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The effects of resuscitation with different plasma products on endothelial permeability and organ injury in a rat pneumosepsis model

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Abstract

Background Endothelial injury and permeability are a hallmark of sepsis. Initial resuscitation of septic patients with crystalloids is associated with aggravation of endothelial permeability, which may be related either to low protein content or to volume. We investigated whether initial resuscitation with different types of plasma or albumin decreases endothelial dysfunction and organ injury in a pneumosepsis rat model compared to the same volume of crystalloids.

Study design and methods Sprague–Dawley rats were intratracheally inoculated with *Streptococcus pneumoniae*. Twenty-four hours after inoculation, animals were randomized to 2 control groups and 5 intervention groups ($n = 11$ per group) to receive resuscitation with a fixed volume (8 mL/kg for 1 h) of either Ringer's Lactate, 5% human albumin, fresh frozen plasma derived from syngeneic donor rats (rFFP), human-derived plasma (hFFP) or human-derived solvent detergent plasma (SDP). Controls were non-resuscitated ($n = 11$) and healthy animals. Animals were sacrificed 5 h after start of resuscitation ($T = 5$). Pulmonary FITC-dextran leakage as a reflection of endothelial permeability was used as the primary outcome.

Results Inoculation with *S. Pneumoniae* resulted in sepsis, increased median lactate levels (1.6–2.8 mM, $p < 0.01$), pulmonary FITC-dextran leakage (52–134 $\mu\text{g mL}^{-1}$, $p < 0.05$) and lung injury scores (0.7–6.9, $p < 0.001$) compared to healthy controls. Compared to animals receiving no resuscitation, animals resuscitated with rFFP had reduced pulmonary FITC leakage (134 vs 58 $\mu\text{g/mL}$, $p = 0.011$). However, there were no differences in any other markers of organ or endothelial injury. Resuscitation using different human plasma products or 5% albumin showed no differences in any outcome.

Conclusions Resuscitation with plasma did not reduce endothelial and organ injury when compared to an equal resuscitation volume of crystalloids. Rat-derived FFP may decrease pulmonary leakage induced by shock.

Keywords Albumin, Endothelial dysfunction, Plasma, Resuscitation, Sepsis, Shock, Transfusion

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Background

A hallmark of sepsis is the presence of a dysregulated inflammatory host immune response with ensuing endothelial injury and hyperpermeability [1–3]. Under healthy conditions, the inner endothelial cell wall is lined with a matrix of proteoglycans and glycoproteins, termed the glycocalyx [4]. This structure is integral in regulating vascular permeability, facilitating leukocyte migration [5] and maintaining coagulation homeostasis [6]. During sepsis, constituents of the glycocalyx such as syndecan-1 and thrombomodulin are shed into the circulation, which is associated with worse patient outcomes [2, 7].

The primary initial treatment for patients with septic shock consists of fluid resuscitation to ensure adequate tissue perfusion [8]. However, low protein-content fluids, such as crystalloids, may harm the endothelial barrier and glycocalyx, as shown *in vitro* [9, 10]. In septic patients, fluid resuscitation with crystalloids is dose-dependently associated with an increase in circulating levels of constituents of the glycocalyx [11], although it is not clear whether this association is merely reflecting disease severity. High protein-content fluids, such as albumin and plasma, may be less harmful [12]. Albumin 4%, when compared to crystalloids, reduced endothelial dysfunction, inflammation and oxidative stress in a rat endotoxemia model [13]. Moreover, the use of albumin as an add-on fluid therapy in sepsis may confer benefit in those with baseline hypo-albuminemia [14], although results of clinical trials on albumin indicated no benefit on mortality [15]. Plasma might be even more promising [12, 16]. In an experimental sepsis model, plasma reduced mortality, lung edema and syndecan-1 shedding compared to crystalloids [17]. Observational clinical data suggests that plasma is associated with reduced markers of glycocalyx degradation in septic shock [18]. In addition, as solvent detergent plasma (SDP) is depleted from cellular debris, this plasma product may confer a superior benefit on the endothelium than fresh frozen plasma (FFP), as suggested in a trial in patients undergoing aortic surgery and in observations in critically ill pediatric patients [19, 20].

Alternatively to protein content, the observed benefit of plasma may be related to the use of less volume, as plasma has a 3–4 times higher volume expansion capacity than crystalloids. The preclinical shock models comparing plasma to crystalloids have used significantly higher volumes of crystalloids [17, 21, 22]. As no studies have evaluated the effects of the same volume of fluids on endothelial and organ injury in sepsis, it is unclear whether benefit is due to the type of fluid or the difference in infused volume.

The primary aim of this study was to investigate the effects of initial resuscitation with a similar fixed-volume of crystalloids, and rat-derived fresh frozen plasma (rFFP)

on shedding of glycocalyx constituents, endothelial permeability and organ injury in a rat pneumosepsis model. We hypothesized that resuscitation with rFFP, would limit pulmonary endothelial permeability, reducing organ injury compared to crystalloids. In addition, we investigated the effects of different human-derived protein-rich plasma products, including human-derived fresh frozen plasma (hFFP), human-derived solvent detergent plasma (SDP) and 5% human albumin on endothelial and organ injury. We hypothesized that SDP would be superior to FFP or albumin.

Methods

General information

This study was approved by the Animal Care and Use Committee of the Amsterdam University Medical Centers, location AMC at the University of Amsterdam, the Netherlands. The procedures were performed in accordance with the European Parliament directive (2010/63/EU) and the national law of the Experiments on Animals Act (Wod, 2014). 72 male Sprague Dawley rats (Envigo, Indianapolis, IN, USA), 12–13 weeks of age and weighing around 400 g, were group housed in standard cages under normal conditions of a 12:12-h light:dark cycle receiving food and water *ad libitum* for at least 7 days before the experiment. Studies were conducted and reported following the ARRIVE guidelines (Additional file 2: Appendix S2) [23].

Pneumosepsis model

Streptococcus pneumoniae (serotype 3, ATCC 6303; Rockville, MD, USA) were cultured on Colombian Agar with 5% sheep blood plates (COS) (Biomerieux Benelux B.V, Zaltbommel, the Netherlands). After 12 h of growth, 5–6 separate colonies were isolated and transferred to 20 mL brain heart infusion (BHI) broth. After overnight incubation at 37 °C, the culture was centrifuged for 10 min at 2700 g, 20 °C (5804 R, Eppendorf AG, Hamburg, Germany). Afterwards, medium was removed and bacteria were washed with sterile 0.9% saline and centrifuged a second time for 10 min at 2700 g, 20 °C (5804 R, Eppendorf AG, Hamburg, Germany). Using a spectrophotometer (Secoman S250), optical density (OD) was measured. Assuming that an OD₆₂₀ of 0.350 = 1×10^8 colony forming units (CFU), inoculum was made using sterile 0.9% NaCl. Prepared inocula were plated on COS plates in serial tenfold dilutions and grown overnight to quantify.

Following anesthesia using 5% isoflurane/60% FiO₂ mix, rats underwent intratracheal inoculation with $3\text{--}5 \times 10^8$ CFU *S. Pneumoniae* (ATCC6303) (BSL-2) in 100 µl sterile 0.9% NaCl. In pilot experiments, this dose range induced pneumosepsis, defined as lactatemia with

growth of *S. pneumonia* in lung and blood cultures, in all animals. Then, animals were transferred to their cages and allowed to wake up (Additional file 1: Fig. S1). During the experiments the following exclusion criteria were used: animals receiving too low ($<3 \times 10^8$ CFU) or high ($>5 \times 10^8$ CFU) amounts of *S. pneumoniae*. Animals without growth of *S. pneumonia* in either lung or blood cultures. Animals that died before start of intervention. Exclusion criteria were established before start of experiments.

Twenty-four hours after inoculation ($T=0$), animals were randomized to 5 intervention and 2 control groups ($N=11$ per group) with opaque envelopes using a 1:1 allocation. Intervention groups received resuscitation with either Ringer's lactate (RL), 5% human albumin, rat-derived fresh frozen plasma (rFFP), human-derived fresh frozen plasma (hFFP) or human-derived solvent detergent plasma (SDP). Positive controls ($n=11$) underwent all procedures while not receiving any resuscitation. Healthy controls ($n=6$) underwent all procedures but were sham inoculated with 100 μ L sterile NaCl 0.9%. Researchers were not blinded to group allocation during conduct of the experiment and data analyses. A 21G venflon was inserted in the tail vein under anaesthesia with 1–3% isoflurane/60% FiO₂ while breathing spontaneously. After obtaining a baseline blood sample ($T=0$). Fluids were administered at 8 ml/kg in 1 h (perfusor[®] fm; B Braun, West Bloomfield Township, MI, USA). Animals received no additional fluids between inoculation and start of resuscitation ($T=0$).

After resuscitation, the catheter was removed and anesthesia was stopped. After 5 h, rats were exsanguinated through heart puncture ($T=5$). Fifteen min before sacrifice, 0.5 mL saline containing 12.5 mg/ml fluorescein isothiocyanate (FITC)-labelled 70 kDa dextran (Sigma Aldrich, St. Louis, MO, USA) was administered through a newly placed tail vein catheter. Immediately after exsanguination, 2 mL of heparinized saline (1250 iE/mL, Leo Pharma BV, Amsterdam) was administered to prevent clotting. Thereafter, laparotomy and thoracotomy were performed, urine was collected by bladder puncture, and the inferior vena cava was cut. The circulation was flushed using 100 ml 0.9% NaCl, administered into the right ventricle, to remove excess intravascular FITC-dextran. Before flushing, the left pulmonary and renal hilum were ligated for wet-to-dry (W/D) ratios. After flushing, both lungs and kidneys were taken out for later assessment.

Animal welfare

Animals were monitored during the whole study period using a modified M-CASS scoring sheet (Additional file 1: Results S2) [17, 24]. Scoring 4 points in any of the

categories was considered a humane endpoint and would result in early termination by heart puncture under anesthesia. No analgesics were administered during the study period. Animals were monitored during predefined moments by the responsible researcher (After inoculation, T12, T24, T26.5 and T29) or animal caretaker (T8, T20). Monitoring frequency was increased when unusual progression of symptoms was witnessed. Autopsy was performed on all animals not reaching the end of the study period to assess cause of death.

Intervention products

Rat fresh frozen plasma (rFFP) transfusion product was prepared from donor rats according to national blood bank standards. In short; rats were anesthetized using 5% isoflurane and whole blood was obtained through cardiac puncture using a 19G syringe containing 10% citrate-phosphate-dextrose (Sigma, USA). Blood was centrifuged at 2000G, 18 °C for 10 min after which plasma was separated centrifuged again at 2000G, 4 °C for 15 min (5804 R, Eppendorf AG, Hamburg, Germany). Thereafter, upper 2/3rd of plasma was taken, snap-frozen in liquid nitrogen and stored at -80 °C. Human albumin (Albuman 200[®], Prothya Biosolutions Netherlands, the Netherlands) was diluted 1:3 using sterile ringer's lactate to a concentration of 5% and stored at -80 °C. Both FFP (Sanquin, the Netherlands) and SDP (Omniplasma[®], Octapharma, Switzerland) products were AB- and retrieved from Sanquin, the national blood bank. Prior to the experiment, all plasma products were aliquoted, snap frozen and stored at -80 °C. At the day of the experiment, required infusion products were quickly thawed in a water bath at 37 °C, after which they were kept at room temperature until infusion.

Measurements

Blood was drawn before resuscitation ($T=0$) and before exsanguination ($T=5$) (Additional file 1: Fig. S1). Blood gas analyses (RAPIDPoint 500, Siemens, Germany) and total blood counts (cell Coulter AC[®]T diff2 Hematology analyser, Beckmann, Germany) were performed at both timepoints. All other biochemical assessments were measured only at termination ($T=5$). Blood was centrifuged twice (2000g, 15 min, acceleration 9, brake 0, 18 °C; 5804 R, Eppendorf AG, Hamburg, Germany), aliquoted, snap frozen in liquid nitrogen and stored at -80 °C till further evaluation.

Pulmonary vascular leakage

To quantify the amount of pulmonary FITC-dextran leakage, the superior lobe of the right lung was thawed and homogenized using a radio immunoprecipitation assay (RIPA) buffer and tissue homogenizer (TH-115,

Omni international, Kennesaw, Georgia, USA). 100 μ L of homogenates were measured for fluorescence using a spectrophotometer (spectramax M2, molecular devices, San Jose, California, USA).

Pulmonary histopathology

The left inferior lobe of the right lung was fixed in 10% formaldehyde and embedded in paraffin, after which 4- μ m-thick sections were cut and stained with haematoxylin and eosin (H&E). A pathologist blinded for treatment allocation evaluated the lung tissue for the presence of oedema, bronchitis, endothelialitis, interstitial inflammation and thrombi formation on a scale of 0–4 (0=absent, 1=mild, 2=moderate, 3=severe, 4=very severe), as described previously [25, 26]. For the full scoring list, see the supplement (Additional file 1: Table S1). No other organs were assessed for histopathology.

Bacterial growth

The right inferior lobe of the lung was homogenized in sterile 0.9% NaCl. Lung homogenates were plated on COS plates in serial tenfold dilutions. Whole blood retrieved during termination ($T=5$) was plated on COS plates, growth of *S. pneumonia* in whole blood was defined as bacteremia.

Parameters of systemic and organ injury

As a reflection of systemic endothelial injury and glycocalyx degradation, soluble levels of syndecan-1 (Elabscience, USA), thrombomodulin (TM) (Lifespan Biosciences, Inc. USA), von Willebrand factor (vWF) (Elabscience, USA) and intercellular adhesion molecule-1 (ICAM-1) (Elabscience, USA) were measured using commercially available ELISA kits according to manufacturer guidelines. ELISA values that were lower than the reference value were set at the lowest detection range. To assess liver and kidney injury, aspartate transaminase (AST), alanine transaminase (ALT), albumin and creatinine were measured by standard enzymatic reactions using spectrophotometric, colorimetric or turbidimetric measurement methods.

Sample size and statistical analyses

The primary outcome was pulmonary leakage of FITC-dextran. In a previous sepsis model, resuscitation with plasma compared to normal saline reduced syndecan-1 levels (21.8 vs 31.0 ng/mL) [17]. These data were extrapolated to a sample size with 7 groups. Using a one-way ANOVA analysis of variance ($V=17.5$) and a common standard deviation of 9, the use of 10 rats had a power of 80% to reach a statistically significant difference in vascular leakage. With an expected mortality of 5% in our model, 11 rats were included per group.

Statistical analysis was done using SPSS Statistics V.26 (IBM). Graphs were made using GraphPad Prism 8 (GraphPad Software). All data were regarded as non-parametric and are shown as median with interquartile range (IQR). Data were tested for differences using the Kruskal Wallis test with post-hoc multiple comparisons. A p value < 0.05 was considered statistically significant. No correction was used for multiple testing. All data collected till the moment of death was used in analyses.

Results

Pneumosepsis model

All animals inoculated with *S. pneumonia* ($N=66$) developed pneumosepsis with growth of *S. pneumoniae* in both lung homogenate and blood cultures and lactatemia when compared to sham inoculated animals ($N=6$) (median: 1.56 [IQR: 0.87–1.69] vs 2.77 [1.96–3.51] mM, $p \leq 0.01$, Table 2). Septic rats had higher weight loss over 24 h compared to healthy control animals (8.1 [7.1–9.1] vs 0.9 [0.3–1.4] %, $p \leq 0.001$). Septic animals had increased levels of potassium and base excess, whereas calcium levels and leucocyte and platelet counts dropped (Table 1). Moreover, septic animals had increased pulmonary oedema (higher W/D ratio) and higher pulmonary histology injury scores (Fig. 1), and elevated systemic parameters of endothelial injury when compared to healthy controls (Figs. 1, 2). Septic animals did not develop hepatic or renal injury when compared to healthy control animals as levels of ALT, AST, albumin and creatinine were comparable between all groups (Table 2).

No differences were seen in pre-resuscitation parameters between the several groups with sepsis (Tables 1, Additional file 1: Table S2). During the experiment, 4 animals died. Two animals died during transfusion (both in hFFP group) and two animals were sacrificed upon reaching humane endpoints (1 after albumin and 1 non-resuscitated animal, Additional file 1: Results S1). All other animals concluded the study ($N=68$). Of note, 5 animals of the hFFP group and 3 animals of the SDP group developed a transient increase of body temperature between 0.5 and 1 °C immediately after start of infusion.

The effect of resuscitation fluids on pulmonary injury

Pulmonary vascular leakage was increased in septic animals compared to healthy controls (134 [105–165] vs 52 [33–112] μ g/mL, $p=0.01$, Fig. 1). Compared to the group resuscitated with Ringer's Lactate, resuscitation with rFFP did not result in differences in any outcome of pulmonary vascular leakage (Fig. 1), wet/dry ratios (Fig. 1), histology scores (Additional file 1: Table S1) and pulmonary bacterial outgrowth (Additional file 1: Fig. S2). Of interest, compared to non-resuscitated controls,

Table 1 Pre-resuscitation parameters ($T=0$)

Parameter	Sham ($n=6$)	NR ($n=11$)	RL ($n=11$)	rFFP ($n=11$)
<i>Hemodynamics</i>				
Heart rate (bpm)	370 (344–385)	360 (348–372)	367 (340–377)	370 (344–380)
Saturation (%)	97 (95–99)	96 (95–98)	98 (97–99)	97 (94–98)
Total amount infused (mL)	0 (0–0)	0 (0–0)	2.79* (2.73–3.06)	2.85* (2.75–2.98)
<i>Blood count</i>				
Hb (mM)	10.1 (10.0–11.1)	11.4 (11.0–12.2)	10.6 (10.0–12.0)	11.7 (9.3–12.1)
Leukocytes ($\times 10^9/L$)	12.0 (7.1–14.6)	1.6* (1.4–2.4)	2.6* (1.1–8.6)	2.5* (1.6–7.5)
<i>Blood gas</i>				
Lactate (mM)	1.98 (1.62–2.05)	2.35* (2.11–2.62)	2.55* (2.37–2.72)	2.59* (2.09–2.92)
pH	7.42 (7.41–7.44)	7.44 (7.42–7.45)	7.43 (7.42–7.45)	7.43 (7.43–7.47)
pCO ₂ (mmHg)	45.1 (44.5–45.5)	48.0 (42.7–52.7)	47.2 (41.1–48.9)	50.2 (43.8–50.8)
BE (mM)	3.2 (2.1–4.6)	5.5* (4.2–7.2)	4.6 (3.6–5.9)	6.5* (5.6–6.9)
HCO ₃ ⁻ (mM)	28.2 (27.2–29.8)	31.2* (29.5–33.7)	30.2* (28.0–32.0)	31.6* (30.2–32.9)
Na ⁺ (mM)	138 (137–139)	138 (137–140)	139 (137–139)	139 (137–140)
K ⁺ (mM)	5.3 (5.1–5.4)	5.6* (5.4–5.8)	5.9* (5.3–6.6)	5.8* (5.6–6.2)
Ca ²⁺ (mM)	1.21 (1.19–1.25)	1.13* (1.10–1.17)	1.12* (1.04–1.17)	1.10* (1.03–1.12)
Glucose (mM)	8.9 (7.5–12.2)	7.6 (7.1–9.3)	8.0 (7.4–8.9)	7.9 (6.8–8.2)

Data are presented as median (inter-quartile range). * $p < 0.05$ when compared to the sham group. BE base excess, bpm beats per minute, Hb haemoglobin, NR No resuscitation, rFFP rat fresh frozen plasma, RL Ringer's lactate

animals resuscitated with rFFP had reduced pulmonary FITC-dextran vascular leakage (134 [105–165] vs 58 [44–89] $\mu\text{g/mL}$, $p=0.01$, Fig. 1). No outcome parameter

of pulmonary or endothelial injury differed between animals resuscitated with SDP, hFFP or 5% albumin (Additional file 1: Figs. S3, S4).

The effect of resuscitation fluids on systemic injury

When compared to animals receiving no resuscitation, animals resuscitated with either Ringer's lactate ($p=0.04$) had increased kidney wet-to-dry ratios, whereas resuscitation with rFFP showed no difference (Table 2). However, when compared to resuscitation with RL, resuscitation with rFFP did not differ in terms of kidney edema. Also, no differences in levels of syndecan-1, TM, vWF and ICAM-1 were found (Fig. 2). No outcome parameter of systemic injury differed between animals resuscitated with SDP, hFFP or 5% albumin (Additional file 1: Fig. S4, Table S3).

Discussion

This study investigated the effects of resuscitation with a fixed volume of fluids with either a low (crystalloid) or a high protein content (plasma), as well as the effects of different types of human-derived plasma products on endothelial and pulmonary injury in an experimental rat model of pneumosepsis.

This study did not show a benefit of resuscitation with rFFP on reducing endothelial permeability or organ edema when compared to resuscitation with crystalloids. This is in contrast to findings of experimental studies using models of hemorrhagic shock and sepsis, wherein plasma resuscitation compared to crystalloid resuscitation decreased endothelial permeability and improved outcome [17, 21, 22]. A potential explanation for a reduced effect in our study, is that in these previous studies, resuscitation volumes between groups differed, with two–threefold higher volumes of crystalloids than plasma, which could even reach 75 ml/kg [17, 21, 22]. In this study, a fixed volume was chosen for all groups to compare the effects of fluid content on endothelial and

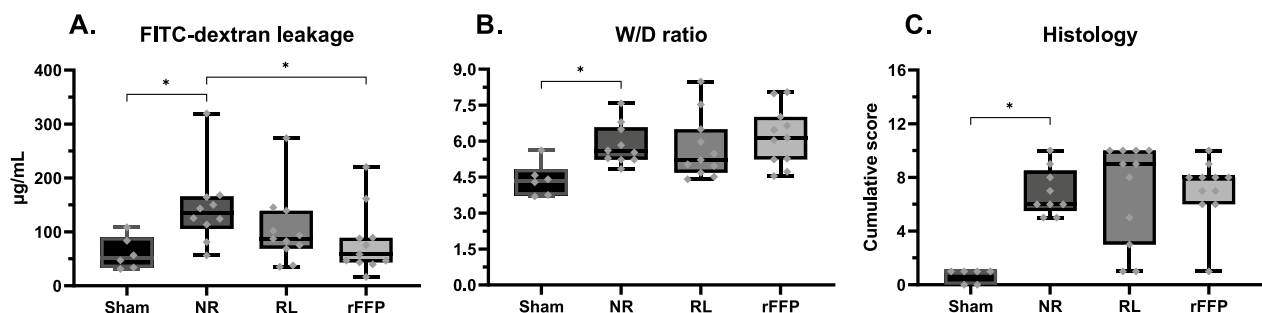


Fig. 1 Markers of pulmonary injury post resuscitation. Data are presented as boxplots with median, interquartile ranges and minimum and maximum values showing all individual data points. * $p < 0.05$. FITC Fluorescein isothiocyanate, NR no resuscitation, rFFP rat fresh frozen plasma, RL Ringer's Lactate, W/D Wet-to-dry

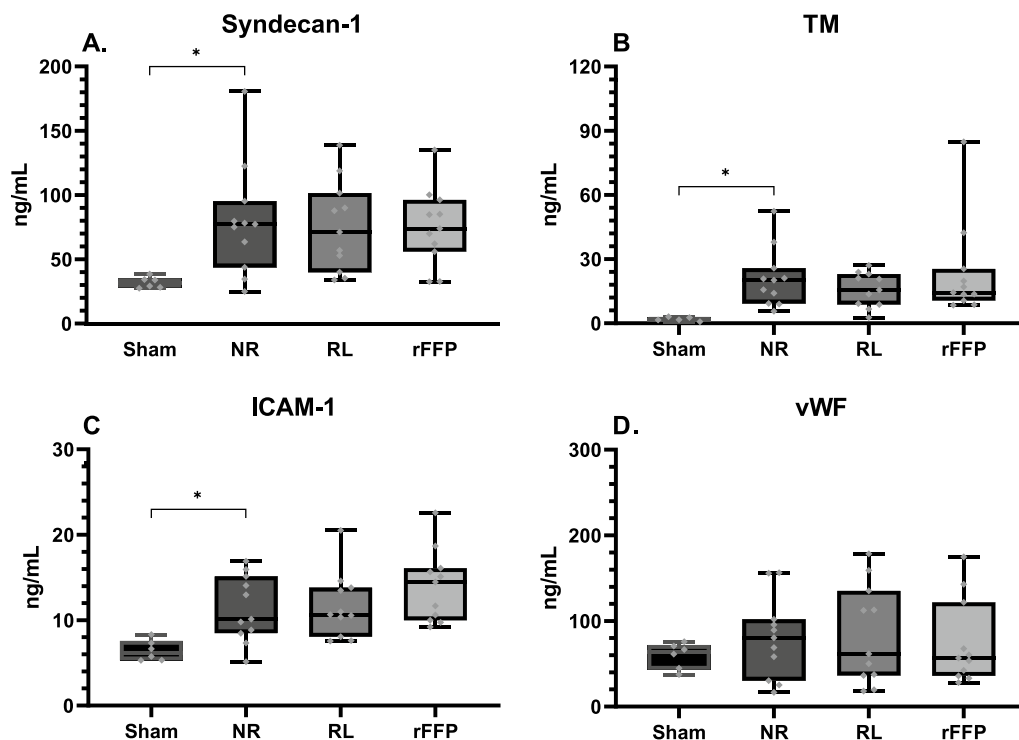


Fig. 2 Markers of endothelial injury post-resuscitation. Data are presented as boxplots with median, interquartile ranges and minimum and maximum values showing all individual data points. * $p < 0.05$. *ICAM-1* intercellular adhesion molecule 1, *NR* no resuscitation, *rFFP* rat fresh frozen plasma, *RL* Ringers Lactate, *TM* Thrombomodulin, *vWF* von Willebrand factor

Table 2 Parameters of systemic organ injury post resuscitation

Parameter	Sham (n=6)	NR (n=10)	RL (n=11)	rFFP (n=11)
Lactate (mM)	1.56 (0.87–1.69)	2.77* (1.96–3.51)	2.24* (2.10–2.71)	2.53* (2.13–2.71)
Hb (mM)	8.9 (8.3–9.4)	10.7* (10.3–11.1)	10.1* (9.4–11.6)	10.5* (9.7–11.3)
Glucose (mM)	10.3 (9.5–11.6)	7.4* (6.1–10.1)	8.2* (7.1–9.4)	7.3* (7.0–7.5)
ALT (U/L)	34.0 (30.8–38.3)	36.5 (30.8–39.5)	35.0 (30.0–38.3)	38.0 (23.0–40.0)
AST (U/L)	64.5 (59.8–73.5)	72.5 (56–78.8)	75.0 (54.0–82.5)	63.0 (56.0–103.5)
Albumin (g/L)	36.5 (33.5–39.0)	35.5 (33.3–39.5)	36.0 (34.0–38.0)	37.0 (38.0–35.0)
Creatinine (μmol/L)	23.5 (19.5–28.3)	23.0 (20.0–25.0)	23.0 (21.8–25)	23.0 (21.0–29.0)
Kidney W/D ratio	3.6 (3.4–4.0)	3.8 (3.6–3.9)	3.9* (3.7–4.1)	3.8 (3.7–3.9)

Data are presented as median (inter-quartile range). * $p < 0.05$ when compared to sham group. *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *Hb* Hemoglobin, *NR* No resuscitation, *rFFP* rat fresh frozen plasma, *RL* Ringer's lactate, *W/D* Wet-to-dry

organ injury, independent of fluid volume. As our data do not suggest that administering protein rich fluids is unequivocally beneficial, we postulate that the beneficial

effects of plasma found in previous experimental studies are largely related to administration of less volume. This would suggest that the amount and not the type of resuscitation fluid is the most important driver for aggravated endothelial injury. Multiple randomized controlled trials in sepsis showed that liberal use of crystalloids results in more kidney injury, respiratory failure and mortality compared to a restrictive approach [27–29]. On the other hand, a recent RCT found no advantage of a restrictive fluid resuscitation on mortality, although it should be noted that resuscitation volumes were quite low [30]. A systematic review and meta-analysis from the same authors report an overall very low quality of evidence with no benefit towards any strategy [31]. Therefore, whether administration of large volumes of crystalloids induce adverse outcome by worsening of endothelial function remains a topic of debate.

An alternative explanation for a limited protection from plasma is that the pneumosepsis model may have been too mild and did not cause a low albumin level. In line with this reasoning, albumin reduced 90-day mortality in septic shock patients, while this was not witnessed in patients with sepsis and no shock [32]. In addition, albumin may only benefit the subgroup of patients with hypo-albuminemia [14]. Therefore, protein-rich

fluids such as plasma might only be favorable in the most severely ill septic shock patients and/or in those with low albumin levels. In the current model, even though septic animals all had bacteremia and showed signs of sepsis, most animals receiving no resuscitation and other types of supportive therapy survived 5 h. Thereby, it can be questioned whether resuscitation was indeed necessary. However, the presence of hyperlactatemia in the setting of sepsis reflects a clinical situation which would have triggered resuscitation [8].

An interesting finding is that even in this mild pneumosepsis model, animals receiving rat FFP had less lung leakage than non-resuscitated animals, and rat FFP did not aggravate kidney edema. This may suggest a beneficial effect on shock-induced permeability. However, this may be a chance finding and should be confirmed in follow-up studies.

We hypothesized that SDP plasma would be superior but this study found no difference between FFP and SDP on endothelial or organ injury. Conversely, SDP resuscitation increased kidney edema, while this was not witnessed in animals receiving FFP products. The use of cross-species transfusion products may have played a role, as human plasma products induced a transient increase of body temperature in some animals and 2 animals in the hFFP group unexpectedly died during transfusion.

This study has several limitations. First, as already mentioned, hemodynamic monitoring was absent. Second, due to higher data variability than expected, the used sample size may have been too low to show potential differences between interventions. In addition, we transfused human-derived plasma products to rats possibly resulting in cross-species reactions which could skew advantageous effects. We chose human products as it enabled us to compare the effects between SDP and FFP products. Multiple control groups were added to the model to control for this. However, this does increase the probability of type 1 errors. Rats were followed for 5 h after start of resuscitation. Therefore, long term effects on endothelial and organ injury are missed. Only male rats were used in the current model reducing the translational generalizability of the found results. This choice was made to limit the amount of animals used. Female rats develop less endothelial injury due to estrogen production. Therefore, including female rats would reduce the effect size warranting a larger sample size. Finally, no antibiotics, mechanical ventilation or vasopressors were used in the current model, as we aimed for a model that reflects primary resuscitation upon hospital admittance. Obviously, this choice reduces translational value to patients whom already received other organ support, such as mechanical ventilator. However, this was chosen

deliberately as we aimed to investigate the direct effects of the used resuscitation products on the endothelium reducing possible confounders.

Conclusion

In conclusion, resuscitation rFFP when compared to an equal resuscitation volume of crystalloids did not reduce markers of glycocalyx degradation, endothelial injury and organ injury, but may limit shock-induced pulmonary leakage. In addition, different types of human-derived plasma products including 5% human albumin, FFP and SDP resulted in similar endothelial and pulmonary injury.

Abbreviations

ALT	Alanine transaminase
AST	Aspartate aminotransferase
Bpm	Beats per minute
CFU	Colony forming units
COS	Colombian Agar with 5% sheep blood plates
Hb	Hemoglobin
FITC	Fluorescein isothiocyanate
hFFP	Human-derived fresh frozen plasma
H&E	Hematoxylin and Eosin
ICAM-1	Intercellular adhesion molecule 1
IQR	Interquartile range
NR	No resuscitation
rFFP	Rat-derived fresh frozen plasma
RIPA	Radio immunoprecipitation assay buffer
RL	Ringer's Lactate
SDP	Solvent detergent plasma
TM	Thrombomodulin
vWF	Von Willebrand factor
W/D ratio	Wet dry ratio

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s40635-023-00549-9>.

Additional file 1: Supplementary data.

Additional file 2: ARRIVE guidelines.

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Author contributions

DB, DK and NJ designed the study. DB, AB and JV executed the experiments and collected the data. JR analysed and interpreted histopathological data. All authors critically evaluated the data. DB, DK and NJ wrote the manuscript. All authors critically evaluated the manuscript.

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Availability of data and materials

The data sets generated and/or analysed during the current study are not publicly available but are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

This study was approved by the Animal Care and Use Committee of the Amsterdam University Medical Centers, location AMC at the University of Amsterdam, the Netherlands. The procedures were performed in accordance with the European Parliament directive (2010/63/EU) and the national law of the Experiments on Animals Act (Wod, 2014).

Consent for publication

Not applicable.

Competing interests

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References

- Stephan J, Hofer S, Funke B, Brenner T, Henrich M, Martin E et al (2011) Sepsis and major abdominal surgery lead to flaking of the endothelial glycocalyx. *J Surg Res* 165(1):136–141
- Johansson PI, Stensballe J, Ostrowski SR (2017) Shock induced endotheliopathy (SHINE) in acute critical illness - a unifying pathophysiologic mechanism. *Crit Care* 21(1):25
- Ding R, Meng Y, Ma X (2018) The central role of the inflammatory response in understanding the heterogeneity of sepsis-3. *Biomed Res Int* 2018:5086516
- Moore KH, Murphy HA, George EM (2021) The glycocalyx: a central regulator of vascular function. *Am J Physiol Regul Integr Comp Physiol* 320(4):R508–R518
- Constantinescu AA, Vink H, Spaan JAE (2003) Endothelial cell glycocalyx modulates immobilization of leukocytes at the endothelial surface. *Arterioscler Thromb Vasc Biol* 23(9):1541–1547
- Curry FE, Adamson RH (2012) Endothelial glycocalyx: permeability barrier and mechanosensor. *Ann Biomed Eng* 40(4):828–839
- Fang Y, Li C, Shao R, Yu H, Zhang Q (2018) The role of biomarkers of endothelial activation in predicting morbidity and mortality in patients with severe sepsis and septic shock in intensive care: a prospective observational study. *Thromb Res* 171:149–154
- Evans L, Rhodes A, Alhazzani W, Antonelli M, Coopersmith CM, French C et al (2021) Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock 2021. *Crit Care Med* 49(11):e1063–e1143
- Cheung-Flynn J, Alvis BD, Hocking KM, Guth CM, Luo W, McCallister R et al (2019) Normal Saline solutions cause endothelial dysfunction through loss of membrane integrity, ATP release, and inflammatory responses mediated by P2X7R/p38 MAPK/MK2 signaling pathways. *PLoS ONE* 14(8):e0220893
- Zeng Y, Adamson RH, Curry FR, Tarbell JM (2014) Sphingosine-1-phosphate protects endothelial glycocalyx by inhibiting syndecan-1 shedding. *Am J Physiol Heart Circ Physiol* 306(3):H363–H372
- Hippensteel JA, Uchimido R, Tyler PD, Burke RC, Han X, Zhang F et al (2019) Intravenous fluid resuscitation is associated with septic endothelial glycocalyx degradation. *Crit Care* 23(1):259
- Milford EM, Reade MC (2019) Resuscitation fluid choices to preserve the endothelial glycocalyx. *Crit Care* 23(1):77
- Kremer H, Baron-Menguy C, Tesse A, Gallois Y, Mercat A, Henrion D et al (2011) Human serum albumin improves endothelial dysfunction and survival during experimental endotoxemia: concentration-dependent properties. *Crit Care Med* 39(6):1414–1422
- Caironi P, Tognoni G, Masson S, Fumagalli R, Pesenti A, Romero M et al (2014) Albumin replacement in patients with severe sepsis or septic shock. *N Engl J Med* 370(15):1412–1421
- Melia D, Post B (2021) Human albumin solutions in intensive care: a review. *J Intensive Care Soc* 22(3):248–254
- van den Brink DP, Kleinvelde DJB, Sloos PH, Thomas KA, Stensballe J, Johansson PI et al (2021) Plasma as a resuscitation fluid for volume-depleted shock: potential benefits and risks. *Transfusion* 61(S1):S301–S312
- Chang R, Holcomb JB, Johansson PI, Pati S, Schreiber MA, Wade CE (2018) Plasma resuscitation improved survival in a cecal ligation and puncture rat model of sepsis. *Shock (Augusta, Ga)* 49(1):53–61
- Straat M, Müller MC, Meijers JC, Arbous MS, Spoelstra-de Man AM, Beurskens CJ et al (2015) Effect of transfusion of fresh frozen plasma on parameters of endothelial condition and inflammatory status in non-bleeding critically ill patients: a prospective substudy of a randomized trial. *Crit Care* 19(1):163
- Stensballe J, Ulrich AG, Nilsson JC, Henriksen HH, Olsen PS, Ostrowski SR et al (2018) Resuscitation of endotheliopathy and bleeding in thoracic aortic dissections: the VIPER-OCTA randomized clinical pilot trial. *Anesth Analg* 127(4):920–927
- Camazine MN, Karam O, Colvin R, Leteurtre S, Demaret P, Tucci M et al (2017) Outcomes related to the use of frozen plasma or pooled solvent/detergent-treated plasma in critically ill children. *Pediatr Crit Care Med* 18(5):e215–e223
- Kozar RA, Peng Z, Zhang R, Holcomb JB, Pati S, Park P et al (2011) Plasma restoration of endothelial glycocalyx in a rodent model of hemorrhagic shock. *Anesth Analg* 112(6):1289–1295
- Torres LN, Chung KK, Salgado CL, Dubick MA, Torres Filho IP (2017) Low-volume resuscitation with normal saline is associated with microvascular endothelial dysfunction after hemorrhage in rats, compared to colloids and balanced crystalloids. *Crit Care* 21(1):160
- Percie du Sert N, Ahluwalia A, Alam S, Avey MT, Baker M, Browne WJ et al (2020) Reporting animal research: Explanation and elaboration for the ARRIVE guidelines 20. *PLOS Biol* 18(7):e3000411
- Huet O, Ramsey D, Miljavec S, Jenney A, Aubron C, Aprico A et al (2013) Ensuring animal welfare while meeting scientific aims using a murine pneumonia model of septic shock. *Shock* 39(6):488–494
- van Manen L, Maas A, Roelofs J, Vlaar APJ, van Bruggen R, Juffermans NP (2020) The effect of washing of stored red blood cell transfusion units on post transfusion recovery and outcome in a pneumosepsis animal model. *Shock* 54(6):794–801
- de Beer F, Lagrand W, Glas GJ, Beurskens CJ, van Mierlo G, Wouters D et al (2016) Nebulized C1-esterase inhibitor does not reduce pulmonary complement activation in rats with severe streptococcus pneumoniae pneumonia. *Cell Biochem Biophys* 74(4):545–552
- Hjortrup PB, Haase N, Bundgaard H, Thomsen SL, Winding R, Pettit V et al (2016) Restricting volumes of resuscitation fluid in adults with septic shock after initial management: the CLASSIC randomised, parallel-group, multicentre feasibility trial. *Intensive Care Med* 42(11):1695–1705
- Andrews B, Muchemwa L, Kelly P, Lakhi S, Heimbürger DC, Bernard GR (2014) Simplified severe sepsis protocol: a randomized controlled trial of modified early goal-directed therapy in Zambia. *Crit Care Med* 42(11):2315–2324
- Andrews B, Semler MW, Muchemwa L, Kelly P, Lakhi S, Heimbürger DC et al (2017) Effect of an early resuscitation protocol on in-hospital mortality among adults with sepsis and hypotension: a randomized clinical trial. *JAMA* 318(13):1233–1240

30. Meyhoff TS, Hjortrup PB, Wetterslev J, Sivapalan P, Laake JH, Cronhjort M et al (2022) Restriction of intravenous fluid in ICU patients with septic shock. *N Engl J Med* 386(26):2459–2470
31. Meyhoff TS, Møller MH, Hjortrup PB, Cronhjort M, Perner A, Wetterslev J (2020) Lower vs higher fluid volumes during initial management of sepsis: a systematic review with meta-analysis and trial sequential analysis. *Chest* 157(6):1478–1496
32. Zou Y, Ma K, Xiong JB, Xi CH, Deng XJ (2018) Comparison of the effects of albumin and crystalloid on mortality among patients with septic shock: systematic review with meta-analysis and trial sequential analysis. *Sao Paulo Med J* 136(5):421–432

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