Polymyxin B-Immobilized Fiber Hemoperfusion in Patients with Sepsis

Tsukasa Nakamura, MD; Takaharu Matsuda, MD; Yoshimasa Suzuki, MD; Hisataka Shoji, PhD; Hikaru Koide, MD

Drs. Nakamura and Matsuda are with the Department of Medicine, and Dr. Suzuki is with the Department of Surgery, Misato Junshin Hospital, Saitama. Dr. Shoji is with the Artificial Organs Department, Toray Medical Co., Ltd., Tokyo. Dr. Koide is with the Division of Nephrology, Department of Medicine, Koto Hospital, Tokyo, Japan.

Objective. *The use of polymyxin B-immobilized fiber (PMX-F) treatment has been demonstrated to reduce chemical mediators of sepsis. The objective of the present study was to evaluate the effects of PMX-F treatment in patients with sepsis.*

Methods. *The design of this study was prospective. Between January 1997 and April 2002, 314 patients were treated for severe sepsis or septic organ failure. Of these patients, 206 were treated by PMX-F hemoperfusion, during which standard supportive care was continued without alteration. The remaining 108 patients were treated by conventional means. Serum levels of mediators—including endotoxin, interleukin-6, tumor necrosis factor-α, soluble interleukin-2 receptor, endothelin-1, and platelet factor-4—were measured before and immediately after PMX-F treatment.*

Results. *The post-treatment 2-week survival rate was 69% in the PMX-F treatment group and 36% in the conventional treatment group ($p < 0.01$). In the PMX-F treatment group, the sepsis severity score, the Goris score for the number of failed organs, and the Acute Physiology and Chronic Health Evaluation (APACHE) II score in survivors were significantly less than those in non-survivors ($p < 0.01$). In the survivors, all mediators were significantly decreased after PMX-F treatment ($p < 0.01$); however, there were no significant changes in the mediators in non-survivors.*

Conclusion. *These data suggest that PMX-F hemoperfusion is superior to conventional treatment in patients with sepsis and is effective in reducing endotoxin and blood inflammation mediators in surviving septic patients.*

Sepsis is a common cause of admission to intensive care units, probably because of the severity of illness of hospitalized patients and the persistently high incidence of nosocomial infections.[1] A central event in the pathophysiologic cascade of sepsis is the excessive systemic release of proinflammatory cytokines in response to a microbial invasion.[2] Attenuating the systemic effects of these mediators is a logical goal of adjuvant therapy in sepsis.

However, the effectiveness of treatments targeting single mediators during established sepsis has been disappointing.[3] Danner et al.[4] reported the importance of reducing plasma endotoxin levels to achieve a good outcome for septic patients, but did not mention any correlation with clinical parameters. Recently, the polymyxin B-immobilized fiber (PMX-F) cartridge has been used clinically as one therapeutic intervention against sepsis.[5] We reported previously in small-scale studies[6-8] that PMX-F treatment was, in fact, effective in patients with sepsis.

The aim of the present study was to conduct a relatively large clinical trial to assess the value of PMX-F treatment for sepsis. We evaluated changes of various parameters as a response to direct hemoperfusion in patients with severe sepsis.

PATIENTS AND METHODS

Study Design

The design of the study was prospective. Between January 1997 and April 2002, 314 septic patients were admitted to our hospitals or, in some cases, to intensive care units if they were already hospitalized at the time sepsis developed. Conventional treatments, including resuscitation, antibiotics, and organ support, were continued and remained unchanged. The PMX-F regimen was given to patients who were confirmed to have an infection or positive results of a plasma endotoxin assay, and who demonstrated the systemic inflammatory response syndrome (SIRS) and at least 1 organ failure.[9]

Multiple organ failure (MOF) was diagnosed according to the MOF diagnostic criteria proposed by Goris et al.[10] Impairments of the kidney, liver,

respiratory system, central nervous system, cardiovascular system, coagulation, and the gastrointestinal system were estimated by the Goris definition.[10] The severity of infection was judged according to the septic severity scores (SSS).[11] The severity of the critical illness was evaluated by the number of failed organs, the Acute Physiology and Chronic Health Evaluation (APACHE) II, and the Goris score. Patients younger than 18 years or older than 85 years were excluded.[9] Informed consent was obtained from each patient or from responsible family members.

Patients

We selected 206 septic patients for PMX-F hemoperfusion. The remaining 108 patients received various conventional treatments including antibiotic therapy, administration of gamma globulin and vasopressors such as catecholamines, and hemodynamic monitoring. The conventional treatment group served as historical controls; however, the severity of sepsis in this group was slightly less than in the PMX-F treatment group. The patients were randomly divided into the two groups.

Table I. Underlying conditions of the PMX-F and conventional treatment groups.

	PMX-F Treatment (n = 206)	Conventional Treatment (n = 108)
neoplasm	46	32
collagen disease	28	14
diabetes	44	30
renal disease	36	15
liver disease	26	18
cardiovascular disease	76	45
respiratory disease	62	30
CNS disease	38	14
postoperation	68	44
trauma	38	22

CNS: central nervous system; PMX-F: polymyxin B-immobilized fiber.
The numbers shown represent the number of patients, some of whom had more than 1 underlying condition.

Methods

In the present study, the conventional treatment group did not receive hemoperfusion. The PMX-F treatment methodology and the hemoperfusion column used have been described previously.[5-9] PMX-F therapy was performed twice within a 24-hour interval, as previously reported.[6-8] Access to the circulation for direct hemoperfusion with PMX-F was obtained via a double-lumen catheter (Arrow International, Inc., Reading, PA, U.S.A.) inserted into the femoral vein by Seldinger's method. In patients undergoing hemoperfusion, we used a shunt access for the PMX-F treatment. Direct hemoperfusion was carried out for 2 hours at a flow rate of 100 ml/min, as reported previously.[6-8]

The survival rate, improvement in the Goris score, and the number of failed organs were assessed 2 weeks after the treatment.[9] Various parameters were monitored before and immediately after direct hemoperfusion. Body temperature, blood pressure, heart rate, and the PaO_2/FiO_2 ratio were also recorded.

Blood endotoxin levels were

Table II. Patient characteristics of the PMX-F and conventional treatment groups.

	PMX-F Treatment (n = 206)	Conventional Treatment (n = 108)
age (years)	62.8 ± 12.9	66.4 ± 14.3
sex (male/female)	132/74	67/41
endotoxin detected	82%*	76%
culture-positive	82%	80%
MOF	92%*	84%
failed organs (number)	4.2 ± 0.9*	3.6 ± 0.7
SSS	53.5 ± 8.5*	50.4 ± 8.3
APACHE II score	24.6 ± 6.8	24.0 ± 5.3

MOF: multiple organ failure; SSS: septic severity score; APACHE: Acute Physiology and Chronic Health Evaluation
$p < 0.05$ (PMX-F treatment group vs. conventional treatment group)

determined by an "endospecy" test (Seikagaku Kogyo, Tokyo, Japan; normal upper limit, 10 pg/ml; sensitivity, 0.003 EU/ml [1 pg/ml = 0.0029 EU]). Plasma interleukin (IL)-6 levels were measured by an enzyme-linked immunosorbent assay (Quantikine, R&D Systems, Minneapolis, MN, U.S.A.; normal upper IL-6 limit, 10 pg/ml). The TNF-α assay kit was from Amersham (Buckinghamshire, U.K.; normal upper limit, 5.0 pg/ml). The serum soluble IL-2 receptor assay kit was from T Cell Science (Cambridge, MA, U.S.A.; normal upper limit, 450 U/ml). The plasma platelet factor (PF-4) assay kit was from Diagnostic Stago (Asnieres, France; normal upper limit, 20 ng/ml). Plasma endothelin-1 was assayed by radioimmunoassay using polyclonal anti-rabbit antibody (Pe- ninsula Lab, Belmont, CA, U.S.A.; normal upper limit, 2.3 pg/ml).

The effects of PMX-F on the severity of sepsis and MOF (SSS, Goris score, and APACHE II score) were compared prior to and 2 weeks after treatment.

Table III. Infection sites in patients with sepsis.

	PMX-F Treatment (n = 206)	Conventional Treatment (n = 108)
respiratory system	70	38
abdominal cavity	45	28
urinary tract	28	10
bile tract	18	7
cardiovascular system	18	6
central nervous system	8	5
wound	7	4
others	12	10

The numbers shown represent the number of patients.

Statistical Analysis

Statistical analysis was done using the Mantel-Haenszel test.[9] Data are expressed as the mean ± SD. A statistical analysis was done with regard to survival rate in the PMX-F group vs. the conventional group 2 weeks post-treatment. The values obtained before and after treatment were analyzed using Wilcoxon's nonparametric *t*-test in those cases in which there was correspondence to pre-treatment values. A p-value of < 0.05 was considered significant.

RESULTS

As shown in *Table I*, the underlying conditions were varied and multiple. Patient characteristics are shown in *Table II*, and infection sites are shown in *Table III*. *Table IV* shows the organisms that caused the infections.

Survival rates 2 and 4 weeks after PMX-F treatment were 69.4% and 68.0%, respectively. Those after conventional treatment were 36.1% and 32.4%, respectively (PMX-F group vs. conventional treatment, $p < 0.01$). The Goris score improved significantly, from 6.1 ± 1.4 to 3.4 ± 0.6 ($p < 0.01$) 2 weeks after PMX-F treatment. The number of failed organs also improved, from 4.2 ± 0.9 to 2.2 ± 0.6 ($p < 0.01$).

Data for survivors and non-survivors at 2 weeks after PMX-F treatment are shown in *Table V*. The changes in blood pressure, heart rate, body temperature, and the PaO_2/FiO_2 ratio in survivors and non-survivors are shown in *Figure 1*.

In the survivors, blood pressure was significantly increased ($p < 0.01$), heart rate was significantly decreased ($p < 0.05$), body temperature was significantly decreased ($p < 0.01$), and the PaO_2/FiO_2 ratio was significantly

Table IV. Organisms causing the infections* in patients with sepsis.

	PMX-F Treatment (n = 206)	Conventional Treatment (n = 108)
E. coli	56 (27%)	22 (20%)
K. pneumonia	32 (16%)	14 (13%)
P. aeruginosa	84 (41%)	32 (30%)
B. cepacia	34 (17%)	15 (14%)
P. vulgaris	28 (14%)	10 (9%)
E. cloacae	38 (18%)	22 (20%)
H. influenza	16 (8%)	4 (4%)
P. mirabillis	32 (16%)	16 (15%)
Candida	22 (11%)	10 (9%)
Gram-positive bacteria	42 (20%)	20 (19%)

**Combined infections were detected in some patients. The numbers shown represent the number (and percentage) of patients.*

increased ($p < 0.01$). In non-survivors, these parameters showed little change.

Blood mediator levels of endotoxin, IL-6, TNF-α, endothelin-1, soluble IL-2 receptor, and PF-4 before and after PMX-F treatments were compared between survivors and non-survivors. All mediators were significantly improved in survivors after PMX-F treatment ($p < 0.01$) (*Figure 2*). By contrast, these mediators showed little change after PMX-F treatment in non-survivors.

Table V. Disease severity and survival outcome 2 weeks after PMX-F treatment.

	Survivors (n = 143)	Non-Survivors (n = 63)
SSS	48.6 ± 8.5	61.7 ± 8.8*
Goris score	5.2 ± 1.7	7.6 ± 1.9*
failed organs (number)	3.9 ± 0.9	5.5 ± 0.9*
APACHE II score	25.2 ± 3.2	26.8 ± 5.4*

$p < 0.01$ (survivors vs. non-survivors)

DISCUSSION

In the present study, we found that plasma endotoxin adsorption with polymyxin B has a beneficial effect on the outcome and symptoms of patients with severe sepsis when compared with conventional treatment. An improvement in both the Goris score and the number of failed organs was apparent after 2 weeks in all patients who survived more than 2 weeks.

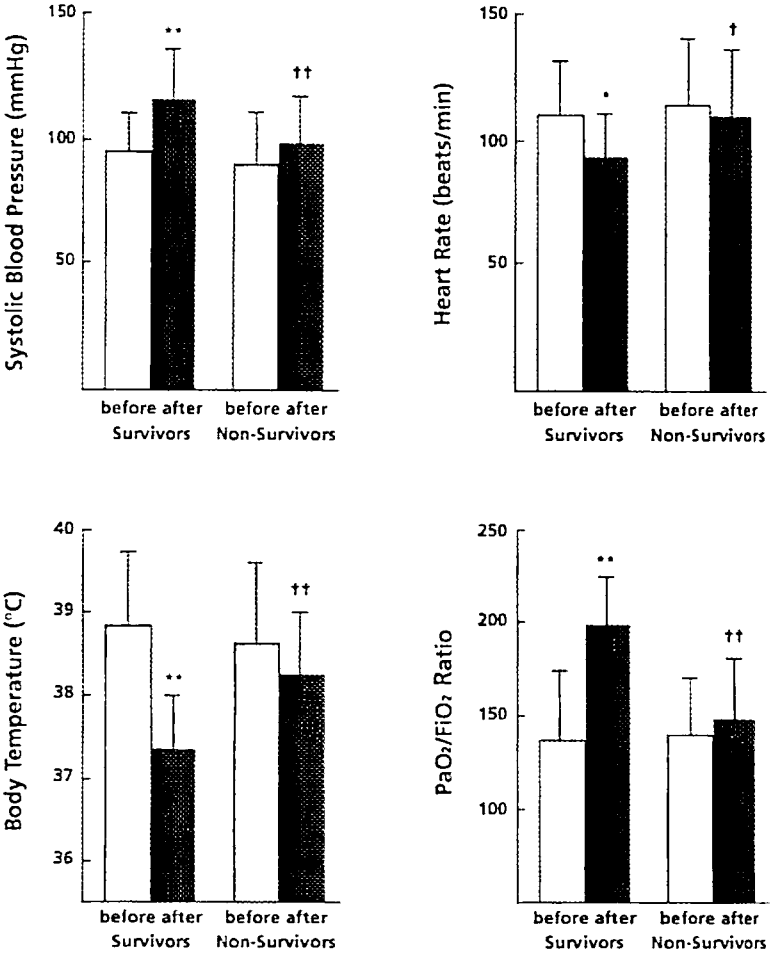

*Figure 1. Levels of clinical parameters before and immediately after PMX-F treatment in survivors and non-survivors. *$p < 0.05$, **$p < 0.01$ (before vs. after); †$p < 0.05$, ††$p < 0.01$ (survivors vs. non-survivors)*

The mechanisms underlying the efficacy of endotoxin adsorption in patients with severe sepsis are still unclear. In small-scale studies, many investigators have reported that several mediators play pivotal roles in sepsis or its progression to MOF.[2,5-9] In the present study, blood IL-6, TNF-α, endothelin-1, soluble IL-2 receptor, and PF-4 levels before treatment were significantly higher in non-survivors than in survivors; while PMX-F treatment reduced these levels in survivors, it did not in non-survivors. The influence of rapid reduction in plasma endotoxins may be found not only in monocytes, lymphocytes, and platelets, but in the endothelium as well.

TNF-α has a major proinflammatory role in the pathogenesis of sepsis and MOF, stimulates the production of many other pro-inflammatory and anti-inflammatory mediators, and is the first cytokine to be released systemically.[12] IL-6, which has been found consistently in the circulation of septic patients,[2] is a pleiotropic cytokine with potent biological effects encompassing stimulation of B- and T-lymphocytes, and plasma IL-6 levels have been found to closely correlate with the severity and outcome of sepsis.[13]

Endothelin-1 has an important

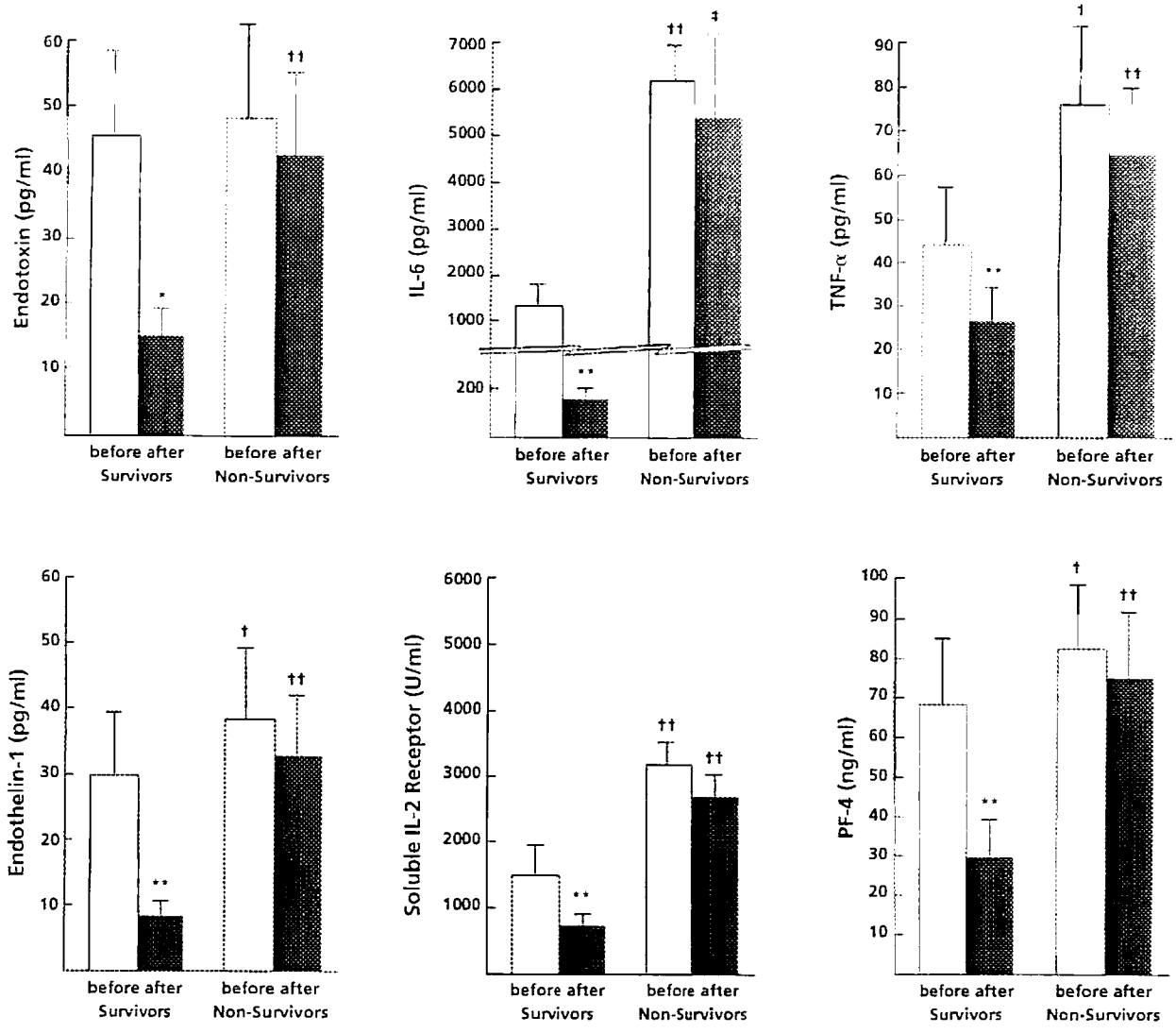

Figure 2. Levels of various mediators before and immediately after PMX-F treatment in survivors and non-survivors.
**p < 0.05, **p < 0.01 (before vs. after);*
†p < 0.05, ††p < 0.01, ‡p < 0.001 (survivors vs. non-survivors)

role in modulating immune and hemodynamic events. Recently, we reported that endothelin-1 may play pathophysiological roles in the progression of heart failure.[14] Some investigators have reported that increased levels of endothelin-1 are associated with a poor outcome in sepsis syndrome.[15, 16] Brauner et al.[17] reported that endothelin-1 is an early and sensitive predictor of mortality, and that very early determination of TNF-α and endothelin-1 in sepsis may help to identify patients at higher risk for an adverse outcome. We showed in the present study that plasma endothelin-1 levels are increased in septic patients and that PMX-F treatment reduced these levels in survivors but not in non-survivors.

The soluble IL-2 receptor is released into body fluids mostly from T- and B-lymphocytes that seem to have an ancillary role in the pathogenesis of sepsis.[18] Our present data lead us to agree with the previous suggestion that determination of IL-2 receptor levels has diagnostic and prognostic values in a redundant clinical course of patients with sepsis.[19] PMX-F treatment was effective in reducing soluble IL-2 receptor levels in survivors.

Platelet activation has been estimated by measuring increases in plasma levels of platelet α-granule proteins including PF-4. Our present data imply that platelets were

activated in patients with sepsis. The increased PF-4 levels may not only be indicators of platelet activation, but may play further roles in septic shock syndrome. Sepsis causes various activation reactions, including blood coagulation and fibrinolysis, in addition to platelet activation.

CONCLUSION

In summary, our current results indicate that septic patients treated with PMX-F have a better chance of survival than do patients treated with conventional methods. Blood endotoxin could be one of the therapeutic targets for the treatment of sepsis. Plasma endotoxin reduction by PMX-F treatment correlated with a reduction in the various mediators in surviving septic patients whose endotoxins were adequately removed.

References

1. Alberti C, Brun-Buissoh C, Burchardi H, Martin C, Goodman S, Artigas A, Sicignaho A, Palazzo M, Moreno R, Boulme R, et al. Epidemiology of sepsis and infection in ICU patients from an international multicentre cohort study. *Int Care Med* 2002; 28:108-121.
2. Schluter B, Raufhake C, Erren M, Schotte H, Kipp F, Rust S, Van Aken H, Assmann G, Berendes E. Effect of the interleukin-6 promoter polymorphism (-174G/C) on the incidence and outcome of sepsis. *Crit Care Med* 2002; 30:32-37.
3. Bone RC. Sir Isaac Newton, sepsis, SIRS, and CARS. *Crit Care Med* 1996; 24:1125-1127.
4. Danner RL, Elin RJ, Hosseini JM, Wesley RA, Reilly JM, Panllo JE. Endotoxemia in human septic shock. *Chest* 1991; 99:169-173.
5. Shoji H, Tani T, Hanasawa K, Kodama M. Extracorporeal endotoxin removal by polymyxin B immobilized fiber cartridge: Designing an antiendotoxin efficacy in the clinical application. *Ther Apher* 1998; 2:3-12.
6. Nakamura T, Suzuki Y, Shimada N, Ebihara I, Shoji H, Koide H. Hemoperfusion with polymyxin B-immobilized fiber attenuates the increased plasma levels of thrombomodulin and von Willebrand factor from patients with septic shock. *Blood Purif* 1998; 16:179-186.
7. Nakamura T, Ebihara I, Shimada N, Koide H. Changes in plasma erythropoietin and interleukin-6 concentrations in patients with septic shock after hemoperfusion with polymyxin B-immobilized fiber. *Int Care Med* 1998; 24:1272-1276.
8. Nakamura T, Ebihara I, Shoji H, Ushiyama C, Suzuki S, Koide H. Treatment with polymyxin B-immobilized fiber reduced platelet activation in septic shock patients: Decrease in plasma levels of soluble P-selectin, platelet factor 4 and ß-thromboglobulin. *Inflamm Res* 1999; 48:171-175.
9. Tani T, Hanasawa K, Kodama M, Imaizumi H, Yonekawa M, Saito M, Ikeda T, Yagi Y, Takayama K, Awano I, et al. Correlation between plasma endotoxin, plasma cytokines, and plasminogen activator inhibitor-1 activities in septic patients. *World J Surg* 2001; 25:660-668.
10. Goris JA, Boekhorst TPA, Nuytinck JKS. Multiple-organ failure. *Arch Surg* 1985; 120:1109-1115.
11. Stevens LE. Gauging the severity of surgical sepsis. *Arch Surg* 1983; 118:1190-1192.
12. Cole L, Bellomo R, Hart G, Journois D, Davenport P, Tipping P, Ronco C. A phase II randomized, controlled trial of continuous hemofiltration in sepsis. *Crit Care Med* 2002; 30:100-106.
13. Borden EC, Chin P. Interleukin-6: A cytokine with potential diagnostic and therapeutic roles. *J Lab Clin Med* 1994; 123:824-829.
14. Nakamura T, Suzuki S, Ushiyama C, Shimada N, Koide H. Effect of phosphodiesterase III inhibitor on plasma concentration of endothelin-1 and tumor necrosis factor in patients with acute heart failure. *Acta Cardiol* 2002; 57:19-21.
15. Weitzberg E, Lundberg J, Rudehill A. Elevated levels of endotoxin in patients with sepsis syndrome. *Circ Shock* 1991; 33:222-227.
16. Takakuwa T, Endo S, Nakae H, Kikuchi M, Suzuki T, Inada K, Yoshida M. Plasma levels of TNF-α, endothelin-1 and thrombomodulin in patients with sepsis. *Res Commun Chem Pathol Pharmacol* 1994; 84:261-269.
17. Brauner JS, Rohde LE, Clausell N. Circulating endothelin-1 and tumor necrosis factor-α: Early predictors of mortality in patients with septic shock. *Int Care Med* 2000; 26:305-313.
18. Miossec P. Soluble interleukin-2 receptors: Clinical value and biological significance. *Presse Med* 1991; 20:997-1001.
19. Delogu G, Casula MA, Mancini P, Tellan G, Signore L. Serum neopterin and soluble interleukin-2 receptor for prediction of a shock state in Gram-negative sepsis. *J Crit Care* 1995; 10:64-71.