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HEMOPERFUSION USING THE LPS-SELECTIVE MESOPOROUS POLYMERIC ADSORBENT IN SEPTIC SHOCK: A MULTICENTER RANDOMIZED CLINICAL TRIAL

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ABSTRACT—Extracorporeal hemoperfusion (EHP) may improve the course and outcomes of patients with septic shock by targeting cytokines or bacterial endotoxins (lipopolysaccharide [LPS]). Here, we present the results of a multicenter randomized controlled trial (clinicaltrials.gov/ct2/show/NCT04827407) to assess the efficiency and safety of Efferon LPS hemoperfusion cartridges engineered for multimodal targeting LPS, host-derived cytokine, and damage-associated molecule pattern molecules. Patients with intra-abdominal sepsis (IAS) and septic shock (Sepsis-3) were subjected to EHP procedures (n = 38). Control patients with IAS and septic shock (n = 20) were treated using conventional protocols without EHP. The primary end point was resolution of septic shock. Secondary end points included MAP, vasopressor drug dose, partial pressure of arterial oxygen/fraction of inspired oxygen ratio, Sequential Organ Failure Assessment score, length of stay in the intensive care unit, and satisfaction with device use by a 5-point Likert scale. Clinical laboratory tests for a blood cells count, lactate and creatinine concentration, nephelometry test for C-reactive protein, immunochemiluminescent test for procalcitonin, and immunoenzyme analysis for IL-6 concentration were used to monitor the EHP effect versus the control group. Data were analyzed followed the intention-to-treat approach. Wilcoxon STATA 16.0 (StataCorp, College Station, TX) and Excel 2019 with XLStat 2019 add-in (Addinsoft, Paris, France) were used for statistical analysis of the results. The Fine and Gray method of competing risks was used to analyze the primary end point and other data representing the time to event. EHP resulted in a significant and rapid increase in MAP and partial pressure arterial oxygen/fraction of inspired oxygen ratio, progressive decline in norepinephrine doses, and multiorgan deficiency, as evaluated by Sequential Organ Failure Assessment scores. Importantly, EHP led to significantly rapid cumulative mechanical ventilation weaning compared with the control group (subdistribution hazard ratio, 2.5; $P = 0.037$). Early 3-day mortality was significantly reduced in the Efferon LPS versus control group; however, no significant improvements in survival in 14 and 28 days were revealed. Laboratory tests showed rapidly decreased levels of LPS, procalcitonin, C-reactive protein, IL-6, creatinine, leukocytes, and neutrophils only in the Efferon LPS group. Results demonstrate that EHP with Efferon LPS is a safe procedure to abrogate septic shock and normalize clinical and pathogenically relevant biomarkers in patients with IAS.

KEYWORDS—Abdominal sepsis; efferon LPS; endotoxin; hemoperfusion; multiple-organ failure; septic shock

ABBREVIATIONS—CRP—C-reactive protein; DAMP—damage-associated molecular patterns; EAA—endotoxin activity assay; EHP—extracorporeal hemoperfusion; IAS—intra-abdominal sepsis; LOCF—last observation carried forward; MV—mechanical ventilation; PAMP—pathogen-associated molecular patterns; PCT—procalcitonin; PMX—polymyxin B (endotoxin-binding ligand); RRT—renal replacement therapy; sHR—subdistribution hazard ratio

INTRODUCTION

Sepsis is a life-threatening illness caused by an altered immune response to an infection (1,2). Sepsis is diagnosed in 19.4 to 31.5 million patients worldwide, and more than 5 million die (3). The anatomical source of infection is essential for sepsis outcomes (4). Patients with intra-abdominal sepsis (IAS) complicating laparotomy, bowel ischemia, peritonitis, and intestinal perforation have the worst prognosis (5). Septic shock in IAS is associated with the highest mortality rate, ranging from 30% to 80% (6). Therefore, the development of versatile and rational treatments of septic shock in IAS is one of the most important challenges of critical care medicine (2,7).

Bacterial endotoxins, represented by LPSs, which constitute up to 75% of the outer membrane of gram-negative bacteria, are primary triggers in the pathogenesis of sepsis. LPS activates TLR4 or caspase-11, which trigger signaling mechanisms that initiate NF-κB expression (7,8). As a result, additional inflammatory mediators are released abundantly into the bloodstream, causing damage to the vascular endothelium, including that of small vessels, with the development of circulatory failure and

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reduced perfusion pressure. Damage-associated molecular patterns (DAMPs), including extracellular DNA released from dying cells, further promote proinflammatory response cascades by upregulating the gene expression of cytokines, chemokines, coagulation factors, complement, acute phase proteins, and nitric oxide synthase (7–9). Together, these processes cause organ cell damage and play a key role in the pathogenesis of multiple-organ failure and collapse, which are characteristics of septic shock.

State-of-the-art treatment of sepsis, including life-threatening septic shock, involves supporting vital organ function and controlling the site (or sites) of infection by source control and adequate antibiotic therapy (2). Early reduction of blood levels of pathogenically significant PAMP and DAMP molecules used along with the current intensive treatment methods is considered an effective way to recover from septic shock (10). Over the past few decades, numerous experimental and clinical studies have investigated the efficacy of extracorporeal blood purification methods in the treatment of sepsis and septic shock (11–14). The elimination of LPS and other PAMPs and DAMPs has been suggested to reduce the intensity of triggering and maintaining septic pathways, and to prevent immune exhaustion by improving the function of immunocompetent cells and increasing survival.

Over the past decade, considerable attention has been paid to the use of different types of extracorporeal blood purification for the treatment of sepsis. However, the current version of the Surviving Sepsis Campaign guidelines recommends against the use of polymyxin B hemoperfusion for the treatment of sepsis (2), "leaving the door open" for other types of blood purification interventions. In this regard, researchers are increasingly focusing on finding new solutions for selective hemoperfusion aimed at removing both endotoxins and a wider range of molecular targets (cytokines and extracellular DNA), and conducting high-quality clinical trials with a high level of evidence. Furthermore, most recent analytical reviews emphasize the need for systematic clinical studies on novel sorbent-based hemoperfusion technologies (15,16).

Recently, an Efferon LPS hemoperfusion device containing a multimodal polymeric adsorbent capable of simultaneous removal of LPS and endogenous inflammatory mediators (cytokines, etc.) was developed and approved for clinical use in Russia (17,18). The rationale behind this original approach is reliance on (*a*) intrinsic mesoporosity to bind small and middle-sized molecules (cytokines and DAMP molecules including extracellular DNA) and (*b*) surface-immobilized LPS-selective ligand to bind endotoxin molecules.

This study aimed to evaluate the efficacy and safety of hemoperfusion with Efferon LPS in patients with IAS complicated by septic shock. We hypothesize that this treatment is safe and results in septic shock resolution.

MATERIALS AND METHODS

Study design

This multicenter, randomized controlled trial was conducted at four clinical institutions in Moscow. The study design (https://clinicaltrials.gov/ct2/show/NCT04827407) was approved by the Interdisciplinary Ethics Committee "Bioethics" (Protocol No. 142 of February 11, 2021; Fig. 1).

Patients and treatment

Adult patients with IAS and septic shock, diagnosed according to the Sepsis-3 criteria (1) (subsequently confirmed by bacteriological testing), within the first 12 h after the start of vasopressor infusion and within 24 h after surgical intervention, were included in the study. Exclusion criteria were age less than 18 years, pregnancy, acute bleeding, granulocytopenia, and thrombocytopenia. For the detailed inclusion and exclusion criteria, refer to Appendix 1, http://links.lww.com/SHK/B673. All patients received basic intensive therapy for septic shock according to the Surviving Sepsis Campaign 2016 guidelines (2).

Data collection

Patient clinical status and demographic and anthropometric data were assessed upon admission to the intensive care unit. After patients were included in the study, we assessed the severity of disease according to the Acute Physiology and Chronic Health Evaluation II (APACHE II) score, Sequential Organ Failure Assessment (SOFA) score, and hemodynamic and gas exchange parameters such as MAP, use of vasopressor drugs, and partial pressure of arterial oxygen (PaO_2)/fraction of inspired oxygen (FiO_2) ratio. The platelet, leukocyte, lymphocyte, and neutrophil counts and levels of lactate, C-reactive protein (CRP), procalcitonin (PCT), and IL-6 were evaluated at baseline and 72 h after inclusion in the study. Endotoxin levels were determined using a kinetic chromogenic limulus amebocyte lysate test (Appendix 2, http://links.lww.com/SHK/B673). The satisfaction of the investigators with the use of hemoperfusion was assessed using a 5-point Likert scale (19). Microbiological monitoring results, duration of mechanical ventilation (MV), and need for renal replacement therapy (RRT) were evaluated.

Intervention

A single-use Efferon LPS hemoperfusion cartridge, containing a polymeric adsorbent, was used. The polymeric matrix of the sorbent consisted of macroporous hypercrosslinked polystyrene beads with a large specific surface area of 700 to 900 m^2/g (these polymers were first obtained and described by Davankov and Tsyurupa (20)). Cytokines and other DAMP molecules are adsorbed into internal pores by a nonspecific hydrophobic mechanism (van der Waals interactions). A synthetic ligand of the conserved LPS domain, Lipid A covalently immobilized on the surface of the hypercrosslinked matrix (21).

Thus, the intrinsically porous matrix and surface-immobilized LPS-selective ligand provide an adsorbent with "multimodal" type of action, preferentially targeting both bacterial and host-derived pathogenic molecules, which are two types of dissimilar therapeutic targets.

Patients who met the inclusion criteria and signed an informed consent form were randomized 2:1 using the Interactive Web Response Systems. No later than 24 h after patient inclusion, extracorporeal hemoperfusion (EHP) treatment was performed (Efferon LPS group) or standard therapy was used (control group). Hemoperfusion using Efferon LPS was performed twice at 24-h intervals. The extracorporeal circuit was rinsed with 1,000 mL 0.9% NaCl solution containing 5,000 IU unfractionated heparin. The duration of hemoperfusion was at least 4 h, using a standard dialysis catheter at a blood flow rate of 100 to 160 mL/min. Anticoagulation was performed with unfractionated heparin.

End Points

The primary end point was resolution of septic shock (the time interval from the start of vasopressor support to the sustained discontinuation of vasopressor support over 4 h was calculated).

Secondary end points (evaluated in 72 h) included MAP, vasopressor drug dose, PaO_2/FiO_2 ratio, SOFA score, length of stay in the intensive care unit, and satisfaction with device use (5-point Likert scale; Appendix 3, http://links.lww.com/SHK/B673).

In addition, outcomes were assessed using 3-, 7-, 14-, and 28-day mortality rates; total hospital mortality; and duration of MV. The subgroups of surviving patients and the requirements for RRT were compared. Endotoxin levels, routine blood tests, and markers of systemic inflammation were assessed 0 and 72 h later (Fig. 1 and Appendix 3, http://links.lww.com/SHK/B673).

Statistical analysis

We used STATA 16.0 (StataCorp, College Station, TX) and Excel 2019 with XLStat 2019 add-in (Addinsoft) for statistical analysis of the results. Data are presented as medians (1Q, 3Q). Data analysis followed the intention-to-treat approach. The Wilcoxon exact test was used for paired samples, and the Mann-Whitney *U* test was used for unpaired samples. When analyzing longitudinal data, two approaches were used: censoring of patients dropped out because of death and imputation of the last observation instead of missing data, the so-called last observation carried forward method (LOCF) (22). The nonstandardized effect size for repeated measurements, 0- and 72-h time points, and Δ_{RM} were also calculated, and the data are presented as the median (1Q, 3Q) of the individual

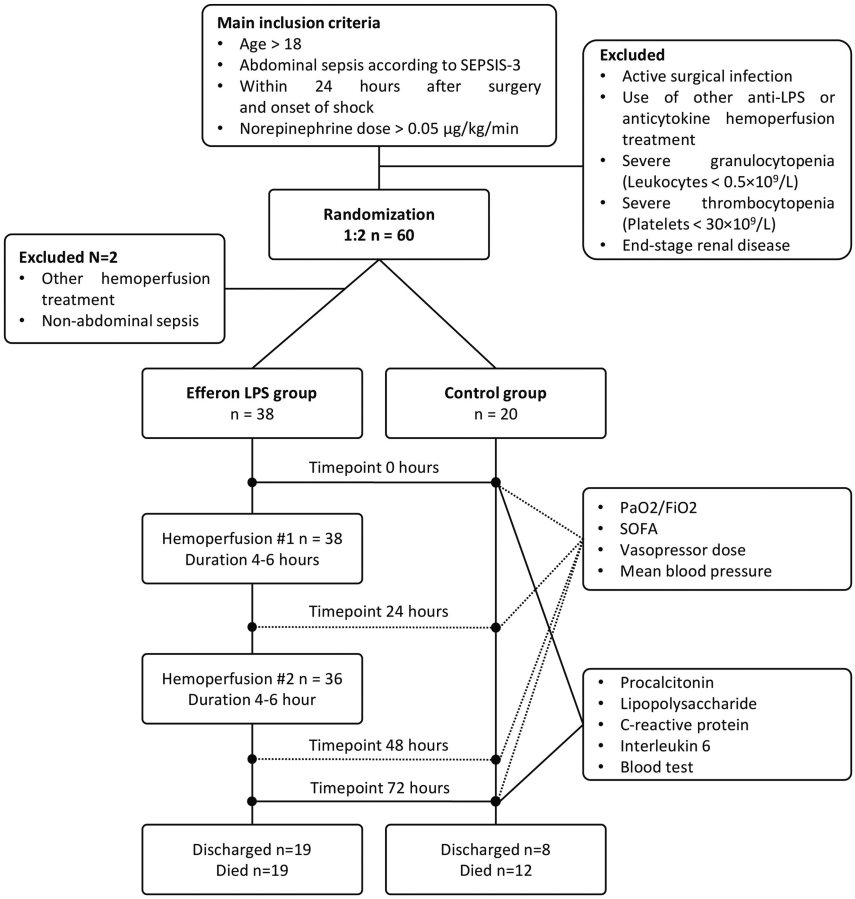


FIG. 1. **Trial flow diagram.**

changes in the parameters. Fisher exact test was used to compare the rates of unrelated samples. The Fine and Gray method of competing risks was used to analyze the primary end point and other data representing the time to event (23). The originally scheduled use of the Kaplan-Meier method or the Cox model was suboptimal for analyzing data in terms of the time interval to "septic shock resolution" because the alternative event "death during septic shock" should be regarded not as a noninformative censoring but rather as a competing risk one (24). All results were considered statistically significant at $P < 0.05$.

RESULTS

Of the 60 patients with abdominal sepsis and septic shock included in the study and randomized 2:1 (40 patients in the Efferon LPS group and 20 in the control group), March 2021 to May 2022, 2 patients in the main group were withdrawn from the study because of protocol deviation. One patient was diagnosed with nonabdominal sepsis, and the other was additionally treated with anticytokine hemoperfusion using CytoSorb cartridges (Cytosorbents Inc., Princeton, NJ).

In the Efferon LPS group (Table 1), the median age was 53 years; in the control group, it was 66 years. The percentages of women were 57.9% and 35%, respectively; the APACHE II score was 24 points in both groups; and the SOFA scores were 7.0 and 7.5 points, respectively. The groups did not differ significantly in age, sex, body mass index, APACHE II, and SOFA scores (Appendix 4, Fig. 3S, http://links.lww.com/SHK/B673), hemodynamic status, or the PAO_2/FIO_2 ratio. All patients received vasopressor support; 97.4% of patients in the Efferon LPS group and 85% of patients in the control group required MV ($P = 0.114$), and 73.7% and 60% required RRT, respectively ($P = 0.373$). Blood, urine, and abdominal fluid cultures did not differ significantly between groups. Gram-negative organisms were isolated in 50% of the Efferon LPS group and in 55% of the control group, whereas mixed species were found in 45% and 40%, respectively.

Thirty-six patients underwent two hemoperfusions using Efferon LPS, whereas two patients underwent only one hemoperfusion because of lethal outcomes (Table 2). The time from development of septic shock to hemoperfusion was 5.2 (3.0, 12.2) h, and between the first and the second hemoperfusion, it was 24.5 (23.3, 26.0) h. The median duration of hemoperfusion was 300 (300, 360) min for the first treatment and 300 (250, 300) min for the second treatment. Efferon LPS treatment was performed in combination with RRT in 15.8% and 19.4% of patients, and the doses of unfractionated heparin were 870 (500, 1,000) and 750 (500, 1,200) IU/h, respectively.

The safety of hemoperfusions

During hemoperfusion, the following accidents were observed: a patient developed delirium 240 min after hemoperfusion (with underlying barbiturate withdrawal), another patient had reduced duration of the first hemoperfusion (180 min) because of catheter displacement, and one extracorporeal circuit clotting was identified 120 min after hemoperfusion. Thus, the rate of extracorporeal circuit clotting during hemoperfusion was less than 2%.

TABLE 1. **Baseline patient characteristics in the study groups**

Variables	Efferon LPS (n = 38)	Control (n = 20)	*P*
Age, y	53 (41, 70)	66 (47, 75)	0.561
Sex, M/F	16/22	13/7	0.167
Body mass index, kg/m^2	25.8 (22.7, 33.5)	31.1 (28.7, 32.8)	0.157
Mechanical ventilation, n (%)	37 (97.4)	17 (85)	0.114
Vasopressor support, n (%)	38 (100)	20 (100)	1.0
Renal replacement therapy, n (%)	28 (73.7)	12 (60)	0.373
APACHE II score	24 (22, 26)	24 (23, 25)	1.0
SOFA score	7.0 (7, 9)	7.5 (6, 11)	0.827
MAP, mm Hg	63 (56, 71)	63 (58, 81)	0.460
Norepinephrine dose, μg kg^{-1} min^{-1}	0.74 (0.40, 0.90)	0.60 (0.28, 0.87)	0.358
Patients refraction to norepinephrine, dose ≥0.5 μg kg^{-1} min^{-1}, n (%)	25 (66)	11 (55)	0.570
P_{AO_2}/F_{IO_2} ratio, mm Hg	273 (233, 293)	239 (209, 286)	0.258
Causes of septic shock			
Intestinal perforation, n (%)	10 (26.3)	8 (40)	0.373
Perforated peptic ulcer, n (%)	8 (21.1)	2 (10)	0.468
Perforated colonic diverticula, n (%)	5 (13.2)	4 (20)	0.705
Urinary tract infection, n (%)	6 (15.8)	3 (15)	1.0
Biliary sepsis, n (%)	4 (10.5)	1 (5)	0.650
Infected necrosis severe acute pancreatitis, n (%)	3 (7.9)	2 (10)	1.0
Acute appendicitis, n (%)	2 (5.3)	0	0.540
Microorganisms*			
Gram negative, n (%)	19 (50)	11 (55)	0.787
Gram positive, n (%)	1 (2.6)	0	1.0
Mixed, n (%)	17 (44.7)	8 (40)	0.786
No growth, n (%)	1 (2.6)	1 (5)	1.0

Data are presented as median (1Q, 3Q).

*Microorganisms were isolated from blood, urine, and abdominal fluid cultures. The significance of the differences was assessed using the nonparametric Mann-Whitney test and Fisher exact test.

APACHE II, Acute Physiology and Chronic Health Evaluation II; F_{IO_2}, fraction of inspired oxygen; M/F, male/female; n, number of patients; P_{AO_2}, partial pressure arterial oxygen; RRT, renal replacement therapy; SOFA, Sequential Organ Failure Assessment.

In the Efferon LPS group, there was a significant decrease in platelet count after 72 h (Δ_{RM} = −32 [−164, +21] × 10^9/L, P = 0.022); in the control group, the effect size was comparable at Δ_{RM} = −38 (−64, +17) × 10^9/L (Efferon LPS group vs. control group, P = 0.582). However, all changes in platelet count were within the normal range in both groups (Table 3).

Clinical effects of hemoperfusion with Efferon LPS

Septic shock resolved in 26 of the 38 patients in the Efferon LPS group (68%) compared with only 9 of the 20 patients in the control group (45%) (P = 0.098). The duration of septic shock (time to vasopressor withdrawal) in the surviving patient cohorts differed significantly between the treatment groups, with a median time to blood pressure normalization of 57 (37, 80) h in the Efferon LPS group and 101 (58, 197) h in the control group (subdistribution hazard ratio [sHR], 2.20; 95% confidence interval [CI], 1.11–4.34; P = 0.029; Fig. 2).

Further evaluation revealed strong and clinically significant effects of hemoperfusion, manifesting as an increased likelihood of shock halting and recovery.

Thus, the use of Efferon LPS resulted in a significant increase in MAP from 63 (56, 71) to 86 (69, 91) mm Hg (P < 0.001) as early as 24 h after the start of hemoperfusion (Fig. 3A). The rise in blood pressure continued further, and by 72 h, the MAP increased to 94 (84, 103) mm Hg (P < 0.001). In the control group, the increase in MAP was less significant, from 63 (58–80) mm Hg at 0 h to 67 (62–77) mm Hg at 24 h (P = 0.229). Significant differences in MAP versus time point 0 were observed only at the final time point of the study (72 h; Fig. 3A). Doses of vasopressor drugs (adjusted to the 2020 Vasoactive Inotropic Score

TABLE 2. **Parameters and accidents during hemoperfusion using the Efferon LPS**

Variables	First hemoperfusion (n = 38)	Second hemoperfusion (n = 36)
Time from septic shock to the first hemoperfusion, h	5.2 (3.0, 12.2)	
Time from first to second hemoperfusion, h		24.5 (23.3, 26)
Blood flow rate, mL/min	140 (120, 150)	140 (120, 150)
Duration of treatment, min	300 (300, 360)	300 (246, 300)
Combination with RRT, n (%)	6 (15.8)	7 (19.4)
Heparin dose, U/h	870 (500, 1,000)	750 (500, 1,200)
Accidents	Catheter displacement: 1	Thrombosis: 1 Delirium: 1

Data are presented as median (1Q, 3Q).

n, number of patients; RRT, renal replacement therapy.

TABLE 3. **Changes in patient parameters**

Variables	Group	Baseline	At 72-h time point	Effect size charge to baseline, Δ_{RM}
LPS, EU/mL	Efferon LPS (n = 37)	0.19 (0.11, 0.53)	0.12 (0.07, 0.33)	**–0.03* (–0.18, +0.00)**
	Control (n = 18)	0.17 (0.07, 0.51)	0.14 (0.07, 0.42)	+0.01 (–0.23, +0.12)
	P	0.702	0.651 (0.649)*	0.328
Lactate, mmol/L	Efferon LPS	3.2 (2.6, 4.2)	1.3 (1.1, 2.2)	**–1.4** (–2.7, –1.05)**
	Control	3.0 (2.3, 4.9)	1.9 (1.7, 3.8)	**–1.1* (–2.2, –0.4)**
	P	0.802	0.222 (**0.023**)	0.249
Creatinine, μmol/L	Efferon LPS	166 (112, 253)	107 (78, 159)	**–34** (–116, –12)**
	Control	178 (92, 272)	89 (81, 264)	–26 (–44, –8)
	P	0.942	0.963 (0.385)	0.449
Total bilirubin, μmol/L	Efferon LPS	19 (9.1, 26.0)	18 (10.3, 26.4)	–1.3 (–6.4, +4.0)
	Control	19 (10.7, 28.1)	15 (10, 27.4)	–3.3 (–7.8, +1.8)
	P	0.865	0.964 (0.981)	0.855
PCT, ng/mL	Efferon LPS	14.9 (9, 31.3)	5.7 (2, 10.4)	**–5.05** (–15, –2.1)**
	Control	6.4 (9, 31.3)	4.9 (2.1, 15.1)	–0.03 (–2.4, +2)
	P	0.068	0.962 (0.872)	**0.035**
CRP, mg/L	Efferon LPS	232 (193, 335)	154 (115, 217)	**–51** (–124, –24)**
	Control	232 (160, 316)	175 (146, 279)	–13 (–59, +11)
	P	0.740	0.105 (**0.035**)	**0.017**
IL-6, pg/mL	Efferon LPS (n = 37)	586 (132, 1758)	251 (107, 563)	**–117* (–1,462, +152)**
	Control (n = 18)	422 (143, 981)	449 (78, 1,050)	–22 (–390, +58)
	P	0.612	0.591 (0.930)	0.459
Leucocyte count, $\times 10^9$/L	Efferon LPS	17.6 (10.4, 22.3)	11.8 (8.5, 15.2)	**–3** (–8.7, –1.2)**
	Control	14.2 (11.2, 17.3)	18.6 (9.2, 19.7)	–1.6 (–3.1, 4.4)
	P	0.309	0.133 (0.154)	**0.017**
Neutrophil count, $\times 10^{9/}$L	Efferon LPS	15.3 (8.4, 20.4)	9.2 (6.9, 12.8)	–2.5 (–9.2, –1.3)
	Control	12.4 (9.3, 14.6)	15.7 (7.9, 16.6)	–1.4 (–2.1, +4.5)
	P	0.238	0.166 (0.179)	**0.010**
Lymphocyte count, $\times 10^9$/L	Efferon LPS	1.04 (0.54, 1.36)	0.80 (0.45, 1.32)	–0.24 (–0.70, +0.20)
	Control	1.04 (0.6, 1.73)	1.38 (0.99, 1.63)	+0.02 (–0.47, +0.48)
	P	0.450	**0.034** (**0.033**)	0.408
Platelet count, $\times 10^9$/L	Efferon LPS	236 (138, 330)	167 (110, 263)	**–32* (–164, +21)**
	Control	200 (81, 319)	152 (81, 302)	–38 (–64, +17)
	P	0.565	0.680 (0.764)	0.582
APTT, s	Efferon LPS	30.0 (24.1, 37.2)	32.4 (30.4, 44.8)	**+4.7* (–2.9, +11)**
	Control	31.4 (27.5, 38.2)	32.8 (28.3, 37.7)	+4.3 (–1.7, +5.8)
	P	0.511	0.477 (0.333)	0.222
RRT requirement, n/N (%)	Efferon LPS	28/38 (74)	11/33 (33)	**–15/26*** (–58)**[a]
	Control	12/20 (60)	5/12 (42)	–2/13 (–15)[a]
	P[b]	0.373	0.738 (0.271)	0.14

Significant differences are marked in bold.

Data are presented as medians (1Q, 3Q), and *P* values were calculated using the Mann-Whitney test (*P* value according to the LOCF method). Δ_p is the absolute value of the repeated measurements effect, that is, the change in the parameter value calculated as the patient value at 72 h minus the patient value at 0 h. Data are also presented as Me (1Q, 3Q).

[a] Data are presented as the ratio of the number of patients weaned from RRT to the number of patients on RRT, for which both values are available (survivors at the 72-h point).

[b] Fisher exact test.

APTT, activated partial thromboplastin time; CRP, C-reactive protein; PCT, procalcitonin; RRT, renal replacement therapy.

*$P < 0.05$ according to the Wilcoxon exact sign test.

**$P < 0.001$ according to the Wilcoxon exact sign test.

***$P < 0.05$, according to the McNemar exact test for paired proportions.

norepinephrine dose (25)) progressively decreased in the Efferon LPS group, and difference versus control group became significant as early as after 48 h from the start of the hemoperfusion (reduction from 0.74 to 0.16 μg kg^{-1} min^{-1}, $P = 0.027$; Fig. 3B). By contrast, in the control group, norepinephrine dose increased significantly from 0.60 to 0.73 μg kg^{-1} min^{-1} after 24 h and returned to baseline values after 48 h (Fig. 3B).

Furthermore, in the Efferon LPS group, the PaO_2/FiO_2 ratio increased significantly during the follow-up period, and its values at any specific time point were significantly higher than those in the control group (Fig. 3C). When analyzing the severity of multiple-organ dysfunction based on the SOFA score, we found that in the Efferon LPS group, the median value of the index after 24 h was unchanged versus time point 0 (7 points), and a significant decrease was observed at 72 h (down to 3 points; Fig. 3D). In the control group, however, the mean SOFA value initially was 7.5 points, and at 24 and 48 h, it increased to 9 points with a further drop to 5 points only at 72 h. The differences between the groups were significant after 24 h of therapy (Fig. 3D, $P = 0.043$).

Notably, as a result of the high mortality in the control group at time points of 48 and 72 h (30% and 40%, respectively), there was an apparent "improvement" in median group values due to dropout (censoring due to death) of the most critically ill patients (rather than an improvement in each patient's performance). In

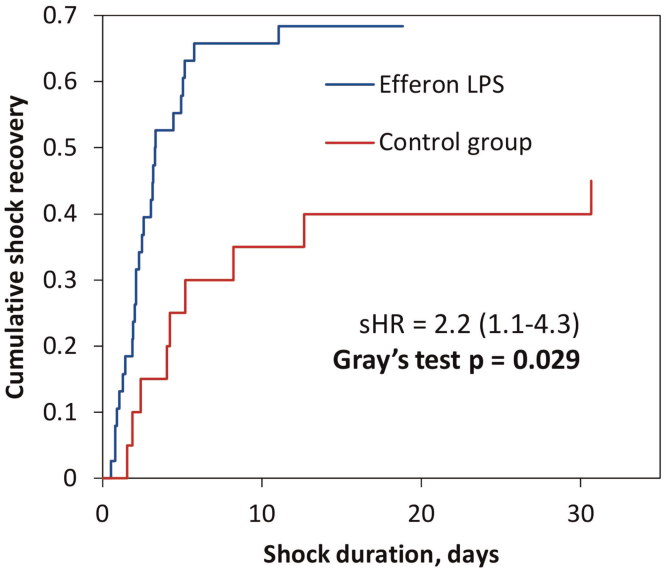


FIG. 2. **Cumulative incidence curves of septic shock duration in the study groups.** sHR indicates subdistribution hazard ratio.

addition, the statistical power of the intergroup comparison test decreased because of the reduced number of patients. To increase the statistical power of the analysis and ensure a less biased estimation of intergroup differences with dropout due to death, we used the LOCF approach (22). The estimation of the median values using the LOCF method is shown as a dotted line in Figure 3. This shift was most clearly observed in the SOFA score and norepinephrine dose graphs. In fact, the patients in the control group did not show a decrease in the severity of multiple-organ failure or in the required vasopressor dose at the 72-h time point. Intergroup differences detected using the LOCF method were significant at 24, 48, and 72 h for all four parameters (Fig. 3, LOCF values).

Analysis of treatment outcomes (Fig. 4A) revealed that early 3-day mortality was significantly reduced in the Efferon LPS group compared with the control group (13% and 40%, respectively; $P = 0.012$). There was not significant trend toward decreased hospital mortality, including 28-day mortality, in the Efferon LPS group compared with the control group (47% vs. 55%, $P = 0.783$), and a similar trend was observed in cumulative hospital mortality (sHR, 0.6; 95% CI, 0.3–1.4; $P = 0.250$).

MV is a routine life support method frequently used in patients with septic shock. In our study, a vast majority of patients were on MV at point 0 (37 of 38 in the Efferon LPS group and 17 of 20 in the control group). As shown in Figure 4B, the duration of MV among the survivors was lower in the Efferon LPS group than in the control group (2.6 [1.3, 5.8] and 4.8 [2.0, 12.5] days, respectively), and they were more likely to be weaned (21 of 37 [57%] and 5 of 17 [29%] days, respectively; sHR, 2.5; 95% CI, 1.1–5.5; $P = 0.037$).

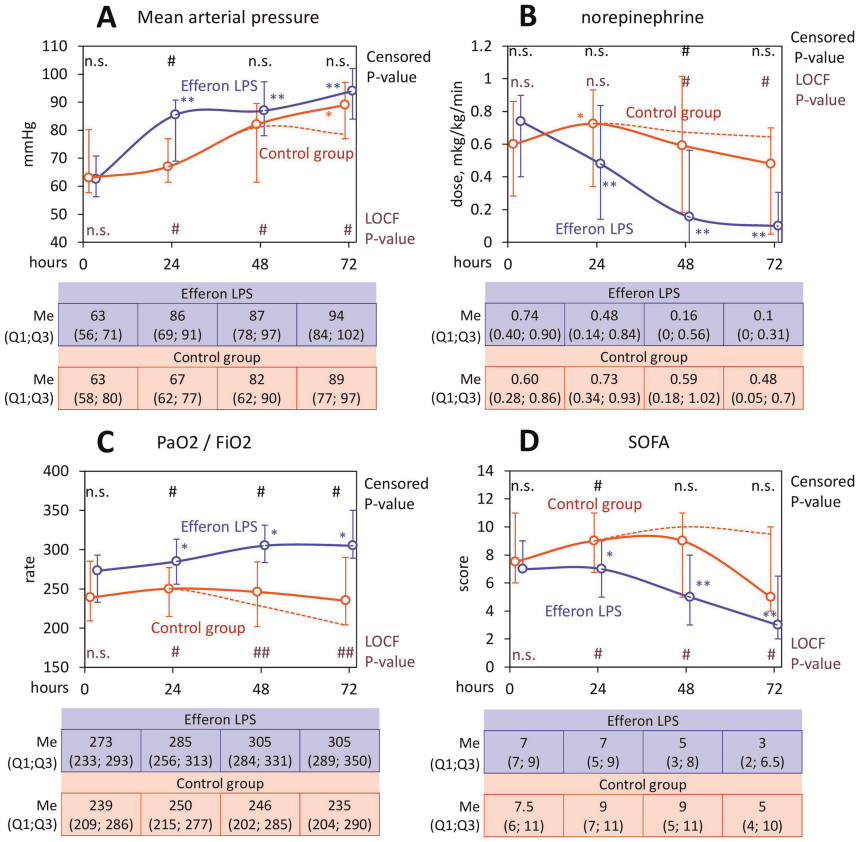
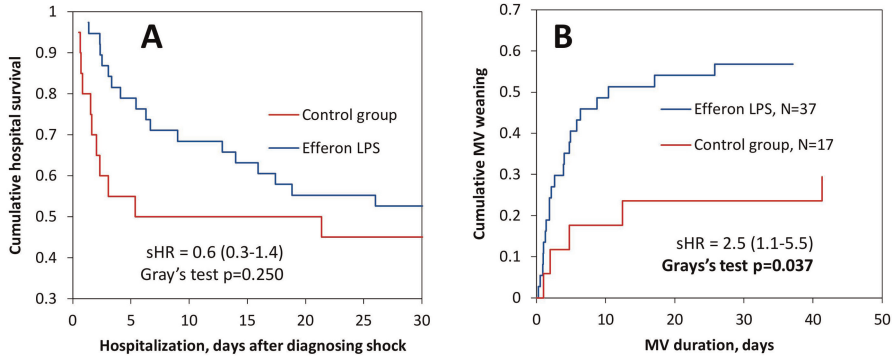


FIG. 3. **Changes in key parameters of disease severity during treatment.** #$P < 0.05$ and ##$P < 0.001$, Mann-Whitney exact intergroup test. *$P < 0.05$ and **$P < 0.001$, Wilcoxon exact sign test (hour 0 comparison). Censored P values were obtained after death-related censoring, LOCF P values were obtained using LOCF method (dotted line). A, Changes in MAP. B, Changes in vasopressor support. C, Changes in respiratory index. D, Changes in organ dysfunction severity according to SOFA scale. LOCF, last observation carried forward; n.s., not significant; SOFA, Sequential Organ Failure Assessment.



Survival, n (%)

Days	3	7	14	28
Efferon LPS	33 (87)	27 (71)	25 (66)	20 (53)
Control group	12 (60)	10 (50)	10 (50)	9 (45)

FIG. 4. **A, Cumulative incidence curves of hospital survival after diagnosing shock.** B, Incidence curves of MV duration in patients weaned from mechanical ventilation. MV indicates mechanical ventilation.

Effects of hemoperfusion on clinical and laboratory parameters

Clinically relevant laboratory parameters were studied in the patients during treatment. Table 3 summarizes the clinical and laboratory parameters before hemoperfusion, with no significant differences between the Efferon LPS and control groups.

However, comparing the pretreatment and posttreatment data, a significant improvement was observed in patients receiving hemoperfusion. Thus, the levels of LPS, PCT, CRP, IL-6, creatinine, leukocytes, and neutrophils significantly decreased in the Efferon LPS group (Table 3). Endotoxin levels decreased from 0.19 to 0.12 EU/mL when assessed 72 h after the use of Efferon LPS. The median change in LPS level was $\Delta_{RM} = -0.03$ (−0.18, +0.00) ($P = 0.027$). As can be seen from the data presented, the median change in LPS was 0–0.18 EU/mL in 50% of cases and more than 0.18 EU/mL in 25% of cases, whereas no LPS reduction was observed in 25% of cases.

Alternatively, in the control group, a different effect was observed with the mean decrease in LPS level from 0.17 to 0.14 EU/mL. The median change was $\Delta_{RM} = +0.011$ (−0.23, +0.12; $P = 0.328$), which means that most surviving patients (n/N = 6/11) exhibited a trend in increasing the LPS level. This suggests that the decrease in median LPS level in the control group may be associated with death (censoring) in patients with initially high LPS levels.

As seen from the Table 3, hemoperfusion resulted in decreased molecular markers of systemic inflammation and sepsis severity: PCT from 14.9 to 5.7 ng/mL ($P < 0.001$), CRP from 232 to 154 mg/L ($P < 0.001$), IL-6 from 586 to 251 pg/mL ($P = 0.046$), and creatinine level from 165.5 to 107.0 μmol/L ($P < 0.001$). Lactate level decreased significantly in both groups after 72 h: from 3.2 to 1.3 mmol/L ($P < 0.001$) in the Efferon LPS group and from 3.0 to 1.9 mmol/L ($P = 0.015$) in the control group. Leukocyte counts decreased in the Efferon LPS group from 17.6 to 11.8 $\times$ 10^9/L ($P < 0.001$) and neutrophil counts from 15.3 to 9.2 $\times$ 10^9/L ($P < 0.001$), whereas the effect size in the hemoperfusion and control groups was significantly different (for leukocytes, $\Delta_{RM} = -3$ [−8.7, −1.2] and −1.6 [−3.1, 4.4], respectively [$P = 0.017$]; for neutrophils, $\Delta_{RM} = -2.5$ [−9.2, −1.3] and −1.4 [−2.1, +4.5], respectively [$P = 0.010$]). Meanwhile, the levels of total bilirubin and percentage of lymphocytes did not change significantly after the two hemoperfusions.

Hemoperfusions yielded a significant decrease in platelet count from 236 to 167 $\times$ 10^9/L ($P = 0.024$) and an increase in APTT from 30.0 to 32.4 s ($P = 0.010$) by the 72-h time point. The requirement for RRT support in the survivors at the 72-h time point significantly decreased in the Efferon LPS group from 74% to 33% ($P < 0.001$), in contrast to the control group.

When analyzing satisfaction with Efferon LPS use according to the Likert scale, the following data were obtained: 29% of the physicians rated the efficacy and safety of the treatments as excellent, 45% as good, 18% as satisfactory, and 8% did not respond.

Our findings demonstrate a strong beneficial effect of hemoperfusion with Efferon LPS on the levels of pathogenetically specific and significant biomarkers of septic shock.

DISCUSSION

In a multicenter randomized controlled Lipopolysaccharide Adsorption in Septic Shock (LASSO) study, hemoperfusion using the Efferon LPS device was safe and associated with significant improvements in hemodynamic and gas exchange parameters as well as with reduced organ dysfunction in septic shock. The use of Efferon LPS resulted in more frequent and faster resolution of septic shock than in the control group. The results obtained in this clinical study are consistent with the findings of numerous studies aimed at evaluating the effectiveness of selective endotoxin adsorption using various adsorption cartridges. Most of these studies used Toraymyxin PMX-20R (Toray Medical Co Ltd, Tokyo, Japan) as a hemoperfusion device, where polymyxin B (PMX; endotoxin-binding ligand) was immobilized on polystyrene fiber (26,27).

The Early Use of Polymyxin B Hemoperfusion in Abdominal Septic Shock 2 study reported the results of PMX hemoperfusions in 357 patients over the previous 5-year period in 57 centers in Europe and Asia (28). Abdominal infections were the most frequent cause of sepsis and septic shock (44%). The hospital mortality rate was 50%, which was similar to that in our study. When comparing centers in Europe and Asia, differences in hospital

mortality rates were observed, with mortality rates of 46.8% and 65%, respectively.

In 2018, the results of the Effect of Targeted Polymyxin B Hemoperfusion on 28-Day Mortality in Patients With Septic Shock and Elevated Endotoxin Level study (29) on patients with septic shock showed an endotoxin activity assay (EAA) score higher than 0.6. In the primary analysis, the 28-day mortality did not differ between the main and control groups (37.7% and 34.5%, respectively). The authors found no differences between the groups with regard to the EAA levels at baseline and at 48 and 72 h. In a subsequent *post hoc* analysis, after the exclusion of patients with high EAA levels (>0.9), a significant reduction in 28-day mortality was observed in the PMX group (26.1% vs. 36.8% in the control group) (30).

Treatment with the anticytokine adsorber CytoSorb has also been shown to improve hemodynamic parameters and reduce hospital mortality in septic shock in an observational trial (35.7% vs. 61.9% in control [$P = 0.015$] and 34.9% vs. 42.8% [$P < 0.001$], respectively) (31).

Nevertheless, the authors of the meta-analyses believe that the results obtained using different sorbents are not yet valid because of the small cohort sizes. Meanwhile, such studies have been universally considered highly promising, because no other reliable, high-tech, and pathogenesis-oriented methods for reducing extremely high mortality in septic shock have been proposed thus far in a clinical setting (15,16).

Hemoperfusion using Efferon LPS resulted in significantly increased 3-day survival compared with that in the control group (87% and 60%, respectively; $P = 0.012$) with tapering over time. We found no significant reduction in cumulative hospital mortality (Fisher test) or risk of hospital mortality (Gray's test). This effect of delayed mortality in the hemoperfusion group could be explained by the need to maintain longer life support in the hemoperfusion group. However, this was not the case in this study. In addition to reduced early mortality, there is significant and rapid stabilization of hemodynamics, earlier and more frequent resolution of septic shock, and successful weaning from MV and RRT. The lack of a significant difference in the cumulative and hospital mortality when using hemoperfusion compared with the control group during a longer intensive care unit stay may be due to the impact of other lethal conditions/complications manifesting relatively later and, most importantly, independent of hemoperfusion. An increase in the statistical power is required to reveal the mechanisms underlying the protective effect of hemoperfusion using Efferon LPS, which provides a rationale for further research.

Limitations

Our study has several limitations.

First, the inadequate power of the study did not allow for conclusions regarding the impact on the cumulative hospital survival of patients. Although cumulative survival was not among the end points of our study, measurement of this parameter is essential for clinicians. Even with expected survival rates of 40% and 60% and group sizes of 20 and 38, the power of Fisher exact test is only 0.22 (the probability of finding a significant effect in the study sample if it actually exists in the general population), and reaching a power of 0.8 requires recruitment of 79 and 158 patients into groups, respectively. The use of the Fine and Gray "time-to-event" survival model did not significantly increase the statistical power of this study.

Second, at 72 h after hemoperfusion initiation, 87% of the patients in the Efferon LPS group and 60% in the control group survived ($P = 0.012$). This high percentage of utmost severely affected dropouts created a characteristic bias in the outcome trends at the 72-h time point ("survivor bias") and negatively affected the significance and validity of the results.

Third, the Efferon LPS group included two patients who had only one hemoperfusion, although the study design implied two hemoperfusions with the Efferon LPS device.

CONCLUSIONS

The use of hemoperfusion with Efferon LPS was associated with significant improvement in patients with septic shock: (1) Hemodynamic parameters: an increase in MAP and a decrease in the need for high-dose norepinephrine (both after 24 h) and dramatically shorter shock duration among survivors (57 vs. 101 h, respectively) (2) Respiratory function: a lesser duration of MV among the survivors (2.6 vs. 4.8 days), increased cumulative MV weaning, and an increase in PaO_2/FiO_2 ratio (starting 24 h post-EHP) (3) Renal function: decreased serum creatinine levels and reduced RRT requirements (4) Decreased multiple-organ deficiency severity starting 24 h post-EHP as evidenced by decreasing the SOFA score (5) Decreased levels of inflammatory markers PCT, CRP, IL-6, leukocyte, and neutrophil counts, accompanied by a reduction in bacterial LPS levels in 75% of patients.

Statistically significant improvements in vital parameters were accompanied by trends in the successful resolution of septic shock (68% vs. 45% in the control group at 72 h) and increased 3-day hospital survival versus the control group (87% and 60%, respectively; $P = 0.012$). Trend toward decreased hospital mortality, including 28-day mortality, in the Efferon LPS group compared with the control group was not significant (47% vs. 55%, $P = 0.783$).

New studies with increased statistical power are warranted to assess the effects of hemoperfusion on hospital and 28-day mortality rates.

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