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Interim analysis and trial termination

Two prespecified interim analyses were planned after approximately 30% and 60% of participants had completed 28-day follow-up. An asymmetric two-sided group sequential design was used with bounds derived from a Hwang–Shih–DeCani spending function ($\gamma = -4$ for the efficacy bound; $\gamma = -2$ for the futility bound; one-sided $\alpha = 0.025$; power = 80%). The futility bound was non-binding. The test statistic (Z) was computed as the standardized difference in 28-day mortality proportions (HA minus ST), such that negative values indicate lower mortality in the HA group. The prespecified efficacy and futility Z boundaries are summarized in **Supplementary Table 1.**

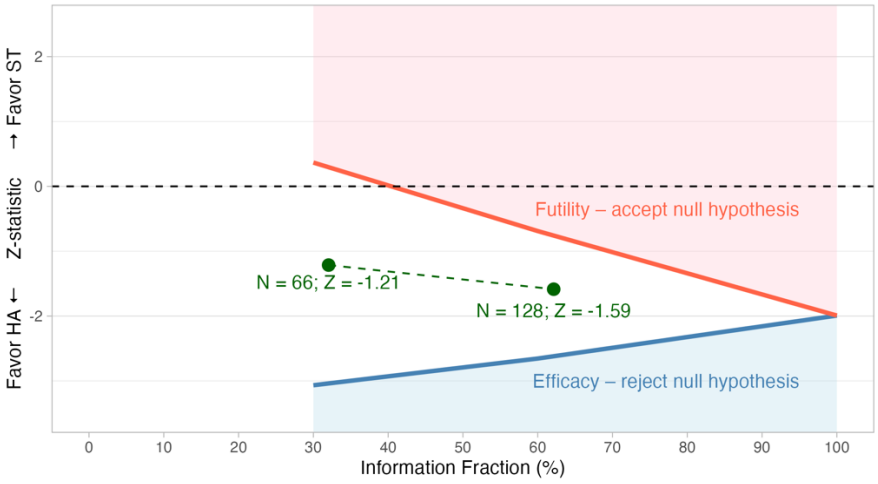
Supplementary Table 1. Prespecified efficacy and futility Z boundaries for the group sequential design.

Analysis	Information	Efficacy Z	Efficacy one-sided P threshold	Futility Z
First interim	30%	−3.07	0.0011	0.367
Second interim	60%	−2.66	0.0040	−0.689
Final	100%	−1.99	0.0232	−1.99

First interim analysis. After 66 participants had completed follow-up (information fraction 32%), 28-day mortality was 53.1% in the ST group (17/32) and 38.2% in the HA group (13/34). The observed Z statistic was −1.21 (one-sided P = 0.112), which did not cross the prespecified efficacy boundary (Z = −3.07; one-sided P threshold = 0.0011) or the futility boundary (Z = +0.37). The trial continued per protocol.

Second interim analysis. After 128 participants had completed follow-up (information fraction 62%), 28-day mortality was 58.5% in the ST group (38/65) and 44.4% in the HA group (28/63). The observed Z statistic was −1.59 (one-sided P = 0.056; two-sided P = 0.113), which did not cross the prespecified efficacy boundary (Z = −2.65; one-sided P threshold = 0.0040) or the non-binding futility boundary (Z = −0.69). Therefore, no formal stopping was recommended at this stage, and the trial was planned to continue. **Supplementary Figure 1** illustrates the observed Z-statistic trajectory relative to the prespecified boundaries.

However, the enrollment rate was slower than expected, with 62% of planned enrollment achieved after 55 months. Due to slow recruitment and exhaustion of available funding, the investigators and sponsor decided to terminate the study at this point. The data presented in this report represent the final analysis of the terminated trial.



Supplementary Figure 1. Z-statistic trajectory from the prespecified interim analyses relative to the group sequential design boundaries.
The x-axis represents the information fraction (percentage of the planned total sample). The y-axis displays the Z statistic (negative values favor the HA group). The red line denotes the nonbinding futility boundary (Hwang–Shih–DeCani spending function, gamma = −2); crossing into the pink-shaded region would indicate potential futility. The blue line denotes the efficacy boundary (gamma = −4); crossing into the blue-shaded region would warrant discussion of early stopping for benefit. Green dots indicate the observed Z statistics at the two interim analyses: first interim (N = 66; Z = −1.21; information fraction = 32%) and second interim (N = 128; Z = −1.59; information fraction = 62%). At both analyses, the observed Z statistic fell between the boundaries.
Abbreviations: HA, hemoadsorption; ST, standard treatment.

Outcome Measure Definitions

Primary outcome

Twenty-eight–day mortality was defined as death from any cause occurring on or before day 28 from hour 0. Vital status at day 28 was confirmed from medical records for participants who remained hospitalized and by telephone follow-up for those discharged before day 28.

Secondary outcomes

ICU mortality was defined as death occurring prior to ICU discharge, with no fixed time limit. For participants transferred to a non-participating ICU or step-down unit, vital status was ascertained only up to the point of transfer; no further follow-up was conducted beyond that point, unless the transfer occurred before day 28. Any resumption of organ support before day 28 resets the count to zero from the date of resumption; the final value reflects the number of days free of that support from the last successful liberation to day 28.

Hospital mortality was defined as death occurring prior to hospital discharge, with no fixed time limit. Similarly, for participants transferred to non-participating hospitals, vital status was ascertained only up to the point of transfer, unless the transfer occurred before day 28.

Organ support-free days were calculated as the number of days alive and free of the respective organ support through day 28, measured from hour 0. Patients who died on or before day 28 were assigned a value of 0, irrespective of their organ support status at the time of death, in accordance with the pre-defined penalization of non-survival.[1] For patients who were never placed on a given organ support, the full 28 days were credited from day 1. For all organ support-free day outcomes, liberation was considered successful only if the participant remained free of the respective support for the remainder of the 28-day observation period without resumption; any resumption before day 28 resulted in a value of 0.

Ventilator-free days were counted from the day of successful extubation or liberation from positive pressure ventilation. Non-invasive ventilation was not counted as mechanical ventilation or positive pressure ventilation. Patients who underwent tracheostomy were considered liberated after 48 consecutive hours free of positive pressure ventilation delivered through the tracheostomy, regardless of whether the tracheostomy remained in situ.

RRT-free days were counted from the day of successful discontinuation of any mode of acute renal replacement therapy. For patients with pre-existing chronic dialysis who required intensification of renal support during the study period, the date of liberation was defined as the date on which the dialysis regimen returned to the participant's outpatient prescription dose and modality (for example, transition from continuous RRT back to intermittent hemodialysis or peritoneal dialysis at the outpatient dose)

Vasopressor-free days were counted from the day of successful discontinuation of all vasopressor agents.

ICU-free days were counted from the day of ICU discharge, defined as transfer out of intensive care-level monitoring and treatment, whether to a participating or non-participating unit. Intermediate care and step-down units were not considered ICU-level care for this outcome.

Shock reversal at hour 6 was defined as achievement of a mean arterial pressure ≥ 65 mmHg, accompanied by either a reduction in lactate of ≥ 20% from the hour-0 value or a mean urine output of > 0.5 mL/kg/h over the preceding 6 hours.[2] Both the blood pressure and the perfusion criterion (lactate reduction or urine production) had to be present simultaneously. Patients who died before hour 6 were classified as not having achieved shock reversal.

Vasoactive-inotropic score was calculated as:[3]

VIS = dopamine (mcg/kg/min) + dobutamine (mcg/kg/min) + [epinephrine (mcg/kg/min) × 100] + [norepinephrine (mcg/kg/min) × 100] + [milrinone (mcg/kg/min) × 10]

Vasopressin was not available in Thailand during the study period and was not included in the calculation.

Norepinephrine-equivalent dose was calculated as a sensitivity analysis to isolate vasopressor effect from inotropic support, using the following conversion:[4]

Norepinephrine-equivalent dose (mcg/kg/min) = Norepinephrine dose (mcg/kg/min) + epinephrine dose (mcg/kg/min) + [dopamine dose (mcg/kg/min) / 150]

Vasopressin was not available in Thailand during the study period, and phenylephrine was not used in the participating centers. They were therefore not included in the calculation.

Adverse events

- **Hypotension during hemoadsorption or renal replacement therapy** was defined as a reduction in systolic blood pressure of > 20 mmHg or mean arterial pressure of > 10 mmHg from the pre-session value, or a requirement to increase vasopressor dose by an amount equivalent to ≥ 0.1 mcg/kg/min of norepinephrine, occurring during an active session.
- **Arrhythmias during hemoadsorption or renal replacement therapy** were recorded when they required medical treatment (antiarrhythmics) or electrical therapy (cardioversion or defibrillation). Asymptomatic or self-terminating events not requiring intervention were not recorded as adverse events.
- **Dialysis catheter-related bleeding** was defined as bleeding at the catheter insertion site requiring pharmacological intervention, blood transfusion, or surgical hemostasis.
- **Dialysis catheter-related infection** was defined as a bloodstream infection or local infection at the catheter site attributed to the dialysis catheter by the treating clinician, based on clinical and microbiological criteria.
- **New-onset leukopenia** was defined as a white blood cell count < 4,000/mm³ occurring in participants whose white blood cell count was ≥ 4,000/mm³ at hour 0. Participants with leukopenia at hour 0 were excluded from the denominator.
- **New-onset thrombocytopenia** was defined as a platelet count < 20,000/mm³ occurring in participants whose platelet count was ≥ 20,000/mm³ at hour 0.

Participants with platelet count < 20,000/mm³ at hour 0 were excluded from the denominator.

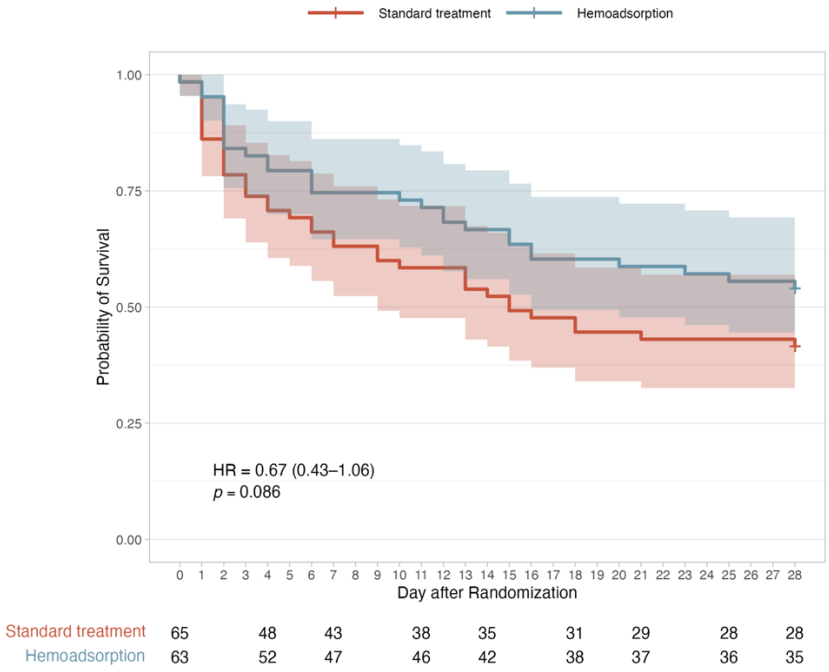
- **New episodes of septic shock** were defined as the requirement to restart vasopressors after a period of successful weaning, accompanied by either a change in antimicrobial regimen or a procedure to control a new or persistent source of infection.

Sensitivity analysis of the primary outcome using the time of randomization as the starting day

The primary analysis used hour 0 as the time origin for survival outcomes, prospectively designated to all outcomes for consistency. As a sensitivity analysis, 28-day mortality was re-analyzed using the time of randomization as the time origin, which represents the conventional anchor for survival outcomes in randomized trials.

Using this approach, 38 of 65 (58%) participants in the ST group and 29 of 63 (46%) in the HA group had died by day 28 (relative risk, 0.79; 95% CI, 0.56–1.10; P = 0.16). Of note, one participant in the HA group was classified as a day-28 death under this analysis but not under the primary analysis, due to the difference in time origin between randomization and hour 0.

The hazard ratio for 28-day mortality in the HA group compared with the ST group was 0.67 (95% CI, 0.43–1.06; P = 0.086; **Supplementary Figure 2**). These results are consistent with the primary analysis, confirming that the choice of time origin does not substantially affect the findings.



Supplementary Figure 2. Kaplan–Meier estimates of survival through day 28, with time anchored to randomization. Survival curves are shown for the ST group (red) and the HA group (blue). The hazard ratio (HR) was for death in the HA group compared with the ST group. The number of participants at risk is displayed below the x-axis at selected time points.

Abbreviations: CI, confidence interval; HA, hemoadsorption; HR, hazard ratio; ST, standard treatment.

Post-hoc Adjusted Cox Proportional Hazards Model

Rationale for covariate adjustment

The prespecified primary analysis was unadjusted. A post-hoc adjusted model was fitted as a sensitivity analysis, using log-transformed Interleukin-6 (IL-6) level and the vasoactive-inotropic score (VIS) measured at hour 0. Covariate selection was based on study design considerations and biological rationale, as well as formal model comparison metrics:

Study design rationale: The preparation period was fixed at 6 hours in the ST group and variable (median 7.5 hours, IQR 5.0–13.0) in the HA group, which was a prospectively designed feature to allow vascular access placement and circuit preparation before hemoadsorption initiation. This asymmetric duration means that the two groups reached hour 0 at different times relative to randomization, potentially introducing individual-level physiological changes that are not fully reflected in summarized characteristics, including evolving inflammatory burden and vasopressor requirements. VIS increased from a median of 37–39 at randomization to 42–46 at hour 0, illustrating the magnitude of physiological change during this interval. Adjustment for IL-6 and VIS at hour 0 therefore accounts for this potential source of variation.

Biological rationale: Both IL-6 and VIS are established independent predictors of mortality in septic shock.[5,6] IL-6 reflects the magnitude of the systemic inflammatory response, which is the primary target of cytokine adsorption with HA-330. VIS quantifies vasopressor dependency, which determines the severity of hemodynamic compromise. Adjustment for these variables accounts for treatment-relevant physiological variation at the start of the treatment period.

Furthermore, IL-6 and VIS at hour 0 are post-randomization measurements selected on design and biological grounds, not on statistical testing of group differences.[7] This model is explicitly labeled post-hoc and contextualized accordingly throughout the manuscript and supplement.

Model specification and results

The primary adjusted model, described in the main manuscript, was specified as:

28-day all-cause mortality ~ Treatment group + log(IL-6) at hour 0* + VIS at hour 0

* IL-6 was log-transformed to improve non-linearity associated with its markedly skewed distribution.

Summary of the model covariates' effects are shown in **Supplementary Table 2**.

Proportional hazards assumption

The proportional hazards assumption was assessed using scaled Schoenfeld residuals for all model terms. The treatment group variable satisfied the proportional hazards assumption (P = 0.409), confirming the validity of the reported hazard ratio as a summary measure of the treatment effect. Log(IL-6) showed a statistically significant time-varying effect on mortality (P = 0.002), consistent with the known attenuation of IL-6's prognostic value as the acute inflammatory phase resolves over time. The global test was significant (P = 0.006), driven

largely by the log(IL-6) term. As the primary interest of this model is the treatment effect estimate, which satisfies the proportional hazards assumption, this violation does not affect the interpretation of the adjusted hazard ratio.

Multicollinearity

Variance inflation factors were computed using a linear proxy model. All values were below 1.03, indicating no meaningful multicollinearity among covariates. Spearman correlation between log(IL-6) and VIS at hour 0 was 0.029, confirming that the two covariates capture independent dimensions of illness severity.

Influential observations

Residuals were evenly distributed around zero for all covariates with no systematic trend, and no individual observation exerted disproportionate influence on the treatment estimate.

Supplementary Table 2. Post-hoc adjusted Cox proportional hazards model for 28-day mortality.

Term	HR	95% CI	P
Treatment group Hemoadsorption vs Standard treatment	0.617	0.391–0.972	0.037
Log(IL-6) at hour 0 (per log unit)[a]	1.100	1.008–1.199	0.032
VIS at hour 0 (per unit)	1.009	1.002–1.015	0.006

Abbreviations: HR, hazard ratio; CI, confidence interval; ST, standard treatment; VIS, vasoactive-inotropic score.

[a] Log(IL-6) is expressed as natural logarithm; the HR represents the increase in hazard per one unit increase in log(IL-6), equivalent to a 2.72-fold increase in IL-6.

Covariate exclusions and sensitivity analyses

For reference, the unadjusted model yielded a hazard ratio of 0.682 (95% CI 0.435–1.070; P = 0.095; AIC = 643.4). The primary post-hoc specification (adding log(IL-6) and VIS at hour 0) significantly improved model fit relative to the unadjusted model ($\Delta\chi^2 = 10.89$, df = 2, P = 0.004; AIC = 636.5), resulting in a hazard ratio of 0.617 (95% CI 0.391–0.972; P = 0.037).

Two additional covariates were evaluated separately as additions to the primary model and excluded on the basis of model metrics. SOFA score at hour 0 did not significantly improve model fit ($\Delta\chi^2 = 2.67$, df = 1, P = 0.103; AIC = 635.8 vs 636.5). Time from randomization to hour 0 also did not significantly improve model fit ($\Delta\chi^2 = 0.76$, df = 1, P = 0.382) and worsened parsimony (AIC = 637.7 vs 636.5). A fully extended model including both additional covariates likewise did not significantly improve fit over the primary specification ($\Delta\chi^2 = 4.28$, df = 2, P = 0.118; AIC = 636.2). Across all five specifications, the treatment

effect was directionally consistent, with all hazard ratios below 1.0 (range of the estimates 0.564–0.682). Full results are presented in **Supplementary Table 3**.

Supplementary Table 3. Treatment effect across model specifications.

Model	Covariates	HR (95% CI)	P value	AIC	$\Delta\chi^2$ (df, P)
Unadjusted	Treatment group	0.682 (0.435–1.070)	0.095	643.4	–
Primary model	Treatment group + log(IL-6) at hour 0 + VIS at hour 0	0.617 (0.391–0.972)	0.037	636.5	10.89 (2; P=0.004) vs unadjusted
Primary model; adding SOFA at hour 0	Treatment group + log(IL-6) at hour 0 + VIS at hour 0 + SOFA at hour 0	0.564 (0.354–0.900)	0.016	635.8	2.67 (1, P=0.103) vs primary
Primary model; adding hours from randomization to hour 0	Treatment group + log(IL-6) at hour 0 + VIS at hour 0 + time randomization–hour 0	0.681 (0.415–1.1200)	0.130	637.7	0.76 (1, P=0.382) vs primary
Full model; adding both parameters	Treatment group + log(IL-6) at hour 0 + VIS at hour 0 + SOFA at hour 0 + time randomization–hour 0	0.648 (0.394–1.060)	0.087	636.2	2.67 (1, P=0.118) vs primary

Hazard ratios were shown for HA group compared with ST group. P values reflect the Wald test for the Treatment group's effect (Hemoadsorption vs Standard treatment) within each model specification.

Abbreviations. HR, hazard ratio; SOFA, Sequential Organ Failure Assessment score; IL-6, Interleukin-6; VIS, vasoactive-inotropic score.

SOFA score changes over time

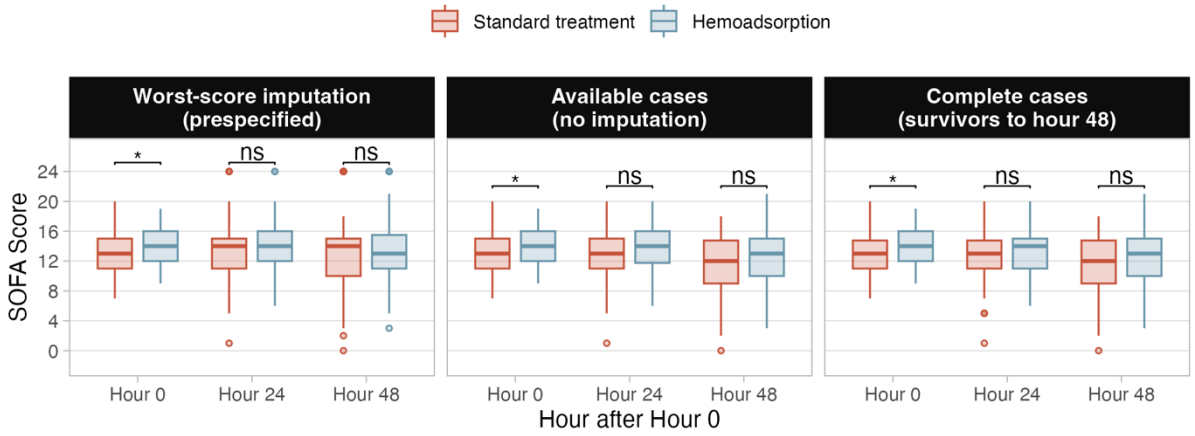
Interpretation of SOFA trajectories in this cohort is subject to the limitation of informative missingness, as patients who died contributed no measurements beyond the time of death.

Sequential Organ Failure Assessment scores were assessed at hours 0, 24, and 48 as a prespecified secondary outcome. Three analytical approaches were used:

- The worst-score imputation approach penalizes death with the maximum possible score (24), consistent with the prespecified analysis plan.
- Available cases approach uses the available data set without any imputation, which may introduce informative dropout due to early deaths.
- Complete cases approach analyzes only participants with complete data set across the three time points (i.e., survived up to hour 48).

While the SOFA score at hour 0 was statistically higher in the HA group compared with ST group, the scores at hours 24 and 48 were comparable. The convergence of results across all three approaches supports the robustness of the null finding.

Results across three analytical approaches are shown in **Supplementary Figure 3**.



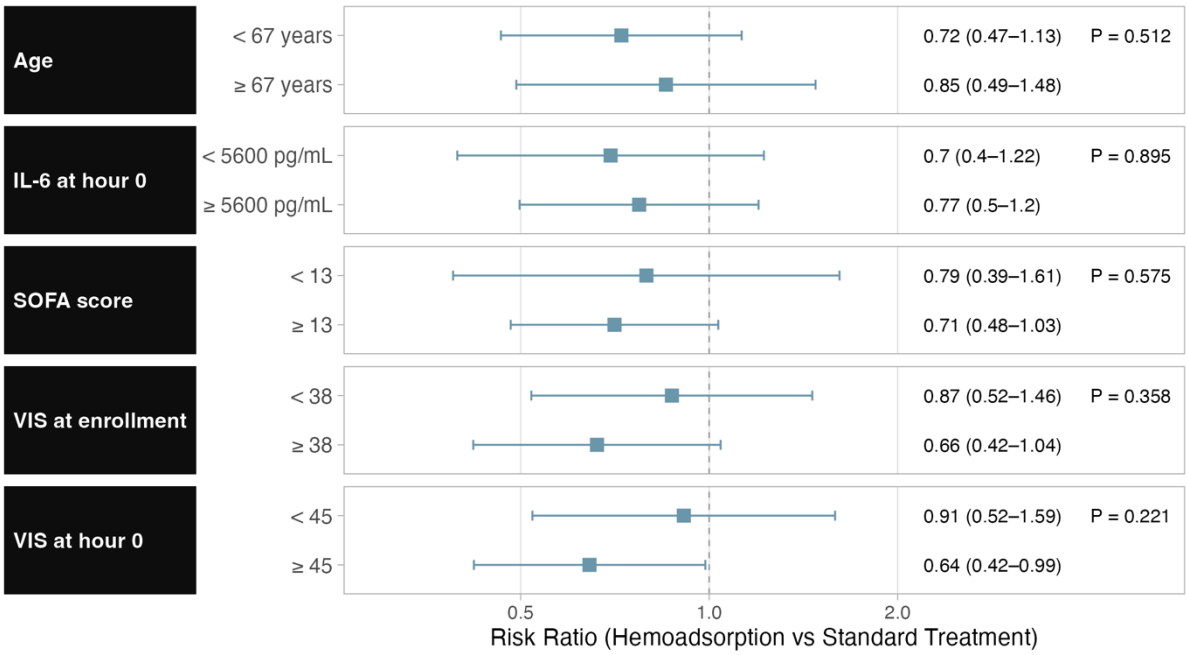
Supplementary Figure 3. SOFA score at hours 0, 24, and 48, shown across three analytical approaches: Worst-score imputation, Available cases, and Complete cases. Data are presented as median with interquartile range. Brackets indicate statistical significance of between-group differences, assessed using Wilcoxon rank-sum tests.

Abbreviations: HA, hemoadsorption; IQR, interquartile range; SOFA, Sequential Organ Failure Assessment; ST, standard treatment.

Subgroup analysis on the primary outcome

The consistency of the treatment effect across prespecified subgroups was examined in a post-hoc exploratory analysis. Participants were stratified by median values of age, baseline SOFA score, IL-6 at hour 0, VIS at enrollment, and VIS at hour 0. Risk ratios for 28-day mortality were estimated within each subgroup, and heterogeneity of treatment effect was assessed using a logistic regression interaction test. Results are shown in **Supplementary Figure 4**.

Point estimates favored hemoadsorption across all subgroups and both strata of each variable. No statistically significant heterogeneity of treatment effect was detected for any subgroup variable. Given the small sample size and the exploratory nature of this analysis, these findings should be interpreted with caution.



Supplementary Figure 4. Subgroup analysis of 28-day all-cause mortality. Risk ratios (RR) with 95% confidence intervals are shown for the hemoadsorption group compared with the standard treatment group. Subgroups were defined by median split of the respective variable. The P interaction value was derived from a logistic regression model including a treatment-by-subgroup interaction term. The vertical dashed line represents RR = 1.0 (no effect). All analyses are exploratory and post-hoc; the trial was not powered to detect subgroup interactions.

Abbreviations: CI, confidence interval; HA, hemoadsorption; IL-6, interleukin-6; RR, risk ratio; SOFA, Sequential Organ Failure Assessment; ST, standard treatment; VIS, vasoactive-inotropic score.

Protocol deviations

Supplementary Table 4. Protocol deviations.

Type of Deviation	Category	ST group	HA group
Minor deviations			
Missed blood sample[a]	Minor	5	4
Major deviations			
First hemoadsorption session initiated after 12 hours from randomization[b]	Major	–	7
Second hemoadsorption session conducted outside the 22–26 hour window[c]	Major	–	11
Hemoadsorption session terminated before 3 hours[d]	Major	–	8 (11 sessions)

Data are presented as number of patients and, where applicable, number of sessions or events. For patients with multiple cited reasons for a deviation, all reasons are recorded; total reasons may therefore exceed total patients.

[a] The numbers reflect participants with at least one missing blood sample at any time point; individual patients may have missed multiple analytes or time points.

[b] Cited reasons: delay in establishing vascular access (n = 2), lack of capable personnel (n = 3), severe hemodynamic instability (n = 2), not specified (n = 2).

[c] Cited reasons: lack of capable personnel (n = 5), transferred out of participating unit (n = 2; one transiently after surgery, one to palliation), not specified (n = 4).

[d] Cited reasons: circuit clotting (n = 4 sessions), severe hypotension refractory to resuscitation (n = 3 sessions), emergency procedure or investigation outside the unit (n = 1 session), not specified (n = 3 sessions).