels of deceased patients. LOS: length of stay; SAPS: Simplified Acute Physiology Score; SOFA: sequential organ failure assessment.

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This discrepancy with our observation of cytokine reduction may lie with the protocol adopted. Vincent *et al.* treated one group with one cycle of Toraymyxin, whereas our patients all underwent 2 cycles of PMX hemofiltration. This feature could represent a pivotal way to assess the real efficacy of Toraymyxin use. However, previously published studies do not permit clear conclusions to be drawn due to the small samples investigated and the lack of specifically designed trials. The use of Toraymyxin has also been proposed for Gram-positive infections [16] and for pathological conditions other than sepsis.[20] However, these data remain to be confirmed since the PMX cartridge was designed specifically for Gram-negative infections.

As mentioned above, the reduction in cytokine levels was associated with decreased vasoactive support and improved respiratory function (Table II), especially in patients surviving at day 28 (Table III). This finding is in agreement with previous data demonstrating that hemofiltration with a PMX column is able to enhance cardiovascular and respiratory function.[14] However, we cannot exclude the possibility that the improved recovery observed may be due to the standard sepsis treatment and not to PMX-hemofiltration therapy alone. Our data suggest that Toraymyxin® treatment may be particularly useful in patients with a plasma cytokine profile including high IL-6 levels, as well as low IL-10 and TNF-α levels.

Several studies have demonstrated that IL-6 is elevated in septic patients and correlates with the severity of illness, but not with mortality or the development of multiple organ dysfunction.[21] Casey *et al.* found that plasma IL-6 concentration was 69% higher in non-survivor septic patients as compared with survivors.[22] In our study, higher pre-treatment levels of IL-6 were correlated with improved clearance after treatment (Table II). Conversely, 2-3-fold higher pre-treatment levels of IL-10 and TNF-α (Table II) in non-survivors compared to survivors might represent a cytokine pattern with a weak response to PMX-cartridge hemofiltration.

The roles of IL-6, IL-10, and TNF-α in sepsis are widely discussed.[8] Despite being highly effective in preclinical trials, therapies directed at antagonizing the role of cytokines in sepsis did not show encouraging clinical results.[23, 24] In a prospective study,[25] CVVH alone was demonstrated to be effective in reducing TNF-α levels in septic patients, but a significant decrease in IL-6 and IL-10 plasma levels was not found. In contrast, Cole *et al.* demonstrated in a randomized controlled trial that TNF-α concentrations did not change after CVVH treatment.[26] Vincent *et al.* did not find a significant difference in plasma cytokine concentrations before and after PMX-treatment. [17] A possible explanation of these conflicting findings could be the use of the sepsis-directed standard therapy, which was unavailable at when most of the studies were performed. With regard to the study by Vincent *et al.*, the use of two cycles of PMX filtration in each patient could have enhanced the efficacy of treatment.

Conclusions

Limitations of the present pilot study need to be considered: small sample size, the absence of a control group without PMX cartridge treatment, the lack of plasmatic endotoxin measurements. Nevertheless, the results highlight the potential beneficial effect of PMX hemofiltration on respiratory/cardiovascular functions. In particular, our findings encourage the use of two cycles of PMX cartridge treatment in cases of Gram-negative sepsis. When used in combination with clinical parameters, serial measurements of IL-6, IL-10, and TNF-α permit us to evaluate the efficacy of treatment. The role of PMX therapy in the treatment of septic patients remains to be evaluated in more numerous, well-designed studies, in order to clarify whether pre-treatment plasmatic cytokine concentration profiles can identify those who will respond best to PMX treatment.

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Conflicts of interest.—In 2007, Alessandro Di Filippo received a grant, paid into institutional funds, to support research on endotoxin concentrations in the context of septic shock from Toray Industries (Tokyo, Japan). This funding was not used to finance the present study. None of the other authors of the manuscript have any conflicts of interest to disclose (financial or non-financial).

Received on November 28, 2008 - Accepted for publication on February 19, 2010.

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