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Endotoxin Adsorption with Polymyxin B Hemoperfusion: Mechanistic Insights and Therapeutic Implications for Septic Shock

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Abstract

Since 1994, polymyxin B immobilized fiber columns (PMX) have been used to remove endotoxins in patients with sepsis and septic shock. Over the past 25 years, this therapy has shown clinical benefits, but large, multicenter randomized trials have yet to demonstrate a clear survival advantage. Following results from a major North American sepsis trial, a new study is currently investigating whether PMX can improve long-term survival in septic patients. Additional insights may come from analyzing large clinical databases. PMX columns have proven effective at adsorbing endotoxins in laboratory studies, and animal experiments have further confirmed their potential. However, the exact way PMX disrupts the sepsis cascade and reduces organ damage is not fully understood. Evidence shows that PMX can enhance antigen expression on monocytes and neutrophils. These immunomodulatory effects, whether through endotoxin removal or other mechanisms, may help explain improvements in organ function observed in patients. Endotoxemia may also contribute to diseases beyond sepsis, and rapid diagnostic tools to detect it could enable more precise treatments and expand the clinical use of endotoxin removal.

Keywords: Septic shock, Endotoxin adsorption, Polymyxin B, Hemoperfusion, Immunomodulation, Idiopathic pulmonary fibrosis

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Introduction

Lipopolysaccharide (LPS), commonly called endotoxin, is a major component of the outer membrane of Gram-negative bacteria. Sepsis remains a serious health threat, especially for older adults, immunocompromised individuals, trauma patients, and those recovering from surgery. A recent international consensus has proposed a more precise classification system, dividing patients into well-defined clinical categories.

This system highlights that sepsis develops when pathogen-associated molecular patterns (PAMPs) trigger the body's immune response. Normally, this response involves a balance of proinflammatory and anti-inflammatory reactions, but when it becomes uncontrolled, organ dysfunction and poor outcomes may result [1]. Sepsis is now described as: (1) an acute, systemic inflammatory condition initiated by infection, and (2) a process in which an abnormal host response contributes to systemic inflammation and septic shock, known as Sepsis-3 [2]. Patients with Sepsis-3 often experience persistent low blood pressure requiring vasopressors and elevated blood lactate levels (>2

mmol/L). If not treated promptly, this can progress to life-threatening multi-organ failure.

Endotoxin has long been considered a potential therapeutic target in sepsis. Attempts to neutralize circulating endotoxin with polyclonal or monoclonal antibodies were explored but did not enter routine clinical use. Another strategy emerged: removing endotoxin directly from the bloodstream using a specialized medical device.

In 1994, researchers developed a selective endotoxin removal column (PMX) containing polymyxin B bound to fibrous material [3]. Polymyxin B is a polycationic antibiotic that binds the lipid A portion of endotoxin, the toxic component conserved across Gram-negative bacteria. Direct intravenous administration of polymyxin B is unsafe due to kidney and nerve toxicity [4, 5], so it was immobilized on a fibrous matrix to allow extracorporeal hemoperfusion (PMX-HP) and safely remove endotoxin from blood circulation.

PMX-HP has been in clinical use in Japan since 1994 and is now available in some countries across Europe, Asia, and North America. A European multicenter pilot study in 2005 confirmed its safety and suggested possible improvements in blood pressure and heart function [6]. Later, three large randomized controlled trials in Italy, France, and North America (2009, 2015, 2018) did not demonstrate a survival benefit at 28 days. However, recent analysis using Japan's Diagnosis Procedure Combination (DPC) database suggested PMX-HP may reduce mortality.

Experimental studies have confirmed the ability of PMX-HP to adsorb endotoxin in vitro and in animal models. Research also indicates immunomodulatory and anti-apoptotic effects, although the full mechanism of action remains unclear.

This review summarizes over 25 years of clinical experience with PMX-HP in sepsis and septic shock. It also traces key milestones in endotoxin research **(Table 1)**, evaluates current evidence, and discusses potential directions for future application.

Table 1. Historical Milestones.

Event	Year(s)	Major Findings
Discovery of microorganisms	1676	Robert Hooke and Antoni van Leeuwenhoek independently identify living microorganisms using microscopy, marking the first observation of microbes.
Establishing the germ theory of disease	1860s	Louis Pasteur (1822–1895) and Robert Koch (1843–1910) show that microorganisms present in infected tissues directly cause disease and can be transmitted between humans and animals.
Ignaz Semmelweis and hand hygiene	1850s	Semmelweis (1818–1865) demonstrates in 1847 that doctors' hands can transmit pathogens causing puerperal fever, and proper handwashing prevents the infection.
Discovery of endotoxin	1892	Robert Koch and Richard Pfeiffer show that roughly 70% of the Gram-negative bacterial cell wall is protease-resistant but lipid-sensitive. Purified endotoxin injected into lab animals proves lethal, fulfilling Koch's Postulates.
Gram staining method	1884	Hans Christian Gram (1853–1938) develops a staining technique to rapidly classify bacteria as Gram-positive or Gram-negative using differential staining and microscopy.
Polymyxin B hemofilters for endotoxin removal	1994	Tohru Tani, Hisataka Shoji, and colleagues create cationic hemofilters that bind circulating endotoxin, removing it from the bloodstream and helping to rescue patients from endotoxemia.
Elucidation of endotoxin structure	2000s	Beutler and team determine the 3D structure of TLR4 as the endotoxin receptor and describe interactions between Lipid A, the core glyco-lipid, and MD2 in signaling endotoxin presence.
Clinical evaluation of endotoxin-targeted therapies	2013–2020	Trials are conducted to assess whether endotoxin removal with filters or monoclonal antibodies improves clinical outcomes.

Historical overview of the anti-endotoxin strategy for the treatment of septic shock

The characterization of bacterial endotoxin, both in terms of structure and function, was a landmark achievement during the late 19th century's age of discovery. Progress in microbiology was pivotal for the broad acceptance of the "germ theory" of disease. At the beginning of the 19th century, the idea that microorganisms caused disease was largely unknown and not widely accepted, but by the century's end, it became recognized as a fundamental cause of illness and death. Early vague concepts, such as "miasma" or "contagion," were gradually replaced with

rigorous, testable scientific methods that included proper controls, sterile technique, and attention to reproducibility. This approach fostered international collaboration among generations of microbiologists working to describe microorganisms systematically in the laboratory. The emphasis on reproducible results established a standard for scientific progress that continues to guide research today.

The combined contributions of scientists like Koch, Pasteur, Panum, and Klebs were critical for microbiology's development. They insisted on applying Koch's laboratory methodology to confirm disease causation, which is now formalized as Koch's postulates. These postulates consist of four criteria: (1) the suspected pathogen must be present when the disease occurs; (2) the disease should not occur in the absence of the pathogen; (3) the disease must be reproducible in experimental animal models; and (4) the causative microorganism must be re-isolated from the animal model and cultured again. While Koch's postulates are not universally applicable [7], they remain a foundational benchmark in microbiology.

A major breakthrough occurred in 1892 when Richard Pfeiffer (1858–1945), a student of Koch [8], was the first to describe bacterial endotoxin. Although exotoxins had been identified earlier, endotoxins differed from toxins such as tetanus or diphtheria. Exotoxins are protein-based, secreted into the extracellular space, and are heat-labile, whereas endotoxins are highly heat-stable.

When separated from other cell wall components, endotoxin comprises roughly 70% of the Gram-negative bacterial cell wall. Another important advance in diagnostic microbiology came in 1884 when Danish physician Hans Christian Gram (1853–1939) developed a staining technique to differentiate bacteria based on cell wall composition, now known as the Gram stain. Bacteria containing endotoxin in their cell walls stain pink after fixation and alcohol decolorization; these are classified as Gram-negative bacteria, such as Pseudomonas aeruginosa. In contrast, bacteria like Staphylococcus aureus or Streptococcus pyogenes retain the stain and appear dark blue.

This straightforward method effectively divides pathogenic bacteria into Gram-negative and Gram-positive groups and continues to serve as a rapid, reliable technique for identifying bacterial pathogens today.

Could bacterial endotoxin serve as a therapeutic target in gram-negative sepsis using antibodies?

Antibodies, whether polyclonal or monoclonal, have been explored as a potential therapeutic strategy for Gram-negative sepsis and septic shock. Both approaches have undergone testing in large multicenter clinical trials. The core glycolipid structure of bacterial endotoxin is immunogenic, allowing polyclonal antibodies to be generated from pooled plasma of blood donors. Endotoxin's inner core, including Lipid A, contains several highly conserved, immunogenic epitopes that make it a suitable target for antibody-based interventions.

This concept was extensively evaluated in the 1980s through multiple large clinical trials, which tested whether high-titer polyclonal antiserum could block pathogen-mediated disease. The outcomes were mixed, and no clear survival benefit was consistently demonstrated [9–11]. Similarly, trials using monoclonal antibodies directed against Lipid A or the endotoxin inner core did not yield significant clinical improvements. Consequently, antibody-based strategies for endotoxin neutralization have largely been set aside until advancements in detection methods or the development of improved, genetically engineered antibodies become available [12].

Design of the polymyxin B immobilized fiber column (PMX)

Polymyxin B is covalently attached to the surface of polystyrene-derived fibers via the primary amino group of its diaminobutyric acid residues [3] **(Figure 1)**. The immobilized polymyxin B molecules are designed to bind the lipid A component of endotoxin through a combination of ionic and hydrophobic interactions. Because the polymyxin B is covalently fixed, it does not enter the bloodstream, allowing clinical use without the drug's known nephrotoxic or neurotoxic effects.

PMX hemoperfusion (PMX-HP) is performed using whole blood circulation at a flow rate of 80–120 mL/min **(Figure 2)**. Unfractionated heparin is typically used as an anticoagulant, whereas in Japan, the short-acting protease inhibitor Nafamostat mesilate is commonly preferred.

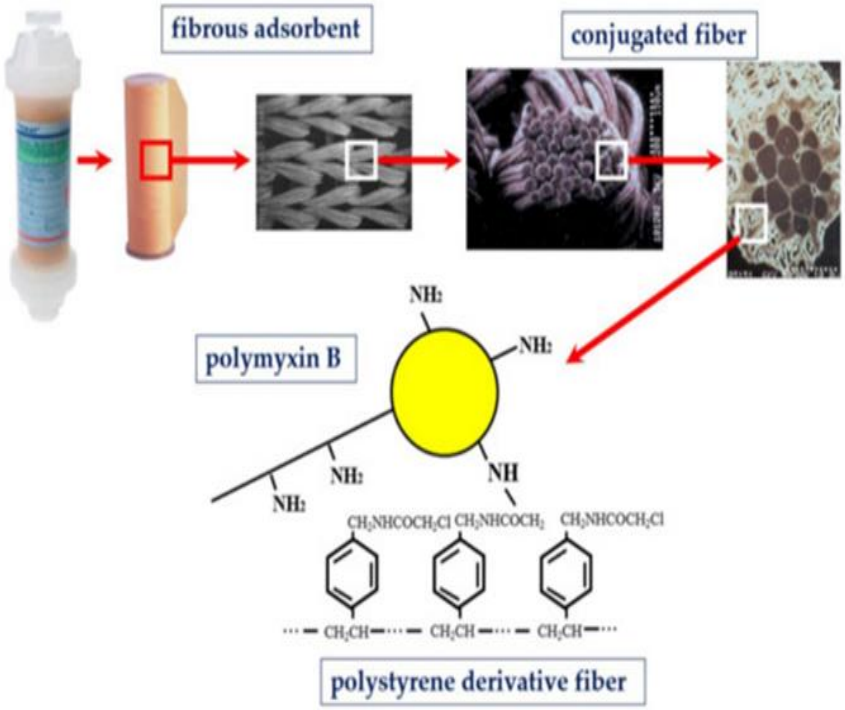

Figure 1. Structure of polymyxin B immobilized fiber column.

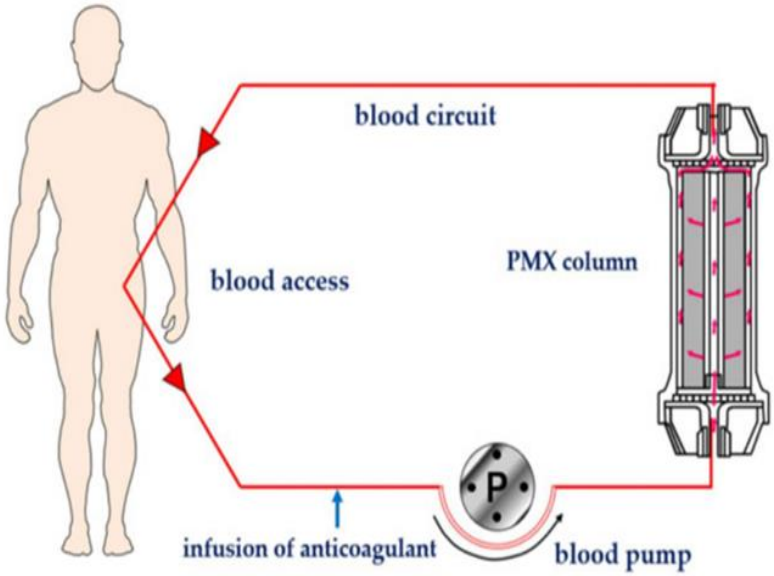

Figure 2. Schematic diagram of hemoperfusion with PMX (PMX-HP). PMX: polymyxin B immobilized fiber column.

Revisiting the endotoxin adsorption capacity in in vitro and in vivo settings

In vitro endotoxin removal experiments with PMX

The capacity of PMX (PMX-20R) to adsorb endotoxin has been examined under controlled in vitro conditions [13]. In these experiments, 1.5 L of bovine serum spiked with LPS from Escherichia coli O111:B4 was circulated through the PMX column for four hours at a flow rate of 100 mL/min. Measurements showed that the LPS concentration, initially 10 ng/mL, decreased to 2–3 ng/mL after perfusion, indicating that approximately 12 μg of endotoxin had been removed from the serum. These results align closely with previous observations, where LPS-spiked bovine serum reached adsorption equilibrium within 2 to 3 hours.

In a further experiment, PMX was tested using 0.5 L of pooled EDTA-anticoagulated whole blood spiked with 100 μg of FITC-labeled E. coli O111:B4 LPS (200 ng/mL) and perfused for 2 hours at the same flow rate. After perfusion, bound LPS was eluted from the column, and fluorescence analysis demonstrated that the PMX column captured an average of 20 μg of LPS. This experiment

confirmed that PMX efficiently removes endotoxin in whole blood settings as well as in serum.

Yamashita *et al*. investigated the duration for which PMX maintains its adsorption capacity before reaching saturation [14]. In this study, LPS was continuously infused into a bovine serum reservoir, gradually increasing the endotoxin concentration over time. Perfusion tests were performed using either PMX (type: PMX-01R) or a control tubing system as a sham procedure. Throughout a 24-hour period, the concentration of LPS in the PMX-treated reservoir consistently remained lower than in the sham control. These findings suggest that the PMX column does not reach saturation even after three hours of continuous perfusion. Therefore, extending PMX perfusion beyond the typical 2–3 hours could be advantageous for maximizing endotoxin removal.

Animal experiments

The effectiveness of PMX in vivo has been evaluated in several animal models of sepsis. Iba *et al*. tested PMX using a non-hypotensive rat model of sepsis induced by intravenous injection of live E. coli [15]. Wistar rats were divided into two groups (n = 7 per group) and underwent either PMX hemoperfusion or perfusion through a dummy column for three hours. The PMX-HP group exhibited lower levels of organ injury markers, including ALT, LDH, and BUN. Additionally, proinflammatory cytokines such as IL-6, TNF-α, and IL-1β were significantly reduced compared to the control group. Microscopic examination of the mesenteric microcirculation revealed better preservation in the PMX-treated animals, and survival rates were markedly improved (93% versus 57% in controls, $p = 0.03$).

Yeh *et al*. examined PMX-HP in a septic pig model induced by fecal peritonitis to study effects on microcirculation [16]. In this model, PMX-HP was applied for two hours. At six hours post-perfusion, the density of small vessels and tissue oxygen saturation in the ileal mucosa were higher in the PMX-treated pigs compared with untreated septic controls. Histologic scoring of the ileal mucosa also demonstrated reduced tissue injury in the PMX-HP group.

Collectively, these animal studies provide evidence that PMX-HP can mitigate microcirculatory dysfunction, improve tissue oxygenation, and reduce histopathological damage in the ileal mucosa under septic conditions associated with endotoxemia. These results strongly support the potential clinical application of PMX-HP for patients with sepsis or septic shock who exhibit elevated circulating endotoxin levels.

Clinical outcomes with PMX indication

Multicenter randomized controlled studies

The EUPHAS trial in Italy was the first multicenter randomized study evaluating the clinical impact of PMX hemoperfusion (PMX-HP) [17]. This trial enrolled patients suffering from severe sepsis or septic shock who required emergency surgery for intra-abdominal infections caused by Gram-negative bacteria. When PMX-HP was combined with standard therapy, patients experienced significant improvements in mean arterial pressure and a reduced need for vasopressors. Mortality at 28 days was also lower in the PMX-HP group (32%, 11/34) compared to those receiving conventional therapy alone (53%, 16/30). However, because the study population was small and a significant survival advantage emerged early, the trial was stopped ahead of schedule. Continuing to withhold PMX-HP from the control group was considered unethical given the high mortality risk. While promising, the early termination sparked debate and prevented a definitive conclusion regarding efficacy.

In contrast, the ABDO-MIX trial in France, which was designed as a prospective, multicenter randomized study, evaluated whether PMX-HP could reduce mortality and organ dysfunction in patients with peritonitis-induced septic shock [18]. The primary endpoint was 28-day mortality. Surprisingly, mortality in the PMX-HP group was 27.7% (33/119) compared to 19.5% (22/113) in the conventional treatment group ($p = 0.14$), and no benefit was seen in organ failure parameters. Unlike EUPHAS, this study did not demonstrate a survival advantage despite enrolling a similar patient population.

Several explanations have been suggested for the disparity between EUPHAS and ABDO-MIX outcomes. In ABDO-MIX, the control group mortality was only 19.5%, substantially lower than the 53.3% observed in EUPHAS, indicating that the trial may have included less severely ill patients. Additionally, only 68% (81/119) of PMX-HP patients completed the two planned hemoperfusion sessions due to clotting of the column or hemodynamic instability, whereas all EUPHAS patients completed both sessions. These limitations underscore that neither trial provides conclusive evidence on the effectiveness of PMX-HP in septic shock, highlighting the need for further rigorous multicenter studies.

The EUPHRATES trial represents the most recent effort in North America to assess PMX-HP [19]. This multicenter, randomized, blinded, sham-controlled study targeted septic shock patients with elevated endotoxin activity (EA ≥ 0.60) as measured by the endotoxin activity assay (EAA). A total of 450 critically ill adult patients were enrolled. The primary outcome was 28-day mortality in the overall cohort and among patients with multiple organ dysfunction score (MODS) > 9. In the overall population, mortality at 28 days was 62.3% survival (84/223) in the PMX-HP group versus 65.5% survival (78/226) in the control group, showing no significant

difference. Among patients with MODS > 9, survival was 44.5% (65/146) in the PMX-HP group compared with 43.9% (65/148) in controls.

Secondary and exploratory analyses revealed that patients treated with PMX-HP experienced greater increases in mean arterial pressure (MAP) by day 3, both in the overall cohort and in those with MODS > 9 (p = 0.02). Moreover, ventilator-free days (VFD) through day 28 were longer in the PMX-HP group among patients with MODS > 9 (p = 0.02). Investigators suggested that PMX-HP might have failed to improve survival due to insufficient dose or duration relative to the patients' high endotoxin burden.

A post hoc analysis by Klein *et al*. focused on patients with EA values between 0.6 and 0.89 [20]. In this subgroup, 28-day mortality was 26.1% (23/88) in the PMX-HP group versus 36.8% (39/106) in controls, representing an absolute reduction of 10.7%. The PMX-HP group also showed longer survival, improved MAP, and increased ventilator-free days.

Romashin *et al*. offered a theoretical explanation for these findings [13]. In patients with EA > 0.9, endotoxin concentrations can exceed 4 ng/mL, translating to a total blood load of over 20 μg in a 5 L blood volume. If endotoxin also distributes into approximately 10 L of extracellular fluid, total endotoxin levels may exceed the adsorption capacity of a single PMX-HP session. The endotoxin burden versus EA value curve shows an asymptotic pattern above EA 0.9, limiting the assay's ability to accurately quantify LPS at these high levels. Consequently, patients with EA > 0.9 may not be suitable for standard PMX-HP dosing in the EUPHRATES protocol. These post hoc findings have generated hypotheses that are currently being tested in the ongoing TIGRIS multicenter randomized controlled trial in the US.

Systematic reviews and meta-analyses on PMX-HP

In the last decade, multiple investigations have assessed the effectiveness of PMX hemoperfusion (PMX-HP) in septic patients. Chang T and colleagues analyzed 17 trials in a comprehensive meta-analysis [21]. Their pooled estimate indicated that PMX-HP reduced overall mortality, with a risk ratio of 0.81 (95% CI, 0.70–0.95; p = 0.007) compared to conventional therapy. The studies were stratified by mortality risk in the control group into low (<0.3), intermediate (0.3–0.6), and high (>0.6) categories. Subgroup analysis revealed that patients in the intermediate-risk group (risk ratio 0.84; 95% CI, 0.77–0.92) and high-risk group (risk ratio 0.64; 95% CI, 0.52–0.78) experienced significant reductions in mortality, whereas no benefit was observed in the low-risk group (risk ratio 1.278; 95% CI, 0.888–1.839). These results suggested that PMX-HP might confer survival advantages particularly in patients with moderate-to-severe disease severity.

Li *et al*. conducted a separate meta-analysis including 13 studies [22]. Their findings also indicated a significant reduction in overall mortality associated with PMX-HP (RR 0.68, 95% CI, 0.51–0.91, p = 0.01). Subgroup analyses highlighted that patients with APACHE II scores below 25 (RR 0.64, 95% CI, 0.52–0.78, p < 0.0001) and those categorized as having sepsis (RR 0.48, 95% CI, 0.32–0.72, p = 0.0003) benefitted most. In contrast to Chang T *et al*., this study indicated that PMX-HP might be particularly advantageous for patients with lower disease severity and emphasized the utility of APACHE II scoring over conventional group mortality for stratification.

Terayama *et al*. reviewed seven randomized controlled trials comparing PMX-HP to standard therapy in severe sepsis or septic shock [23]. Their pooled data showed that PMX-HP was associated with reduced mortality (risk ratio 0.65; 95% CI, 0.47–0.89; p = 0.007; I^2 = 72%). Furthermore, meta-regression analysis suggested a negative correlation between baseline mortality rates and the effect size, implying that patients at higher initial risk were more likely to benefit from PMX-HP treatment.

Conversely, Fujii *et al*. analyzed six RCTs and concluded differently [24]. Their pooled risk ratio for 28-day mortality was 1.03 (95% CI, 0.78–1.36; I^2 = 25%; n = 797), showing no clear advantage for PMX-HP. This analysis included three trials used by Terayama *et al*. and added three additional studies, including two with positive outcomes and the negative EUPHRATES trial [19]. The inclusion of large negative trials appeared to heavily influence the overall result.

Taken together, the meta-analytic evidence remains inconclusive. Variations in study selection, patient characteristics, and baseline severity contributed to the inconsistent outcomes. These findings underscore the need for well-structured RCTs to identify patient populations most likely to benefit from PMX-HP.

Cohort studies utilizing large clinical databases

Observational studies using extensive clinical databases have provided additional insights into PMX-HP efficacy. Iwagami *et al*. analyzed the Japanese DPC database from 2007 to 2012 to assess outcomes in septic shock patients receiving vasopressors and continuous renal replacement therapy (CRRT) in the ICU [25]. Recognizing that acute kidney injury (AKI) is a frequent and severe complication of sepsis, the investigators hypothesized that patients requiring CRRT represent a population most likely to benefit from PMX-HP. Among 3,759 eligible patients, 1,068 received PMX-HP. After propensity score matching, 978 pairs were formed. The 28-day mortality rate was significantly lower in the PMX-HP group compared with controls (40.2% vs. 46.8%; p = 0.003),

suggesting a potential survival benefit in critically ill patients undergoing CRRT.

Similarly, Nakamura *et al*. analyzed data from the Japan Septic Disseminated Intravascular Coagulation (JSEPTIC DIC) study, which included 40 institutions and aimed to evaluate anti-DIC treatments in severe sepsis and septic shock [26]. Of 1,723 eligible patients, 522 received PMX-HP. After propensity score matching, 262 matched pairs were analyzed. Hospital mortality was significantly lower in the PMX-HP group compared to the non-PMX-HP group (32.8% vs. 41.2%; $p = 0.042$), further supporting the potential benefit of PMX-HP in patients with severe septic shock requiring intensive care interventions.

Registry study following the EUPHAS trial in Italy

The EUPHAS 2 study is a multicenter registry designed to evaluate the real-world application of PMX-HP in routine clinical practice (https://www.euphas2.eu, accessed on 18 February 2021). In Phase 1 of the study, data were collected retrospectively from 57 centers between January 2010 and December 2014, encompassing 357 patients (297 from Europe and 60 from Asia) diagnosed with severe sepsis or septic shock due to confirmed or suspected Gram-negative infections [27]. Among these patients, 305 (85.4%) had septic shock, while 52 (14.6%) were classified as severe sepsis. Abdominal infections were the most frequent source (44.0%), followed by pulmonary infections (17.6%), and Gram-negative bacteria accounted for 60.6% of the identified pathogens.

The overall 28-day survival rate was 54.5%, with 60.4% for abdominal infections and 47.5% for pulmonary infections. Notably, patients with abdominal infections who received PMX-HP within 24 hours of septic shock onset had a 28-day survival of 64.5%, closely matching the 68% survival reported in the original EUPHAS study [17]. No life-threatening adverse events associated with PMX-HP were reported, confirming the safety and feasibility of its use in routine clinical settings.

Blood endotoxin levels, measured using the endotoxin activity assay (EA value), were assessed in 132 of the 357 patients (37.0%) across 18 of the 24 participating centers. The median EA value at baseline was 0.77 (0.69–0.90), with 120 patients (90%) showing EA values ≥0.6, indicating that endotoxemia was common in this patient population. Phase 2 of the EUPHAS 2 registry has been ongoing since 2015.

Host response to PMX-HP

Alterations in blood endotoxin levels

The efficacy of PMX-HP in removing endotoxin was assessed in 19 patients by measuring endotoxin levels in the radial artery and at the outlet of the PMX column using the limulus amebocyte lysate (LAL) assay after 24 hours of PMX-HP [28]. In 11 patients, endotoxin concentrations at the PMX outlet were lower than in the radial artery, confirming the column's endotoxin removal capacity at 24 hours. In the remaining eight patients, radial artery endotoxin levels had already normalized (<1.1 pg/mL). Among the 13 patients (68.4%) who showed a reduction in endotoxin after PMX-HP, six (46%) died within 28 days. These patients had very high APACHE II scores, ranging from 29 to 40, suggesting that PMX-HP might have been initiated too late to prevent multi-organ failure. In six patients, endotoxin levels remained elevated after 24 hours. For the entire cohort, the median radial artery plasma endotoxin concentration at the start of PMX-HP was 16.48 pg/mL, decreasing to 1.857 pg/mL after 24 hours, corresponding to a median removal rate of 74.4%.

Novelli *et al*. further explored the clinical utility of EA measurements to identify high-risk post-surgical patients and determine the need for repeated PMX-HP sessions [29]. Thirty-eight patients were enrolled, with 17 patients exhibiting EA values ≥0.6. These patients received standard therapy along with PMX-HP every 24 hours until the EA value dropped below 0.4. Seven patients required two PMX-HP sessions, eight required three sessions, and two required four sessions. EA values consistently decreased following treatment, and all 17 patients survived at 28 days. These findings highlight that PMX-HP effectively reduces circulating endotoxin levels, and repeated sessions may be warranted depending on the patient's endotoxin burden.

Immunostimulatory effects

The host's immune response to infection encompasses both proinflammatory and anti-inflammatory pathways. Key immune cells, including monocytes, macrophages, and neutrophils, orchestrate these responses by generating either acute proinflammatory signals or anti-inflammatory signals, thereby playing a central role in host defense. Drewry *et al*. examined monocyte HLA-DR expression as a prognostic marker in severe sepsis, concluding that HLA-DR expression may more accurately predict mortality and susceptibility to secondary infections than LPS-stimulated TNF-α production in critically ill adult medical and surgical patients [30].

Ono *et al*. investigated how PMX-HP affects monocyte HLA-DR and granulocyte CD16 expression [31]. They enrolled 34 patients who underwent emergency surgery for intra-abdominal infection. In patients with septic shock, HLA-DR expression on monocytes and CD16 expression on neutrophils were markedly lower than in sepsis patients and healthy controls. Negative correlations were observed between the APACHE II severity scores and both HLA-DR expression (%) and CD16 antigen intensity. Ten septic shock patients treated with PMX-HP exhibited significant increases in HLA-DR and CD16 expression post-treatment. Given that HLA-DR mediates

antigen presentation to T cells and CD16 is involved in neutrophil phagocytosis and cytotoxicity, these findings suggest PMX-HP can enhance key immune functions. Although blood endotoxin levels were not measured in this study, the patients' clinical backgrounds and confirmed Gram-negative infections strongly suggested the presence of endotoxemia.

Srisawat *et al*. conducted a randomized controlled trial in patients with severe sepsis or septic shock who had documented elevated endotoxin levels (EA value ≥0.6) [32]. Twenty-nine patients received PMX-HP alongside standard care for two consecutive days, while 30 patients received standard therapy alone. Baseline monocyte HLA-DR expression was similar between groups. By day 3, the median increase in HLA-DR expression was significantly greater in the PMX-HP group compared to controls ($p = 0.027$). Neutrophil activation, assessed via CD11b, remained stable in the PMX-HP group but increased significantly in the control group, suggesting that PMX-HP helps stabilize neutrophil activation. These studies collectively demonstrate that PMX-HP exerts immunomodulatory effects in septic patients, potentially reversing immune suppression and improving clinical outcomes. Further investigations are needed to clarify whether these effects result directly from endotoxin removal or other mechanisms of PMX-HP.

Cellular elements alteration with PMX-HP

Neutrophils

Neutrophils from patients with septic shock (n = 18) exhibited elevated CD11b/CD64 expression and reduced chemokine receptor CXCR1/CXCR2 expression compared to healthy controls [33]. Following PMX-HP, the percentage of neutrophils expressing CXCR1/CXCR2 increased significantly, while CD11b/CD64 expression decreased. Interestingly, circulating cytokine levels, including IL-6, IL-8, IL-10, and HMGB-1, were not altered by PMX-HP, suggesting that the observed effects were cytokine-independent.

Ex vivo experiments using heparinized blood from septic and septic shock patients perfused through PMX columns showed significant reductions in neutrophil and monocyte counts. Flow cytometry indicated that activated neutrophils—characterized by high CD11b/CD64 and low CXCR1/CXCR2—were selectively adsorbed by PMX columns compared with sham controls. This selective removal of activated neutrophils may contribute to correcting immune dysfunction in sepsis and septic shock patients. Other studies have also reported leukocyte adhesion on PMX adsorbents [34, 35]. However, the precise mechanisms underlying this selective neutrophil adhesion remain to be elucidated.

T lymphocytes

CD4+CD25+Foxp3+ regulatory T cells (Tregs) secrete substantial amounts of anti-inflammatory cytokines, including IL-10 and transforming growth factor (TGF-β), while suppressing interferon-γ (IFN-γ) production. Tregs play a pivotal role in the immunosuppressive phase of late sepsis. Ono *et al*. explored the contribution of Tregs in septic shock patients and examined the effects of PMX-HP on Treg reduction [36]. They observed that the proportion of Tregs within the CD4+ T-cell population, along with serum IL-6 and IL-10 levels, was significantly elevated in septic shock patients compared with those without septic shock. Application of PMX-HP led to a notable decrease in both Treg numbers and serum cytokine levels. However, the exact mechanism by which PMX-HP reduces Treg populations remains unresolved.

Apoptotic cells

Cell death in sepsis occurs via apoptosis (programmed cell death) or necrosis. Enhanced apoptosis in immunocompetent cells, such as B cells and CD4+ T cells, contributes to sepsis-associated immunosuppression. Moreover, apoptosis extends to parenchymal tissues in septic conditions. Cantaluppi *et al*. investigated apoptosis in renal tubular and glomerular cells as a contributing factor to acute kidney injury (AKI) during sepsis [37]. Plasma collected from patients before and 72 hours after PMX-HP was co-cultured with human renal tubular cells. Fas (CD95) ligand expression on tubular cells was elevated prior to PMX-HP but significantly decreased 72 hours post-treatment, whereas in the control group, Fas expression remained unchanged. Correspondingly, SOFA and RIFLE scores, reflecting organ injury, were markedly improved after PMX-HP. The reduction in plasma-induced tubular apoptosis was strongly associated with decreased endotoxin levels, suggesting a protective effect of PMX-HP against early kidney injury.

Ito *et al*. conducted histopathological assessments of kidneys, livers, and lungs in a rat cecal ligation and puncture (CLP) model [38]. Hemoperfusion using a sham column served as the control. PMX-HP significantly reduced apoptotic cell counts in renal tubular cells, while reductions were not observed in other organs. These results suggest that endotoxin plays a key role in apoptosis in this bacterial infection model, although the precise mechanism is unclear. Similarly, Mitaka *et al*. reported that PMX-HP may protect against AKI by both inhibiting NF-kB signaling and preventing renal tubular cell apoptosis in rats [39].

Future directions of PMX-HP and endotoxin removal with immune cell alteration

Future efforts should focus on precision medicine, identifying new indications, and clarifying PMX-HP mechanisms of action. Accurate diagnosis of endotoxemia

is critical for selecting patients likely to benefit from PMX-HP. In Japan, the endotoxin-specific limulus amebocyte lysate (LAL) assay is available [40], and endotoxin activity assay (EAA) levels correlate with sepsis severity [41, 42]. Nonetheless, the clinical diagnosis of endotoxemia remains debated, emphasizing the need for a standardized and clinically relevant diagnostic approach.

Since 2006, PMX-HP has been applied in treating acute exacerbation of idiopathic pulmonary fibrosis (AE-IPF) [43, 44]. Japanese IPF guidelines published in 2017 noted limited evidence and advised against routine PMX-HP use in AE-IPF but suggested it may be appropriate for a select subset of patients [45]. While no randomized controlled trials have been conducted for AE-IPF, multiple studies indicate improvements in oxygenation and mortality, potentially through the elimination of activated neutrophils [35]. Acute exacerbations are often triggered by acute events, and although numerous studies exist, the role of infection in AE-IPF remains uncertain. Evaluating endotoxemia in these patients is warranted.

During the 2009 H1N1 pandemic, PMX-HP improved the oxygenation index (PaO2/FiO2) in patients with severe respiratory failure [46, 47]. Additionally, PMX-HP has been applied in critically ill COVID-19 patients [48–51], and recent findings report elevated EA values in these patients [52]. Severe viral infections may thus represent a potential therapeutic target for PMX-HP, though further studies are required to confirm efficacy.

The precise mechanisms through which PMX-HP ameliorates organ dysfunction remain unclear. Further investigation is necessary to determine whether its effects derive primarily from endotoxin removal, immune cell modulation, or a combination of both.

Conclusion

Since its introduction in 1994, PMX-HP has been safely used for septic shock management. Cohort studies utilizing large clinical databases suggest potential survival benefits, though definitive evidence is still awaited. The ongoing TIGRIS multicenter randomized controlled trial aims to establish robust clinical evidence for PMX-HP in septic shock patients with endotoxemia who are likely to benefit. Beyond endotoxin removal, PMX-HP functions as an immunomodulatory device. Future research is required to elucidate its mechanisms and to define optimal patient populations for treatment.

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