# Importance of Endotoxin Clearance in Endotoxemic Septic Shock: An Analysis From the Evaluating Use of PolymyxinB Hemoperfusion in a Randomized Controlled Trial of Adults Treated for Endotoxemic Septic Shock (EUPHRATES) Trial

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**Objectives:** To investigate the relationship between survival and treatment-related reduction in endotoxin activity for patients in the Evaluating Use of PolymyxinB Hemoperfusion in a Randomized controlled trial of Adults Treated for Endotoxemia and Septic shock trial with baseline endotoxin activity assay greater than or equal to 0.60 to less than 0.90 units.

**Design:** Post hoc analysis of a multicenter randomized controlled clinical trial.

**Setting:** Fifty-five tertiary hospitals in North America.

**Patients:** Patients with septic shock and endotoxin activity assay level greater than or equal to 0.60 to less than 0.90 and multiple organ dysfunction syndrome greater than 9.

**Interventions:** Two polymyxin B hemoperfusion treatments or Sham.

**Measurements and Main Results:** One-hundred ninety-four patients were included (88 polymyxin B and 106 Sham). We evaluated the impact of changes in endotoxin activity assay based on comparison to the median reduction from baseline to day 3 and a second method where a target post-treatment endotoxin activity assay level (day 3) was established. The population median reduction in endotoxin activity assay level was 10.4%. In patients with a greater than median reduction, there was trend toward lower mortality with polymyxin B (17.1% vs 33.3%; $p = 0.07$) and a significant increase in mechanical ventilation-free days (20 vs 13.5; $p = 0.04$). The pressure adjusted heart rate showed a significant improvement in the polymyxin B group ($p = 0.02$). For patients who achieved an endotoxin activity assay of less than 0.65 at day 3, the polymyxin B treated group had a trend toward a mortality reduction compared to Sham (16% vs 33%; $p = 0.06$) and a significant increase in ventilation-free day (20 vs 16; $p = 0.05$). Kaplan-Meier analysis showed a 17% reduction in mortality with polymyxin B ($p = 0.04$).

**Conclusions:** These findings suggest that reducing endotoxin activity assay levels with polymyxin B as measured by comparison to a median reduction or when a treatment target is established, may result in improvements in mortality and organ function outcomes. This article is the first to report endotoxin activity assay measurements in response to polymyxin B use versus Sham in patients with septic shock and elevated endotoxin activity assay. These findings are considered to be hypothesis generating and will need to be prospectively validated.

**Key Words:** endotoxin; mortality; polymyxin B hemoperfusion; sepsis

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Endotoxin is a potent trigger for the sepsis inflammatory cascade (1). Elevated levels of endotoxin are measured in septic shock patients with a confirmed Gram-negative infection but also in Gram-positive and fungal or mixed infections as well as in patients with persistent negative cultures (2–4). It is widely reported that endotoxin will translocate across the gut

mucosal membrane in the setting of critical illness (5). The presence of elevated endotoxin activity in septic patients correlates with worsening organ failure (6) and high endotoxin activity assay (EAA) levels are associated with increased mortality (2–4, 7).

The EAA has been used since 2004 to measure endotoxin activity in humans and is based on the ability of its key reagent, an antibody to the highly conserved lipid A epitope of endotoxin to form an antibody-antigen complex in whole blood (8). The antibody has a very high binding affinity, leading to a very high sensitivity. In addition, the antibody does not cross react with Gram-positive or fungal components allowing for a very high specificity. The results are expressed in EAA units where less than 0.39 is low, 0.40–0.59 is an intermediate level, and greater than or equal to 0.60 is a high level. The EAA is the only assay that is approved by the U.S. FDA for measuring endotoxin activity in whole blood. Many therapeutic strategies targeting endotoxin in sepsis have been evaluated and none have shown to impact the course of sepsis in the critically ill (9, 10). The only exception is a novel approach developed in Japan in the 1980s, whereby "blood purification" is achieved using extracorporeal hemoperfusion (11).

Polymyxin B (PMX) is an antibiotic that binds the lipid A component of endotoxin. Its parenteral administration is restricted due to the potential of neuro- and nephrotoxicity. Extracorporeal PMX hemoperfusion was developed to take advantage of PMX's avid endotoxin binding properties while avoiding its systemic toxicity (12). The PMX hemoperfusion cartridge encloses polystyrene-derivative fibers to which PMX is covalently bound. PMX treatment occurs by venovenous extracorporeal hemoperfusion through the cartridge at a flow rate of 80–120 mL/min for 2 hours and is typically administered twice over a 24-hour period (12). It has recently been shown to have a capacity to bind approximately 12 µg of circulating endotoxin per treatment—roughly 24 µg for two treatments (11).

The EAA assay results are not linearly related to endotoxin concentration in blood (13). For example, a reduction in EAA from 0.8 to 0.7 EA units (roughly 2,000 pg/mL reduction) is not equivalent to a reduction from 0.6 to 0.5 EA units (roughly a 100 pg/mL reduction). Thus, simple math cannot be used to calculate the amount of reduction or to compare the amount reduced between two groups.

Although there are hundreds of published articles on the use of the PMX cartridge, the quality of the evidence is generally low. Recently, three randomized multicenter controlled trials have been completed and published with variable results (14–16). The Evaluating the Use of [PMX] Hemoperfusion in a Randomized controlled trial of Adults Treated for Endotoxemia and Septic shock (EUPHRATES) trial included patients with septic shock and high EAA levels (≥ 0.6). Its objective was to test whether adding two PMX treatment would improve mortality at 28 days compared to standard medical therapy alone (16). The study did not demonstrate a difference in mortality at 28 days in the

intention-to-treat population (16), but in a post hoc analysis, a potential mortality benefit was demonstrated in patients with subextreme levels of EAA (< 0.9) (17). The EUPHRATES trial was the only one to capture serial EAA measurements.

Therefore, we performed an exploratory analysis of patients from EUPHRATES and examined whether reducing endotoxin activity levels is associated with improved mortality at 28 days and in other outcomes of interest. In addition, since it has recently been determined that the PMX cartridge method of endotoxin removal can remove approximately 24 µg of endotoxin (presuming two cartridge exposure) and that an EAA level of greater than 0.90 is interpreted as much higher (13), we restricted the analysis to patients with baseline EAA between 0.6 and 0.9 (17).

## MATERIALS AND METHODS

We performed an exploratory analysis on a subpopulation of the EUPHRATES trial as characterized by Klein et al (17). The full protocol and results have previously been published (ClinicalTrials.gov ID: NCT01046669) (16). The patients included in the trial (or substitute decision-maker) provided informed consent. The trial protocol was approved by the institutional research ethics board at each participating site.

### Patients and Definitions

Patients with septic shock and EAA level of 0.60 or greater were enrolled in the trial. Septic shock was defined as treatment with antibiotics for a confirmed or presumed infection, persistent hypotension despite administration of adequate fluid resuscitation, presence of organ dysfunction, and vasopressor therapy for at least 2 continuous hours at protocol described rates. The ICU treating teams were blinded to patient's randomization allocation and post-baseline EAA levels. Klein et al (17) demonstrated a clinically significant reduction in 28-day mortality and improvement in secondary outcomes in patients with baseline EAA levels between 0.6 to 0.9 who received two full treatments per protocol. We chose to further analyze this subgroup of patients.
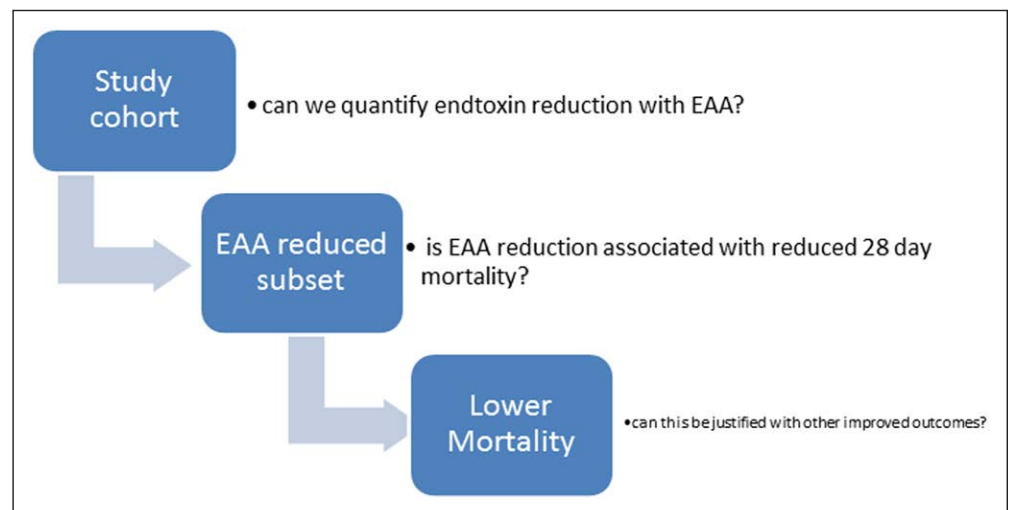


**Figure 1.** Study design algorithm. EAA = endotoxin activity assay.

## Interventions

Patients randomized to the PMX group received two treatments in 24 hours and the Sham group received two Sham hemoperfusion events. The ICU treating medical staff was blinded to the treatment allocation, a second team of nephrologists and dialysis nurses performed the PMX and Sham treatments. The full procedure including the Sham event is detailed in a prior publication (16).

## Endotoxin Activity Assay Analysis

The EAA (Spectral Medical, Toronto, ON, Canada) was measured at baseline, then again at approximately 10 hours after the first PMX cartridge or Sham treatment, at 10 hours after the second PMX cartridge or Sham treatment, and again at 24 hours following the treatment with the second PMX cartridge (day 3). The EUPHRATES study required a baseline minimum level of 0.60 EA units for enrollment (18).

To evaluate the change in endotoxin levels, two methods were used (**Fig. 1**):

1) Calculation of the median reduction in EAA. This was calculated for each patient using the formula of (day 3 EAA–baseline EAA)/baseline EAA. Then the median level was determined using summary statistics.
2) Maximally selected log-rank statistics were used to identify the EAA cutoff for day 3 result that corresponds to the most significant relation with survival, as implemented in the survminer R

## TABLE 1. Comparison of the Study Groups at Baseline

| Variable | Statistic | Polymyxin B | Sham | Total | p |
|---|---|---|---|---|---|
| Patients | n | 88 | 106 | 194 | |
| Age, yr | Mean (SD) | 58.7 (15) | 57.5 (14.4) | 58.04 (14.6) | 0.4357 |
| Gender | Female, n (%) | 33 (37.5) | 40 (37.7) | 73 (37.6) | 0.9731 |
| Race/ethnicity | Asian, n (%) | 2 (2.27) | 6 (5.66) | 8 (4.12) | 0.6537 |
| | Black, n (%) | 8 (9.09) | 8 (7.55) | 16 (8.25) | |
| | Caucasian, n (%) | 72 (81.8) | 81 (76.4) | 153 (78.9) | |
| | Hispanic, n (%) | 3 (3.41) | 7 (6.6) | 10 (5.15) | |
| | Other, n (%) | 3 (3.41) | 4 (3.77) | 7 (3.61) | |
| Endotoxin activity assay baseline | Mean (SD) | 0.735 (0.08) | 0.726 (0.08) | 0.7302 (0.0829) | 0.4606 |
| Multiple organ dysfunction syndrome score | Mean (SD) | 11.7 (1.63) | 11.9 (1.79) | 11.79 (1.72) | 0.6127 |
| Acute Physiology and Chronic Health Evaluation II score | Mean (SD) | 30.6 (7.63) | 29.2 (8.09) | 29.84 (7.9) | 0.2425 |
| Cumulative vasopressor index | Mean (SD) | 6.85 (3.3) | 7.08 (3.06) | 6.974 (3.16) | 0.6988 |
| Mean arterial blood pressure | Mean (SD) | 71.7 (9.23) | 73.5 (10.1) | 72.69 (9.75) | 0.5056 |
| | Stage 0, n (%) | 19 (21.6) | 20 (18.9) | 39 (20.1) | |
| Acute Kidney Injury Network score | Stage 1, n (%) | 9 (10.2) | 17 (16) | 26 (13.4) | 0.6876 |
| | Stage 2, n (%) | 11 (12.5) | 12 (11.3) | 23 (11.9) | |
| | Stage 3, n (%) | 48 (54.5) | 57 (53.8) | 106 (54.6) | |
| Renal replacement therapy use | Yes, n (%) | 19 (21.6) | 28 (26.4) | 47 (24.2) | 0.435 |
| | Intra-abdominal, n (%) | 25 (28.4) | 43 (40.6) | 68 (35.1) | |
| Presumed site of infection | Lung, n (%) | 29 (33) | 38 (35.8) | 67 (34.5) | 0.06822 |
| | Mixed, n (%) | 4 (4.55) | 6 (5.66) | 10 (5.15) | |
| | Other, n (%) | 28 (31.8) | 19 (17.9) | 47 (24.2) | |
| | Missing, n (%) | 2 (2.27) | 0 (0) | 2 (1.03) | |
| | Gram-negative, n (%) | 22 (25) | 13 (12.3) | 35 (18) | |
| Microorganisms | Gram-positive, n (%) | 20 (22.7) | 33 (31.1) | 53 (27.3) | 0.156 |
| | Mixed, n (%) | 13 (14.8) | 22 (20.8) | 35 (18) | |
| | No growth, n (%) | 28 (31.8) | 31 (29.2) | 59 (30.4) | |
| | Other, n (%) | 5 (5.68) | 7 (6.6) | 12 (6.19) | |

For continuous variables, the p values are based on an unequal variance t test between groups. For categorical variables, the p values are based on a χ² test, or Fisher exact test when applicable.

package (https://cran.r-project.org/web/packages/survminer/index.html). This process is used to estimate cut points based on optimized statistical relationships (19).

## Outcomes

The primary endpoint was mortality at 28 days post-randomization. Secondary endpoints were mortality over time to 28 days, change in pressure adjusted heart rate (PAR), mechanical ventilation-free days (VFDs), and dialysis free days.

## Statistical Methods

Continuous variables were presented with mean, SD, median, 25–75th interquartile range and analyzed through $t$ test or Wilcoxon rank-sum test, as applicable. Categorical variables are presented as frequencies and percentages by treatment group and were analyzed using chi-square test or Fisher exact test. Survival analysis, with censoring at 28 days, was performed and depicted using a Kaplan-Meier curve. Maximally selected log-rank statistics (https://cran.r-project.org/web/packages/maxstat/vignettes/maxstat.pdf) were used to define the optimal cut-point discriminator between groups with respect to the primary endpoint. Log-rank test was used to compare the survival distributions between treatments. All analyses were performed in R (version 3.6.0; R Foundation for Statistical Computing, Vienna, Austria; http://www.r-project.org), with $p$ value of less than 0.05 considered statistically significant.

## RESULTS

### Patients and Demographics

There were 194 patients with an EAA level 0.6–0.9 and multiple organ dysfunction syndrome (MODS) greater than 9, of which 88 patients were in the PMX group and 106 in the Sham group. The groups had similar demographic and physiologic variables at baseline, in particular for the PMX group versus Sham the mean MODS was 11.7 (± 1.63) versus 11.9 (± 1.79); $p = 0.63$, mean Acute Physiology and Chronic Health Evaluation II score was 30.6 (± 7.63) versus 29.2 (± 8.09); $p = 0.24$, and EAA levels 0.73 (± 0.08) versus 0.73 (± 0.08); $p = 0.46$ (**Table 1**).

### Survival and Change in EAA Level

The median change in EAA at day 3 was calculated to be an overall reduction in EAA by 10.4% (range reduction of 86% to an increase of 49%). We compared outcomes for all patients with the change in EAA level as above or below the median change for the population. For all subjects regardless of treatment arm, when the EAA reduction was greater than the overall median, the 28-day mortality was 26% (25/95). For the Sham group, the median change was (−0.08) and the average change (+0.09), whereas for the PMX group, the median change was (−0.07) and the average is (−0.09). For those who did not achieve at 10.4% reduction the 28-day mortality was 38% (36/96) ($p = 0.1$).

When the patients with a greater than median reduction were separated by treatment allocation, there was a nonstatistically significant yet clinically meaningful difference of 16.2% in favor of

## TABLE 2. Outcome 28-Day Mortality

| Patients or Population | Alive, $n$ (%) | Dead, $n$ (%) | $p$ |
|---|---|---|---|
| Patients | | | |
| PMX | 65 (74) | 23 (26) | 0.11 |
| Sham | 67 (63) | 39 (37) | |
| Change in EAA | | | |
| Above median | 70 (74) | 25 (26) | 0.097 |
| Below median | 60 (62) | 36 (38) | |
| Greater than median reduction | | | |
| PMX | 34 (83) | 7 (17) | 0.07 |
| Sham | 36 (67) | 18 (33) | |
| EAA level on day 3 | | | |
| ≤ 0.65 | 68 (75) | 23 (25) | 0.06 |
| > 0.65 | 62 (62) | 38 (38) | |
| EAA ≤ 0.65 | | | |
| PMX | 36 (84) | 7 (16) | 0.06 |
| Sham | 32 (67) | 16 (33) | |

EAA = endotoxin activity assay, PMX = polymyxin B.

the PMX treated arm (7/41 [17.1%] vs 18/54 [33.3%]; $p = 0.07$) (**Table 2**).

The Kaplan-Meier estimates of the probability of survival (log-rank test) showed a similar trend for all patients when comparing above and below change in EAA level ($p = 0.096$), and when comparing PMX versus Sham in patients with greater than median reduction ($p = 0.06$) (**Fig. 2**).

### Survival and Day 3 EAA Level

We calculated a target day 3 EAA using maximally selected rank statistic. The EAA level on day 3 that is associated with a mortality benefit is 0.65. We then divided the groups between patients who achieved a day 3 level less than or equal to 0.65 and those greater than 0.65.

The 28-day mortality for patients with a day 3 EAA less than or equal to 0.65 was 23 of 91 (25%) and greater than 0.65 was 38 of 100 (38%) ($p = 0.06$) (Table 2).

For patients who achieved a day 3 EAA of less than or equal to 0.65, those in the PMX arm had a 28-day mortality of seven of 43 (16%) versus the Sham arm 16 of 48 (33%) ($p = 0.06$) (Table 2).

Using a Kaplan-Meier survival analysis, we found significant differences in the probability for survival to 28 days between the patients that had day 3 level of less or equal to 0.65 and those greater than 0.65 ($p = 0.05$). For patients who achieved a level of less or equal to 0.65 on day 3, those in the PMX group had a significant survival compared to Sham ($p = 0.04$) (**Fig. 3**).

### Secondary Outcomes Based on Greater Than Median Reduction of Endotoxin

For patients with greater than median EAA reduction, the PMX treated group had significantly more VFD (median 20 vs 13.5 d;
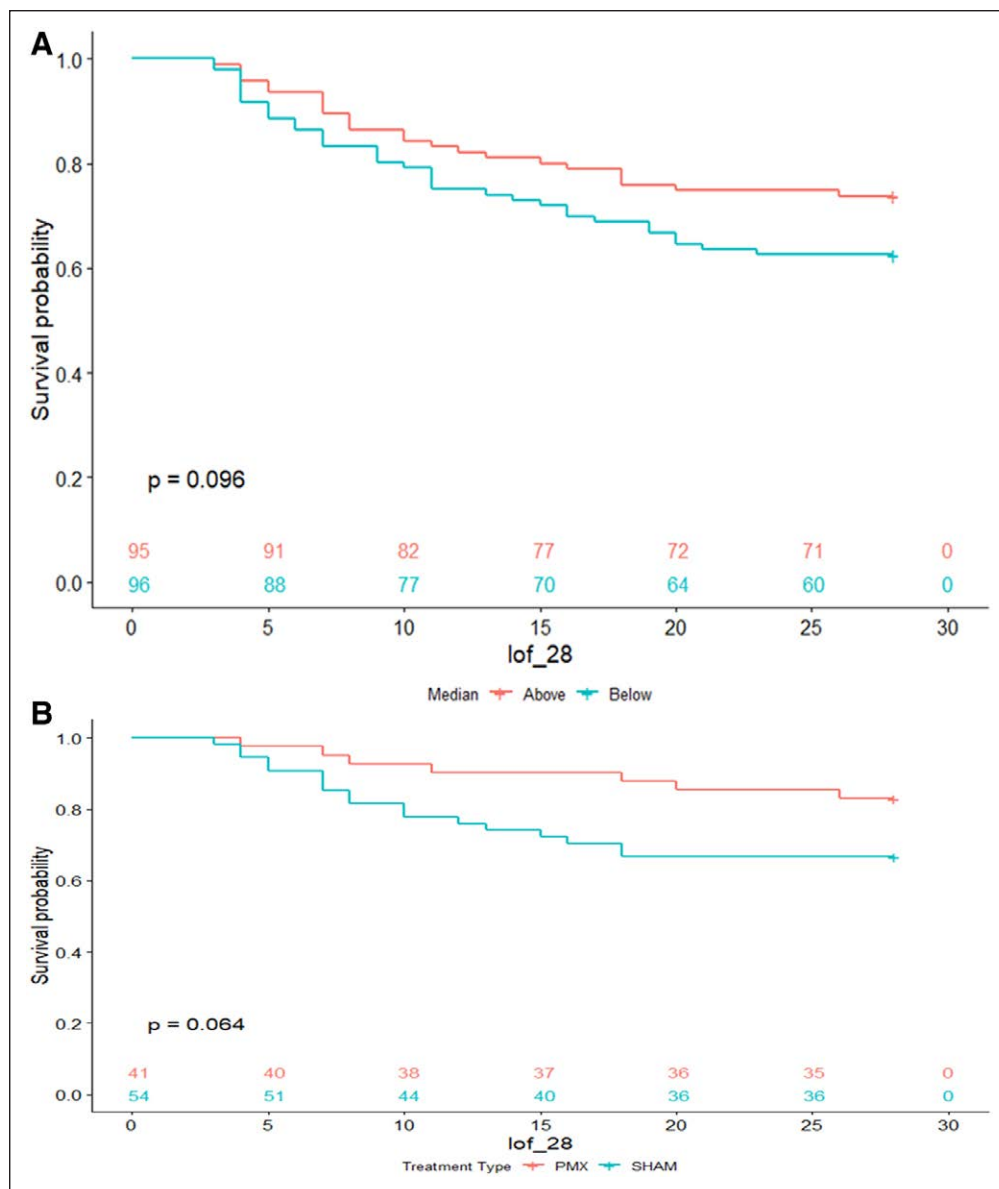
Figure 2. **A**, Kaplan-Meier estimates of the probability of survival to day 28 by above and below median endotoxin activity assay (EAA) reduction groups. *X*-axis is days. The log-rank test with $p = 0.096$. **B**, Kaplan-Meier estimates of the probability of survival to day 28 among subjects with above median EAA reduction, by treatment groups. *X*-axis is days. The log-rank test with $p = 0.064$. PMX = polymyxin B.

## DISCUSSION

The evolution of medicine to allow for the selective targeting of those patients most likely to benefit from a specific therapy has been referred to as "theragnostics or precision medicine" (20). Currently, several oncological treatments are being optimized based on specific mutations or markers in patients (21). However, precision medicine is much broader and also includes the ability to titrate the dose, timing, duration, and other variables of a therapy to maximize therapeutic benefit and minimize side effects (22).

Septic shock with endotoxemia represents a complex, but potentially ideal disease state for this therapeutic approach. Seymour et al (23) recently described four different novel phenotypes of sepsis patients using artificial intelligence and biomarkers wherein the risk of death varied from 5% to 40% from the lowest risk to the highest risk phenotypic group.

Ronco et al (24) have long described the "Peak Concentration Hypothesis" wherein continuous renal replacement therapies particularly at high volumes might be beneficial in cutting the peaks of the concentrations of both pro- and anti-inflammatory mediators, restoring a situation of immunohomeostasis. Recently they have refined this and described "Sequential Extracorporeal Therapy in Sepsis," which incorporates PMX into the "peak concentration" approach along with continuous renal replacement therapy (CRRT) (25).

$p = 0.04$). Dialysis free days was 22 versus 15 days (median PMX vs Sham) ($p = 0.18$). The PAR also showed a significant improvement in the PMX treated group versus Sham, mean (SD) change from baseline: (−2.7 [2.4] vs −1.2 [2.7]; 95% CI, −2.3 to −0.2; $p = 0.02$).

### Secondary Outcomes Based on Treatment Target of Less Than 0.65 EAA Units

For patients with EAA level of less than 0.65 on day 3, there was a significantly higher VFD in PMX treated patients versus Sham (median) 20 versus 16 days ($p = 0.05$). Dialysis free days were not significantly different (median PMX vs Sham) 20 versus 15 days ($p = 0.35$). The PAR was (mean [SD] change from baseline) −2.6 (2.4) versus −1.7 (2.7); 95% CI, −2.0 to 0.1; $p = 0.08$ in PMX and Sham groups, respectively.

In this article, we continue to further unravel the complex dataset of the EUPHRATES trial. We found that patients who achieved reductions in EAA levels or reached a specific EAA goal had a trend improvement in the mortality outcome. Although this result did not achieve statistical significance, the trial was underpowered for this subgroup. Lowering of endotoxin levels was also associated with improved organ function for cardiovascular (PAR) and respiratory systems as measured by less days on a ventilator. This enhances the biologic plausibility to the mechanism wherein endotoxin reduction has the potential to reduce 28-day mortality. The importance of these findings is not only to better identify those patients as appropriate for anti-endotoxin therapy, but to also consider the use of EAA to meaningfully monitor the response to therapy and potentially dose-adjust according to that
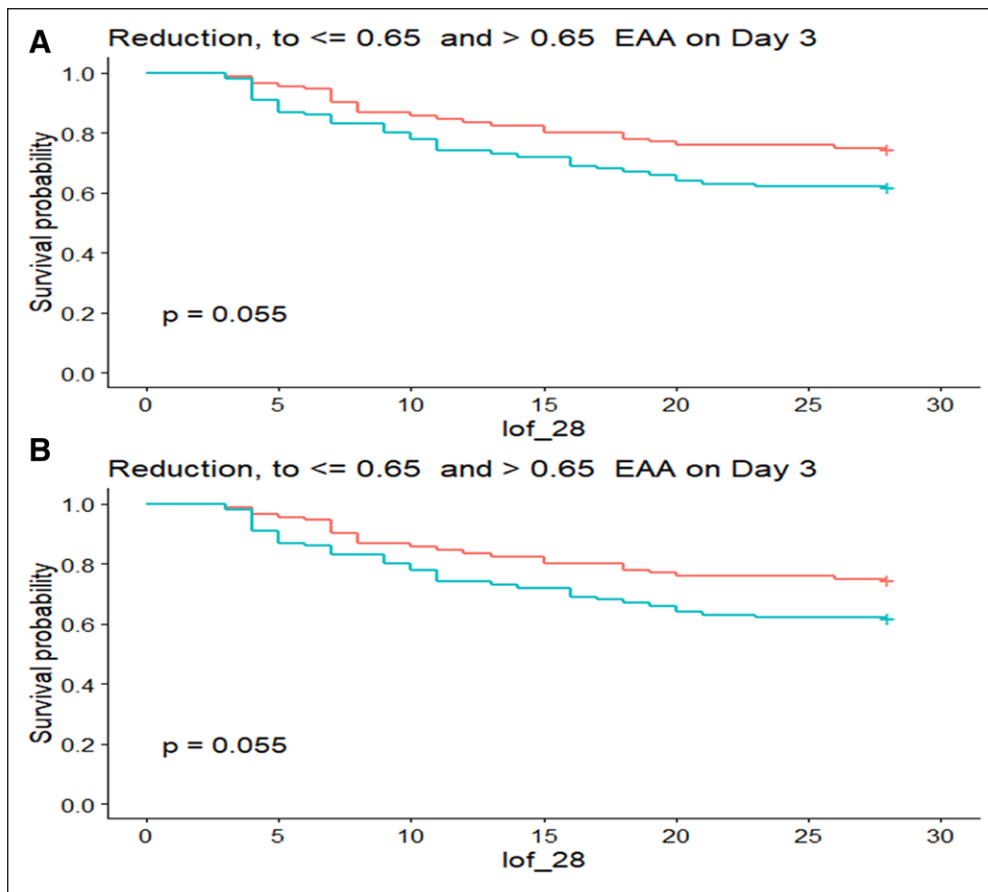
**Figure 3. A**, Kaplan-Meier estimates of the probability of survival to day 28 by day 3 endotoxin activity assay (EAA) cutoff point of 0.65. *X*-axis is days. The log-rank test with *p* = 0.055. **B**, Kaplan-Meier estimates of the probability of survival to day 28 by day 3 EAA cutoff point of 0.65. *X*-axis is days. The log-rank test with *p* = 0.042.

response. In other words, we show herein that two treatments with PMX can reduce endotoxin levels but additional treatments to achieve the required level of reduction might be needed for some patients to reduce mortality more broadly.

The randomized controlled trials conducted so far have been performed with a fixed number of PMX treatments (one or two). Our analysis suggests that this may be an insufficient dose for patients with high levels of endotoxin activity. Furthermore, it may be that endotoxin found in the bloodstream may not represent the totality of its presence in other compartments such as interstitial fluid. The dosing procedure for PMX includes a period of 22–24 hours between PMX cartridge administrations so as to allow endotoxin to re-compartmentalize from extravascular sites.

It is unknown how much is enough when it comes to endotoxin reduction. In a study that looked at a "treat to a target" approach for EAA levels in transplant patients that underwent PMX therapy, 12 out of 28 patients included required more than two treatments to lower the EAA levels to their prespecified target including four patients who required four treatments (26). Importantly, there were no deaths in any of these patients. In another study, 10 out of 17 patients with postoperative septic shock required three or more PMX treatments to lower EAA levels to a prespecified target of 0.4. In that study,

treatment with PMX and lowering of EAA level resulted in significant improvements in hemodynamic variables and all but one survived at 60 days (27).

In another retrospective study of a propensity-matched cohort of critically ill patient septic shock on CRRT, Iwagami et al (28) found that patients that received two PMX treatments had a lower 28-day mortality compared to those that had only one session (35.7% vs 42.6%) suggesting a possible "dose response." It is possible that observational studies like this one from Japan where PMX is widely available and where clinicians use a variable number of treatments based on clinical response could actually better mirror real-world clinical practice of treating to an EAA/clinical response level.

Reductions of endotoxin in patients can occur endogenously through renal and hepatic mechanisms and exogenously via hemo-adsorption (12, 29, 30). In this study, we have found that when the reduction included exogenous removal such as for the PMX group, outcomes were improved. In those patients, endotoxin reduction led to improved cardiovascular organ function and less days on mechanical ventilation. This could allow for greater chance of survival to 28 days.

It is notable that the reduction in EAA units in both PMX and Sham groups is both relatively small in absolute terms and similar between the groups. In considering this, one needs to recognize the logarithmic nature of the EAA dose-response curve where even small differences in EAA represent large biological changes in circulating levels of lipopolysaccharide. Also, with respect to the relatively similar reduction between the groups, there are many complex factors that may play a potential role in this including other potential effects of PMX therapy on other PAMPs/DAMPs/mediators, the complex kinetics of EAA and endotoxin clearance, along with numerous others that could not be considered in the current analysis.

Our article has several limitations. Most importantly, it is an exploratory analysis based on a subgroup of the larger EUPHRATES trial. Therefore, any findings or suggestions need to be confirmed in prospectivetrials designed to answer the specific question of whether targeting a predefined EAA goal would improve outcome. Second, the measurement of EAA levels were consistently measured among patients at 24-hour intervals; however, more frequent measurements could have provided added granularity to the data. A third limitation is that we have not

evaluated possible confounding factors that might have contributed to day 3 levels of EAA. These could include conditions such as inadequate source control of a gut or infective source, a difference in severity of lung injury, inappropriate choices of antibiotics, or the influence of a new hospital-acquired infection or others. The study is also limited in that we did not have information within the study cohort on the use of greater than two PMX cartridges. Also, we did not test for the impact of multiple comparisons in our statistical plan.

## CONCLUSIONS

These findings suggest that PMX enhanced reduction in septic shock patients with pretreatment elevated EAA levels may be associated with improved outcomes. The dosing regimen of PMX therapy may not be "one size fits all" and should be tailored according to measured post-treatment levels, patient's clinical response, or a combination of both. These findings are considered to be hypothesis generating and will need to be prospectively justified.

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