CHAPTER 193

Toraymyxin and Other Endotoxin Adsorption Systems

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OBJECTIVES

This chapter will:

1. Describe the role of endotoxin in sepsis.
2. Discuss diagnostic approaches to endotoxin measurement.
3. Describe the history of endotoxin-targeted therapy in sepsis, from drugs to medical devices.
4. Discuss the characteristics of the Toraymyxin endotoxin adsorption system as well as other endotoxin removal systems.
5. Discuss the clinical data on Toraymyxin.
6. Discuss the preliminary results of the EUPHRATES trial (Evaluating the Use of Polymyxin B Hemoperfusion in a Randomized Controlled Trial of Adults Treated for Endotoxemia and Septic Shock).

ROLE AND MEASUREMENT OF ENDOTOXIN IN SEPTIC PATIENTS

Endotoxin, also known as lipopolysaccharide, is a major cell wall constituent of gram-negative bacteria (Fig. 193.1). The lipid A portion of endotoxin is conserved across bacterial species and is responsible for many of the pathogenic effects observed in sepsis. Endotoxin in the blood comes from several potential sources, a bacterial infection or translocation of bacteria that reside normally in the commensal flora of the gastrointestinal tract during periods of hypotension and impaired gut perfusion in shock.[1–3] Endotoxin causes the release of cytokines such as interleukin-6 and tumor necrosis factor-alpha and activates complement and coagulation factors resulting in multiple organ failure and even death. High levels of endotoxin in the blood are responsible for many of the symptoms seen during sepsis such as fever and elevated white blood cell count. Severe sepsis occurs when the body is overwhelmed by the inflammatory response and body organs begin to fail. Sepsis may develop as a result of infections acquired in the community, such as pneumonia, or may be a complication that develops during the treatment of trauma or cancer or after major surgery. Increased levels of endotoxin can be associated with other conditions in critically ill patients, such as cardiac surgery and burns.

The historical assay for the detection of lipopolysaccharide in non–blood-based or non–plasma-based fluids has been the *limulus* amebocyte lysate (LAL) assay. It is based on the observation in 1956 that killed gram-negative bacteria caused the blood of the horseshoe crab to clot. This test performs well in matrices such as crystalloids, food products, or solutions such as dialysate. In plasma and in whole blood, however, lipopolysaccharide exists in a variety of different forms and binds to lipopolysaccharide-binding protein and other substances including lipoproteins, so various extraction and pretreatment strategies have been developed to attempt to release lipopolysaccharide from its binding sites in whole blood or plasma and to neutralize inhibitors including fungal products and other proteins that confound *LAL* technologies. These changes have not been successful in overcoming all issues, however. In fact the *LAL* test has never been approved by regulatory agencies in North America for clinical use in humans. Nevertheless, some clinical data has been collected with it, demonstrating an association between high levels of endotoxin in sepsis and mortality.

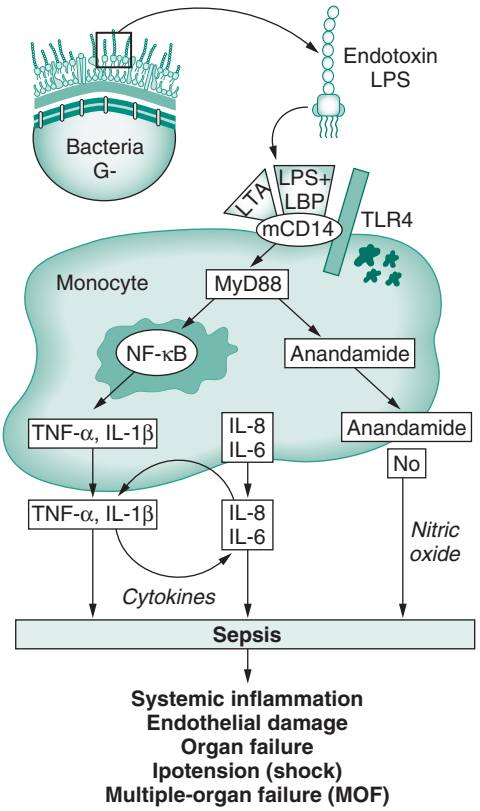

FIGURE 193.1 Role of endotoxin in sepsis. Endotoxin triggers the release of inflammatory mediators and nitric oxide, a major endogenous vasodilator.

The Endotoxin Activity Assay (EAA) was cleared by the U.S. Food and Drug Administration for the measurement of endotoxin in whole blood in 2004 to assess patients for risk of severe sepsis.

The test is done as a rapid assay in whole blood and relies on the following factors:

- Endotoxin reacts with a high-affinity antibody specific to lipid A.
- The antibody-antigen complex activates complement and is amplified by the patient's neutrophils in whole blood.
- The amplification results in an enhanced respiratory burst in the presence of zymosan; the burst is detected by luminal chemiluminescence.
- The magnitude of the priming influence is proportional to the concentration of antigen-antibody complex.

The test is feasible, accurate, and reliable, providing in less than 40 minutes a measurement of blood endotoxin concentration. In a multicenter clinical trial involving approximately 1000 patients, the test was shown to have excellent test characteristics in critically ill patients with suspected sepsis, and its results correlated strongly with adverse outcomes, including death and organ dysfunction.[4] Elevations of endotoxin are associated significantly with the development of a clinical diagnosis of severe sepsis. When endotoxin levels are moderately high, the odds ratio for development of severe sepsis is 2.0; when endotoxin levels are very high, the odds ratio rises to 3.0. Results of this trial suggest that the EAA could be used as a biomarker of the risk for development of sepsis as well as a trigger for targeted endotoxin-directed therapies.

THERAPIES AGAINST ENDOTOXINS: FROM DRUGS TO MEDICAL DEVICES

Many efforts have been made in the last three decades to assess therapies against endotoxin, the primary trigger of the inflammatory process, as a drug target.[5] The idea started in the middle 1970s, when polymyxin B was discovered to be protective against endotoxin-induced hemodynamic shock but at the same time was demonstrated to be extremely toxic to the kidney and the central nervous system.[6]

A number of anti-endotoxin strategies have been tested (e.g., monoclonal antibodies, small molecule Toll-like receptor 4 [TLR4] inhibitors, endotoxin binding proteins), but all have failed to demonstrate reproducible outcomes in septic subjects after early positive results.[5] An interesting emerging solution is the use of extracorporeal therapies by means of devices expressly dedicated to the selective removal of endotoxins. In the last three decades, different researchers and manufacturers have developed different extracorporeal devices.

In 1983 a Japanese scientist named Hisataki Shoji began developing a blood endotoxin removal cartridge (Toraymyxin) that could be applied clinically through direct hemoperfusion of blood against a fiber of polystyrene and covalently bound polymyxin B.[4] Approved first in Japan in 1994 and in Europe in 1998, Toraymyxin is the most widely available device for the removal of endotoxins from blood. It has been used worldwide on more than 100,000 patients with an excellent safety profile.

In 2001 Ullrich et al. tested a device for in vivo endotoxin adsorption based on a cartridge of high-affinity polymethacrylate-bound albumin (Endotoxin Adsorber EN 500, Fresenius Medical Care AG, Bad Homburg, Germany).[7] Results of the pilot study were encouraging, but a large multicenter randomized trial reported by Reinhart in 2004 did not demonstrate a benefit of this device.[8]

Bengsch et al. in 2005 proposed that the use of an apheresis system based on a diethylaminoethyl-cellulose absorber could reduce the plasma concentration of endotoxin in patients with severe sepsis.[9] The treatment is based on 5 to 10 consecutive apheresis treatments of 1.6 L of plasma. The researchers described the experience of a pilot observational trial, reporting encouraging results in terms of reduction in endotoxins as well as consequent reduction in inflammatory mediators. However, this product has not been advanced into commercial use. One product that is marketed predominantly in Scandinavia is the Alteco LPS adsorber system. It works to bind endotoxin through charge-based interactions with a polypeptide and has been studied most extensively in patients undergoing high-risk cardiac surgery. Its adsorption capacity for endotoxin in vitro is substantially less than other devices such as Toraymyxin. Another similar device called oXiris (Baxter Gambro USA) has been tested in several small studies but has not been subjected to rigorous study and not adopted widely into broad clinical use.

TORAYMYXIN SYSTEM: CONCEPT AND MANUFACTURING PROCESS

Toraymyxin is a device principally made of covalently immobilized polymyxin B fiber (PMX-F) used as an adsorbent bed.[4] PMX, a polycationic antibiotic, is well known to bind endotoxin and neutralize its toxicity with extremely high affinity. The binding site of PMX to endotoxin is reported

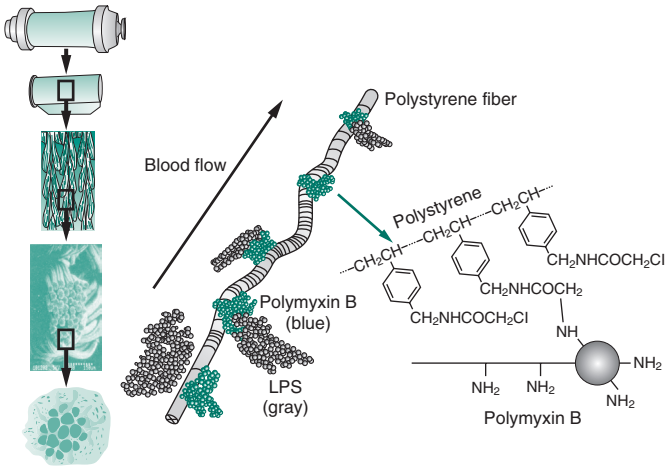

FIGURE 193.2 An adsorbent compartment of a Toraymyxin cartridge; the schema of the cross-section of island-sea type conjugated fiber filament shows an electron micrograph of the cross-section of a fiber filament. The schematic diagram of the immobilization of polymyxin B on the surface of polystyrene-based carrier fiber through a covalent bond.

to be the lipid A portion, with binding via ionic and hydrophobic interactions. Because primary amino groups are positively charged, they appear to play a major role in the ionic binding to the lipid A portion of endotoxin (Fig. 193.2).

The endotoxin adsorption capacity of PMX-F was evaluated in an in vitro setting and was compared with the carrier fiber without immobilized PMX-B.[10] One gram of PMX-F or carrier fibers without PMX was added to a test tube containing 30 mL of calf serum solution with 10 ng/mL purified lipopolysaccharide (*E. coli* 0111 : B4). After 2 hours of incubation, the serum endotoxin level of each test tube was measured with the *LAL* assay.[11] PMX-F sharply reduced the endotoxin level, but the "control" carrier fiber was not effective in lowering endotoxin. The endotoxin adsorption capacity of Toraymyxin also was evaluated. A calf serum solution of purified *Escherichia coli* lipopolysaccharide was circulated through a Toraymyxin cartridge and also a "control" carrier fiber cartridge without PMX affixed at a flow rate of 100 mL/min. After 2 hours of circulation, the endotoxin level was compared; only Toraymyxin could reduce the level of endotoxin.

Jaber et al.[12,13] evaluated the in vitro efficacy of Toraymyxin in a model of 10% human plasma after in vitro characterization of the cytokine-inducing potency of gram-negative bacterial or endotoxin challenges. Heparinized blood was obtained from healthy volunteers. The blood was used to harvest peripheral blood mononuclear cells, and a 10% plasma solution was prepared from the residue. Cytokine production by peripheral blood mononuclear cells incubated with 10% plasma before and after in vitro hemoperfusion was used as the index of endotoxin removal. After 2 hours of hemoperfusion, tumor necrosis factor-alpha production by peripheral blood mononuclear cells was decreased in both samples. These investigators suggested impressive in vitro removal of endotoxin by Toraymyxin.

Various animal studies have been used to assess the biocompatibility of the device[14] as well as to determine the optimal polymyxin concentration, which is 3.0 mg per 1-g of fiber.[3] Biocompatibility was excellent, although a slight reduction in platelet count was noted during the treatment, as has been observed in other canine models of hemodialysis.[14]

Several more recent experiments have shown an excellent safety profile for the device with extremely minimal amounts of PMX detectable in blood after treatment with the column. More recently, Romaschin et al. have performed experiments showing potentially bactericidal properties of the column using blood samples spiked with whole gram-negative bacteria. (author communication)

CLINICAL EXPERIENCE WITH ENDOTOXIN ADSORPTION

Toraymyxin has been used clinically since 1994 in Japan and reimbursed under the Japanese National Health Plan. Patients must fulfill the following three conditions simultaneously for reimbursement for the use of Toraymyxin:

- Endotoxemia or suspected gram-negative infection
- Two or more of the following conditions[15]: fever (oral temperature > 38° or < 36°C), tachycardia (heart rate > 90 beats/min), tachypnea (respirations > 24 breaths/min), and leukocytosis/leukopenia (>12,000 leukocytes/mm^3, <4000 leukocytes/mm^3, or 10% band count)
- Septic shock necessitating vasopressor therapy

Toraymyxin treatment usually is performed for 2 hours at a blood flow rate of 80 to 100 mL/min by direct hemoperfusion. Nafamostat mesylate often is used as the anticoagulant in Japan, whereas heparin is used more commonly in other countries. Since 1994, more than 100,000 patients have been treated with Toraymyxin. The incidence of adverse events is reported as less than 1%; the most common adverse events are thrombocytopenia and line-related complications. There are no known treatment-related deaths reported.

There have been a number of small trials in Japan and Western Europe that have tested the clinical value of PMX-F. A systematic review was done by Cruz et al. in 2007 to describe the published experience with PMX-F.[6] The primary end points of interest were the effects of PMX-F on blood pressure, use of vasopressor drugs, and oxygenation; the other end points of interest were the effects on endotoxin levels and mortality. Of 118 abstracts identified in the literature over the past 10 years, they identified 28 published studies reporting relevant end points.[16–20] This systematic review of the published literature, which involved almost 1400 patients treated in seven countries, demonstrated multiple beneficial effects of direct hemoperfusion with PMX-F in comparison with conventional medical therapy for patients who had sepsis or septic shock (Table 193.1). Briefly, the mean arterial pressure (MAP) increased on average by 19 mm Hg (95% confidence interval [CI], 16–22 mm Hg; $p < .001$) after PMX-F,[16,21] strongly suggesting a clinically significant improvement in hemodynamic status. The pooled estimate also suggests that PMX-F improves gas exchange, as represented by the PaO_2/FiO_2 ratio (weighted mean difference 32 units; 95% CI, 23–41 units).[17] In addition, there appeared to be a beneficial effect on mortality (relative risk [RR], 0.54 relative to standard medical therapy; 95% CI, 0.44–0.67).

This group went on in 2009 to publish in JAMA the Early Use of Polymyxin Hemoperfusion in Abdominal Septic Shock (EUPHAS) trial,[22] a randomized unblinded study of 64 patients in 10 tertiary Italian intensive care units. Importantly, this study demonstrated statistically significant improvements in the primary end points of hemodynamics and organ dysfunction. Specifically, renal function as measured by improvement of serum creatinine, diuresis, and the renal component of the SOFA at 72 hours showed positive trends. Also, the absolute risk of death at 28 days

TABLE 193.1

Summary of Systematic Reviews of the Clinical Effects of Polymyxin B–Immobilized Fiber Column in Sepsis

PARAMETER	NO. OF STUDIES	NO. OF PATIENTS	EFFECT SIZE	95% CONFIDENCE INTERVAL	OVERALL EFFECT (*p* VALUE)
Change in mean arterial pressure (mm Hg)	12	275	+19	15, 22	<.001
Change in dopamine/dobutamine dose (μg/kg/min)	4	96	−1.8	−3.3, −0.4	.01
Change in PaO_2/FiO_2 ratio (units)	7	151	+32	23, 41	<.001
Change in endotoxin level (pg/mL)	17	435	−22	−25.8, −18.1	<.001
Mortality (risk ratio)	15	885	0.54	0.44, 0.67	<.001

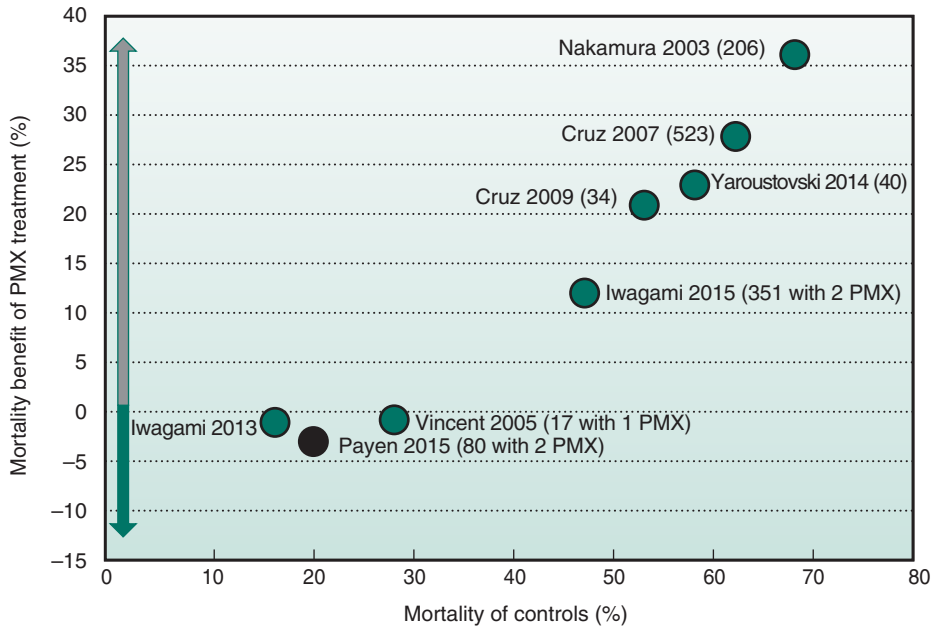

FIGURE 193.3 Association between observed placebo mortality and benefits of PMX-DHP therapy in clinical studies.

improved significantly from 53% in the conventional therapy group to 32% in the PMX-F treated group ($p = .03$). These results, albeit encouraging, are considered controversial because the trial was stopped early after an interim analysis showed the mortality difference, which was a secondary end point. Patients were selected for therapy based on evidence of septic shock from an intraabdominal source to hopefully enrich the patient population with likely endotoxemic patients, but the EAA was not measured. Despite these limitations, EUPHAS may have had a comparative advantage to other sepsis trials that selected patients for anti-endotoxin therapy regardless of source of sepsis or measured levels of endotoxin.

The effects of Hemoperfusion With a Polymyxin B Membrane in Peritonitis With Septic Shock (ABDO-MIX)[23] is a French randomized, controlled, open label multicenter study that evaluated 28-day mortality in patients with septic shock resulting from peritonitis. Eligible patients were randomized to standard of care versus standard care plus PMX-F within 36 hours of abdominal surgery to repair a hollow viscus perforation; 240 patients were enrolled. This study failed to show a difference in mortality between the groups (27.7 vs. 19.5 % in the conventional group [n = 113], $p = 0.14$), but the study had a number of potential limitations that may have contributed to this observation. These include cartridge clotting and failure rates that are much higher than other published trials or clinical experience to date (2 PMX sessions were completed in only 81 of 119 patients [69.8 %]), suggesting technical issues in the implementation of the therapy protocol. Also, the observed composite mortality was substantially less than the estimate included in the sample size calculation (37%), thus decreasing the power of the study to detect a difference.

Recently, Iwagami et al. from Japan published two important retrospective studies using Japanese administrative data from the Japanese National Health Registry. The first evaluated 642 patients with abdominal sepsis who received either one or two sessions of PMX-F. This study failed to show any significant difference in 28-day mortality (17.1% in the treatment group versus 16.3% in the control group $p = .696$).[24] A second study evaluated a cohort of 1068 patients with septic shock who received one or two sessions of PMX-F as well as concomitant treatment with renal replacement therapy. In this study, an important 28-day mortality difference was noted with PMX-DHP therapy (40.2% vs. 46.8%; $p = .003$).[25] Moreover, a dose-response relationship was noted with patients who received one column having a mortality of 43.6% versus 34.5% in those patients who received the complete treatment of two columns. Taken together, the available evidence appears to show an important relationship between the potential benefits of PMX-DHP therapy and severity of illness with those patients at the highest risk of death most likely to benefit from PMX-DHP (Fig. 193.3).

MEASUREMENT AND REMOVAL OF ENDOTOXIN: EUPHRATES A THERANOSTIC TRIAL OF PMX-DHP FOR ENDOTOXEMIC SEPTIC SHOCK

Despite available evidence from Japan and Europe, PMX has not yet been considered for inclusion in international guidelines for sepsis treatment, neither as a rescue therapy in refractory septic shock (for which surviving sepsis guidelines suggest to start considering experimental therapies) nor as a preventive therapy. Because of this, we and others suggested that a definitive randomized trial to assess the clinical benefit of PMX was needed.

Therefore in 2010, the EUPHRATES Trial (Evaluating the Use of Polymyxin B Hemoperfusion in a Randomized Controlled Trial of Adults Treated for Endotoxemia and Septic Shock) Study Protocol for a Randomized Controlled Trial (ClinicalTrials.gov Identifier: NCT01046669) was started, and it was finished in mid-2016.[26] EUPHRATES enrolled 450 patients with vasopressor-dependent septic shock randomized 1:1 to receive two sessions of PMX-DHP 24 hours apart as was done in EUPHAS and consistent with current practice in Japan. However, unique to EUPHRATES was the requirement that all patients randomized to therapy have an Endotoxin Activity Assay (EAA) level > 0.6 and are therefore highly endotoxemic. In addition, this trial is blinded and uses a facade hemoperfusion event in the control group. To ensure an adequate severity of illness after the first interim analysis the Data and Safety Monitoring Board mandated excluding patients with a MODS score of less than 10. Topline results from the EUPHRATES trial were presented at the IRRIV International Vincenza conference in 2017 by Dr. Massimo Antonelli from Italy. The mean APACHEII score in the patients studied was 29.4 in the treatment group and 28.1 in the control group. There were no device-related mortalities noted with an overall very good safety profile for the treatment, and the rate of cartridge clotting was quite low at 8%. No difference in 28-day mortality was observed, either in the full cohort (PMX-F: 84/223 (one patient lost to follow-up) [37.7%] vs. control 78/226 [34.5%]; p=0.49) or in patients with MODS >9 (PMX-HP: 65/146 [44.5%] vs. sham: 65/148 [43.9%]; p=0.92). There was a rise in MAP for the PMX-F treatment arm in both the full cohort and in patients with MODS>9 ([n=450]; rise of 9.4 (17.3) vs. 4.1 (14.4) mmHg; p<0.05) and ([n=295] with MODS >9; rise of 8.0 (15.8) vs. 3.9 (14.1) mmHg; p<0.03]). There were no differences in any other secondary outcomes in these two cohorts.

However, an analysis was done to examine the interaction between EAA and mortality. Based on this a loss of treatment effect was noted for patients with an EAA >0.90. Analyzing the cohort of 194 patients with EAA 0.6 to 0.9 revealed a 10.7% absolute reduction in mortality for patients with EAA 0.6 to 0.9 when treated with PMX-F. This was statistically significant in an adjusted analysis. Differences were also observed in MAP, [PMX-F versus control: increase of 8.9 mmHg (15.0) vs 3.8 mmHg (13.8), p < 0.05] and ventilator-free days (PMX-F group median 20 days vs control median 6 days, p < 0.05).[27] The trial leaders suggest that this result may be explained in part by a recent observation by Romaschin et al. that the EAA becomes asymptotic after 0.9 with levels greater than this potentially representing a massive endotoxin load that vastly exceeds the adsorption capabilities of the column.[28] However, other contributors may have also played a role.

In conclusion, the use of extracorporeal therapy for endotoxin removal in sepsis is biologically plausible and supported by the available evidence. Targeted use of this therapy using the EAA as a tool to identify those patients most likely to benefit should be standard of care when applying this therapy. PMX-F is the most effective and proven method of extracorporeal endotoxin removal. The results of EUPHRATES confirm the unequivocal benefit of use of PMX-F in improving hemodynamics in patients with endotoxemic septic shock. EUPHRATES also suggests an important mortality benefit for this treatment when targeted to patients with an EAA 0.6 to 0.9 in endotoxemic septic shock, reflecting the growing trend towards a personalized medicine approach to disease management. Further trials are needed to better evaluate the potential use of this therapy in other forms of endotoxin-mediated illness.

Key Points

1. The sepsis syndrome generally is caused by circulating endotoxin and its subsequent biologic cascade.
2. Extracorporeal therapy can be recommended only after circulating endotoxin has been detected and measured and the patient's clinical status has been correlated with the endotoxin level.
3. Although drugs have been shown to be rather inefficient in removing endotoxin, extracorporeal removal is becoming an interesting alternative that has displayed some important clinical benefits in recent studies.
4. The EUPHRATES trial confirms the unequivocal benefit of PMX-F therapy in improving hemodynamics and suggests a potential value for a targeted approach to treating endotoxemic septic shock in patients with EAA 0.6 to 0.9

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A complete reference list can be found online at ExpertConsult.com.

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