Volume incompliance and transfusion are essential for transfusion-associated circulatory overload: a novel animal model

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BACKGROUND: Transfusion-associated circulatory overload (TACO) is the predominant complication of transfusion resulting in death. The pathophysiology is poorly understood, but inability to manage volume is associated with TACO, and observational data suggest it is different from simple cardiac overload due to fluids. We developed a two-hit TACO animal model to assess the role of volume incompliance ("first-hit") and studied whether volume overload ("second-hit") by red blood cell (RBC) transfusion is different compared to fluids (Ringer's lactate [RL]).

MATERIALS AND METHODS: Male adult Lewis rats were stratified into a control group (no intervention) or a first hit: either myocardial infarction (MI) or acute kidney injury (AKI). Animals were randomized to a second hit of either RBC transfusion or an equal volume of RL. A clinically relevant difference was defined as an increase in left ventricular end-diastolic pressure (ΔLVEDP) of +4.0 mm Hg between the RBC and RL groups.

RESULTS: In control animals (without first hit) LVEDP was not different between infusion groups (Δ + 1.6 mm Hg). LVEDP increased significantly more after RBCs compared to RL in animals with MI (Δ7.4 mm Hg) and AKI (Δ + 5.4 mm Hg), respectively. Volume-incompliant rats matched clinical TACO criteria in 92% of transfused versus 25% of RL-infused animals, with a greater increase in heart rate and significantly higher blood pressure.

CONCLUSION: To our knowledge, this is the first animal model for TACO, showing that a combination of volume incompliance and transfusion is essential for development of circulatory overload. This model allows for further testing of mechanistic factors as well as therapeutic approaches.

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ransfusion-associated circulatory overload (TACO) is the largest cause of transfusion-related major morbidity and mortality.[1–3] Current understanding of TACO is that volume overload occurs, specifically

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affecting the lungs.[4] Infused volume can overwhelm the heart and circulation, resulting in increased pressure within the vessels. Following Starling's principles, fluid is forced out of the lung capillaries and fill the alveoli.[5] TACO is hallmarked by hydrostatic pulmonary edema, limiting oxygenation capacity of the lung, resulting in hypoxia and respiratory distress. However, to date no studies have investigated the pathophysiology of TACO. There are also no controlled studies investigating the effect of blood products versus conventional fluids on circulatory overload. An animal model of TACO is the first step to investigate mechanisms and test potential treatment or preventative strategies.

Transfusion seems more likely to cause hydrostatic pulmonary edema compared to other infusion fluids, suggesting a different pathophysiology for TACO. In a nationwide hemovigilance study, up to 50% of TACO cases occurred after transfusion of a single blood product.[6] Moreover, the incidence of TACO differs between transfusion products,[7–10] an effect that cannot be explained solely by the infusion of volume. Previous studies in otherwise healthy animals[11] as well as human volunteers[12,13] show that even massive and rapid infusion of crystalloids (30 mL/kg at 100 mL/min) does not result in cardiac overload. In line with this, a retrospective study in critically ill patients found that significantly less volume of blood product was required to develop TACO compared to patients with conventional fluid overload.[14]

Direct pulmonary capillary pressure measurement in the form of left atrial pressure or left ventricular end-diastolic pressure (LVEDP) in patients is invasive.[15] The International Society of Blood Transfusion definition therefore consists of a constellation of indirect signs and symptoms to diagnose TACO. The 2011 guidelines include four or more of the following major criteria within 6 hours after transfusion: acute respiratory distress, acute or worsening pulmonary edema, tachycardia, increased blood pressure, and/or evidence of positive fluid balance.[16] The most recent guidelines, include the same criteria, though with a slightly modified scoring system, and have increased to onset of symptoms to 12 hours after transfusion.[17]

We hypothesized that volume incompliance through a "first hit" is required for TACO to develop, lowering the threshold for a blood transfusion to cause circulatory overload. Risk factors that are strongly associated with the development of TACO include renal and cardiac failure[9,10,14,18,19] which limit hemodynamic accommodation to volume challenges. Furthermore, observational studies showed that pretransfusion volume status is important,[7,10,18] and particularly circulatory overload seems to occur in normovolemic transfused patients.[20]

As direct pulmonary capillary pressure measurement is invasive in patients, we have developed an animal model for TACO in which we can directly measure an increase in LVEDP. We hypothesized that, specifically, volume-incompliant recipients are prone to develop TACO. Furthermore, we aimed to test the hypothesis that TACO is a two-hit syndrome and that

blood transfusions have different effects compared to crystalloids with regard to circulatory overload.

MATERIAL AND METHODS

All experiments were approved by the Dutch national commission for animal experiments (project license: AVD118002017814). All experiments followed the *Guide for the Care and Use of Laboratory Animals*[21] and results are reported following ARRIVE guidelines.[22] Male adult Lewis rats weighing 300 to 350 g were used (LEW/SsNHsd, Envigo). Males were chosen to limit variation within the model due to cardiac[23,24] and renal protective effects of estrogen.[25,26] Animals were housed in an animal care facility, exposed to a 12-hour light–dark cycle, and fed standard rat chow, with ad libitum access to water. Three independent models were used. Rats received a first hit resulting in volume incompliance through either 1) a myocardial infarction (MI) model in the acute setting; 2) an acute kidney injury (AKI) model with a 72-hour delay between first and second hit; or 3) no intervention (control group). Animals were randomized to receive a second hit of either a red blood cell (RBC) transfusion or Ringer's lactate (RL) infusion (Fig. 1). Rats are widely used for hemodynamic studies; Lewis rats are an optimal strain because they have been validated as both an MI and an AKI model in large studies. Overall coronary subservient regions are more consistent in these animals, cardiac infarct size is larger, and survival is higher compared to other strains.[27] A renal ischemia–reperfusion model in Lewis rats showed a consistent time-dependent increase in creatinine with concurrent severity of AKI.[28]

Animal procedures

Control group: an isovolemic anemia transfusion model
Healthy animals were anesthetized employing a brief period of isoflurane 5% and a subsequent bolus injection of a mix of racemic-ketamine (9 mg/100 gr), dexmedetomidine (12.5 μg/100 gr) and atropine (5 μg/100 gr) (KDA) intraperitoneally. An intravenous continuous-rate infusion of ketamine through a tail-vein cannula was used as maintenance anesthesia (5 mg/100 gr/hr). Rats were ventilated via tracheotomy with a ventilator (Babylog 3000, Dräger), with flow and tidal volumes (goal, 6.0 mL/kg) monitored through a differential pressure transducer (Pneumotach, HSE, Holliston). A rat pressure-volume (PV) microcatheter (SPR-838, Millar) was passed through the right carotid artery into the left ventricle. Briefly, the PV catheter was pressure calibrated, and parallel conductance was corrected for using hypertonic saline boluses before termination (NaCl 30%, 10-μL bolus). The volume-cuvette method was used, calibrating for blood conductivity according to previously published protocols[29] at fixed time points, in line with expected blood electrolyte changes: 1) baseline, 2) following isovolemic anemia, 3) after transfusion, and 4) at termination. Arterial blood gas analysis was performed at these time points with use of a blood gas analyzing system (RapidLab 500, Siemens). The right jugular
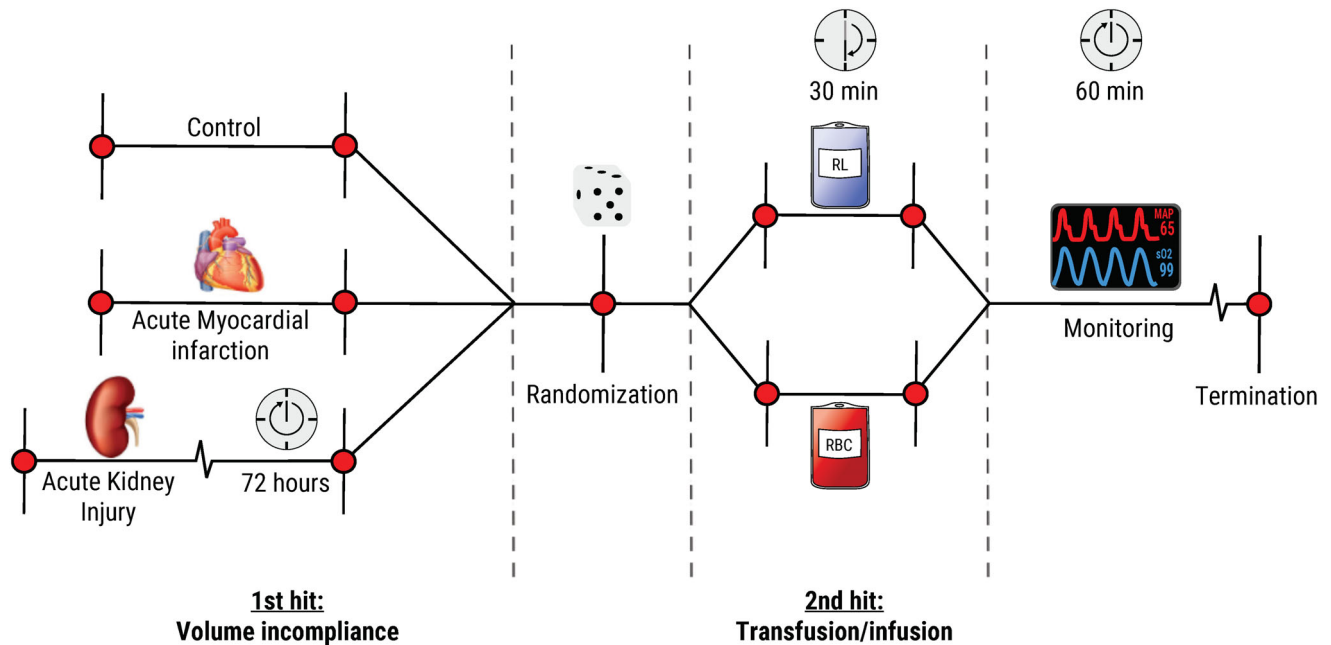
Fig. 1. Two-hit model for TACO investigating RBC transfusion versus RL. A two-hit model for TACO was designed. A first hit resulting in volume incompliance was induced through myocardial infarction in the acute setting or acute kidney injury with 72-hour delay. Controls did not receive a first hit. Animals were randomized to receive a second hit of either crystalloids (RL) or RBC transfusion. [Color figure can be viewed at wileyonlinelibrary.com]

vein was cannulated to record central venous pressure (CVP) and the left carotid artery to measure mean arterial pressure (MAP) and to draw blood. All hemodynamic data were continuously recorded with computer software (LabChart version 6.1, AD Instruments).

Isovolemic anemia was induced by a controlled blood draw of 20% of the estimated circulating volume (ECV) over 15 minutes, replacing blood with an equivalent volume of colloids (Tetraspan 6%, B. Braun Melsungen AG), as pilot experiments using crystalloid as replacement required larger volumes to prevent hypotension. ECV was calculated as 6.5% of animal body weight.[30] Anemia was induced to achieve a clinically relevant anemia transfusion model. Through substitution with colloids, animals remained normovolemic instead of hypovolemic. We hypothesize that pretransfusion volume status is important in the development of TACO. A hematocrit of $30 \pm 2\%$ was targeted, and continued blood substitution was performed until achieved. Subsequently, animals were allowed to stabilize for 30 minutes. All animals received continuous intravenous norepinephrine whereby the dose was titrated to a pretransfusion MAP of 65 mm Hg.

Operators were unaware of treatment allocation until randomization, which occurred after 30 minutes of stabilization. Animals were randomized using a sealed envelope method and were allocated to receive either a transfusion of RBCs or infusion of RL. Block randomization per week, using a 1:1 ratio, was used to ensure that all fresh blood products were used, thereby minimizing the number of donor animals required. Following

randomization, no changes were made to maintenance fluid, norepinephrine dose, or infusion rates of anesthetics. Ventilator pressure settings were fixed for the remainder of the experiment. Based on effect size in pilot studies, a human equivalent of four units of either RBCs or RL was chosen (approx. $4 \times 5\%$ of ECV, assuming an ECV of 6.0 L and units of 300 mL), to be infused at a fixed rate over 30 minutes. After infusion, follow-up was 60 minutes; thereafter, animals were exsanguinated (Appendix S1, available as supporting information in the online version of this paper).

Acute myocardial infarction

Similar to what was described above, under general anesthesia all cannulas were placed and isovolemic anemia was induced. After stabilization, a left anterior thoracotomy ($\pm$2-cm incision through the fourth intercostal rib) was performed and the left anterior descending artery was permanently ligated using a 5-0 Prolene suture.[31] After visual confirmation of blanching and ST elevations on a three-lead electrocardiogram, the thorax was closed, with excess air in the thorax removed through a drain retracted under negative pressure.[32] After thoracotomy, but also when atelectasis was suspected, a lung recruitment maneuver was performed by raising mean airway pressure to 25 cmH$_2$O for five breaths. After stabilization 30 minutes after MI, animals were randomized to either RBCs or RL infusion as described above.

Acute kidney injury model

To induce AKI, healthy animals underwent a 3-cm median laparotomy under general anesthesia (isoflurane with buprenorphine 0.3 mg/kg intraperitoneally [IP]). The renal vascular bundle was identified bilaterally and clamped atraumatically.[28] After 45 minutes of ischemia to each kidney, clips were removed. Ischemia and reperfusion were confirmed by verifying parenchymal color change. The abdomen was closed in two layers with 3-0 vicryl, and animals were awake during the interim period while a new hemodynamic equilibrium was reached. A second dose of buprenorphine IP was given 6 to 8 hours after surgery. After 72 hours, animals were reanesthetized using KDA (same dosage as previous groups) and cannulated; isovolemic anemia was induced, and infusion of study fluids in a randomized manner was performed as described above.

Transfusion product manufacturing, sample collection, and laboratory testing

Buffy-coat reduced RBC products were prepared, following Dutch national blood banking practices and according to methods previously published.[33] Briefly, blood was harvested from donor rats through cardiac puncture (isoflurane anesthesia). Whole blood was collected in a 10:1 ratio in a citrate-phosphate-dextrose solution and was subsequently pooled (as rats lack blood groups). This was centrifuged at 2000 g for 10 minutes at 4°C, the plasma and buffy coat were removed, leaving only the RBC pellet (buffy coat reduction removes 70%-80% of white blood cells). The RBCs were resuspended in a saline-adenine-glucose and mannitol storage solution to a target hematocrit of 60%. RBC product was kept at 4°C in a breathable storage container and transfused to animals within 3 days of harvesting and production. The short interval between storage and transfusion mitigates storage lesion as a confounder because rat RBCs remain of high quality for at least 7 days.[34]

Sample collection is described in Appendix S1, available as supporting information in the online version of this paper. Briefly, after termination, a bronchoalveolar lavage was performed. In the bronchoalveolar lavage fluid (BALF), total protein concentration as well as proinflammatory cytokines interleukin-6 and CINC-3 (homologue of interleukin-8) were determined to exclude transfusion-related acute lung injury. The right lung was used for histopathology and to quantify pulmonary edema by wet-dry (WD) ratio.[35] In the MI model, the heart was excised and quantification of the infarct size was performed to ensure comparable groups.[31] In the AKI model, the kidneys were excised and periodic acid-Schiff stained for histopathology.

Sample size calculation

A clinically significant difference in LVEDP between RBC transfusion and fluids was defined as 4.0 mm Hg. A similar increase in LVEDP was found after transfusion in a study in humans.[36] Based on initial pilot experiments (n = 3 per group; nonrandomized data not shown) we found a difference in LVEDP

between groups of 7.1 mm Hg ±2.8 (mean ± standard deviation). Based on this effect size, an α = 0.05 and β of 80%, we calculated a sample size of four animals per group to find a significant increase in LVEDP between RL and RBCs in the MI model. An additional 50% was added to each group to compensate for dropouts after randomization; animals that dropped out before randomization were replaced. The same sample size was used for the AKI model.

Outcomes

Primary outcome was ΔLVEDP (post- minus pretransfusion LVEDP) comparing RBC infusion to RL infusion. Secondary outcomes include difference in LVEDP at 15, 30, and 60 minutes after transfusion. Pulmonary outcomes were PaO_2/FiO_2 (PF) ratio, WD ratio, histopathology grading of pulmonary edema, BALF total protein, and cytokine concentrations.

Statistical analysis

Statistics were performed using statistical software (R Statistics 3.3.2 with RStudio interface version 1.0.136, RStudio Team). Parameters were inspected for normality with use of histograms and q-q plots. Outliers were detected using Tukey's method, an LVEDP 1.5× outside the interquartile range (IQR) was considered an outlier and excluded from the analysis. Outcomes between the control group and the MI and AKI models were not compared to each other, as these were not timed control experiments; rather, results were analyzed between randomization arms (RBC vs. RL) within respective control, MI, and AKI models. The primary outcome, change in LVEDP comparing pre to posttransfusion, was first analyzed to identify significant increases within a randomization arm using a Wilcoxon signed-rank test. Effect of infusion product on pulmonary capillary pressures was compared using a linear regression model correcting for type of infusion product. Pulmonary and hemodynamic outcomes were compared between randomization arms with the Mann–Whitney U test. A p value of less than 0.05 was the threshold for significance.

RESULTS

A total of 30 animals were used within the following groups: control group (n = 6), MI model (n = 12), and AKI model (n = 12). In the AKI group, after randomization to RBC or RL infusion, one animal died, and one animal was excluded as a statistical outlier (Appendix S2, available as supporting information in the online version of this paper). Isovolemic anemia resulted in a hematocrit drop from baseline 40.0% (IQR, 38.0–42.3) to 29.0% (IQR: 28.8–31.0) after circulating volume replacement (p < 0.001; Fig. 2A). Characteristics at time of randomization are presented in Table 1. Between randomization arms of the control, MI, and AKI models, there were no apparent differences in hemodynamic or respiratory parameters, fluid balance, or anesthesia infusion rates.
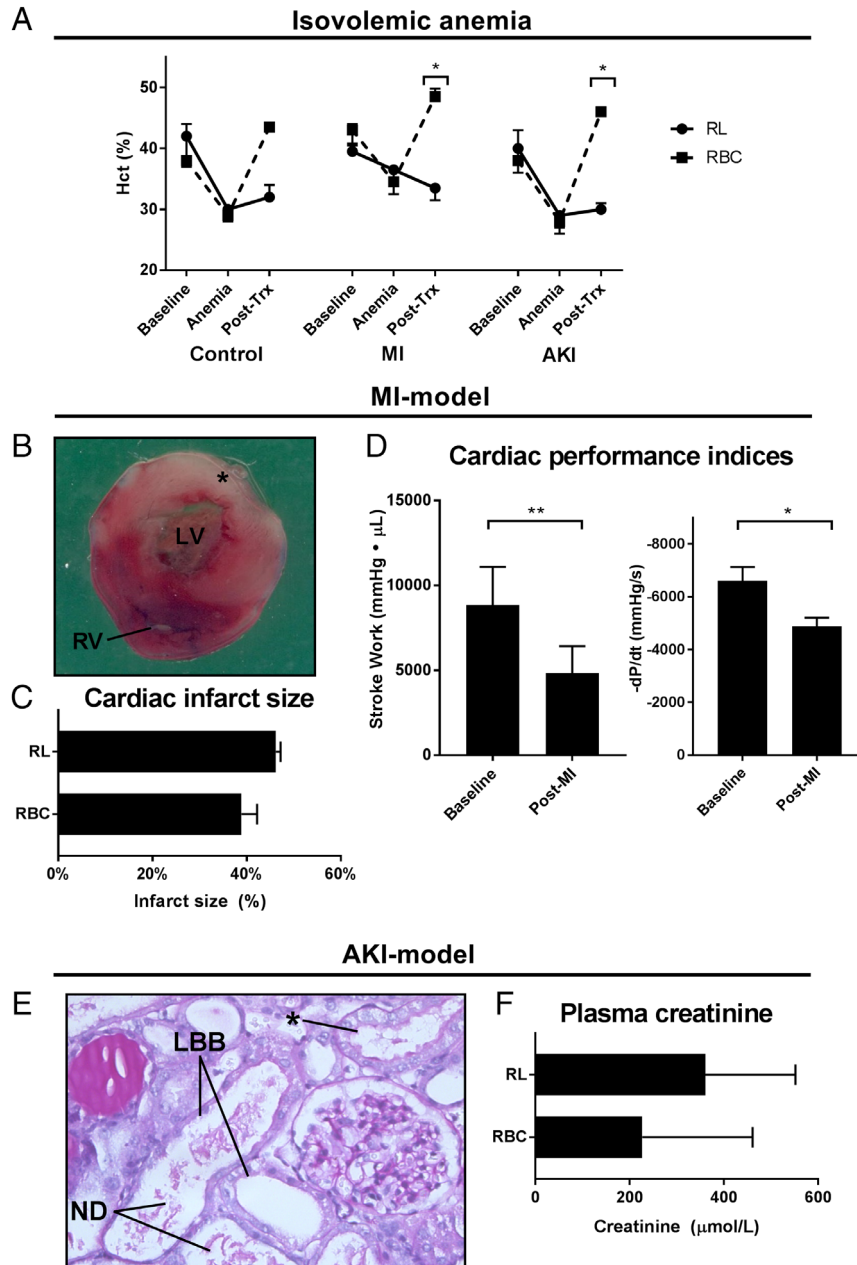
Fig. 2. Validation of anemia and adequate first hit. (A) Hematocrit decreased from baseline after blood is replaced with colloids. Hematocrit subsequently increased significantly only in the transfused groups. **(B)** Macroscopic examination of stained myocardium shows a large left ventricular and partial septal infarction delineated by white tissue (*). **(C)** Proportion of cardiac ventricle infarct volume per randomization arm. **(D)** Stroke work is a measure of cardiac effort, -dP/dt is a measure of myocardial relaxation, and both are decreased during and after ischemia. **(E)** Histopathology of renal parenchyma stained with periodic acid-Schiff staining (PAS-D) in animals with AKI showing loss of brush border (LBB) in areas (compared to an intact brush border (*) and necrotic debris in the tubular lumen (ND). **(F)** Plasma creatinine level per randomization arm. *p < 0.05; **p < 0.01. Hct = hematocrit; LV = left ventricle; RV = right ventricle. [Color figure can be viewed at wileyonlinelibrary.com]

Effect of volume overload in volume-compliant animals

In control animals, that is, those without a first hit, LVEDP did not increase significantly following infusion of RL or blood transfusion. Moreover, in the primary outcome (ΔLVEDP between RBC and RL), there was no difference between randomization arms after RBCs transfusion: +4.4 mm Hg (IQR, 3.27–9.2, ns) vs. RL: +2.8 mmHg (IQR: 2.4–5.7, ns) – Fig. 3A. In

TABLE 1. Prerandomization characteristics					
	Control		MI model		AKI model	
Prerandomization Characteristics	RL (n = 3)	RBC (n = 3)	RL (n = 6)	RBC (n = 6)	RL (n = 5)	RBC (n = 5)
Weight, gr	330 (318-335)	300 (300-305)	340 (335-349)	325 (316-330)	318 (316-322)	320 (318-322)
Heart rate, bpm	218 ± 24	259 ± 32	232 ± 29	254 ± 22	247 ± 18	245 ± 29
CVP, mm Hg	3.0 (2.4-3.6)	3.9 (3.6-4.4)	4.9 (4.6-5.7)	4.1 (3.6-5.4)	3.7 (3.4-3.8)	3.5 (3.2-4.4)
MAP, mm Hg	62.4 ± 8.0	69.5 ± 8.0	64.0 ± 4.8	64.0 ± 5.3	66.5 ± 5.5	72.6 ± 10.57
Cardiac output, mL/min	14.4 (10.6-16.9)	21.5 (19.2-24.6)	17.1 (13.5-19.4)	15.8 (12.5-20.9)	21.5 (20.9-21.6)	19.4 (18.2-21.1)
Ejection fraction, %	...	...	45 (36-51)	49 (49-60)	...	...
Stroke work, mm Hg × μL	5623 ± 2848	6872 ± 1742	4998 ± 2109	4498 ± 2790	7639 ± 1548	5993 ± 2109
RPP, bpm × mm Hg × 10^3	21.2 (20.7-21.5)	22.8 (21.1-25.4)	21.3 (19.6-24.2)	25.1 (24.5-27.4)	26.2 (22.4-27.1)	24.2 (23.3-24.9)
SVRI, dyn × s/cm^5	368 (306-496)	276 (228-278)	283 (236-381)	282 (218-385)	262 (248-274)	248 (239-260)
Fluid balance, mL	1.83 (1.81-1.96)	1.92 (1.83-1.98)	2.44 (2.36-2.54)	2.32 (2.28-2.46)	1.57 (1.53-1.62)	1.63 (1.53-1.64)
Noradrenaline, μg/kg	2.62 (2.17-2.82)	1.00 (0.82-2.82)	6.79 (5.62-7.72)	6.48 (4.07-7.06)	1.08 (0.95-1.86)	0.94 (0.94-0.98)
PaO$_2$/FiO$_2$ ratio	481 (371-488)	454 (453-460)	421 (375-437)	401 (391-406)	501 (493-518)	493 (492-501)

Overview of animal characteristics at randomization before infusion of study fluids. Data is presented as either mean ± SD or median (IQR) as appropriate.

AKI = acute kidney injury; CVP = central venous pressure; MAP = mean arterial pressure; MI = myocardial infarction; RL = Ringer's lactate; RPP = rate pressure product; SVRI = systemic vascular resistance index.

a single-hit model of volume overload the boundary of clinical relevance was not reached.

Effect of volume overload in volume-incompliant animals

The first hits resulted in significantly reduced cardiac and renal function, suggesting that animals were volume incompliant. In the animals of both the MI and AKI models, pulmonary capillary pressure increased only after blood transfusion but not fluid infusion.

MI animals had pronounced infarctions: 42.6% of total ventricular volume, with significant myocardial dysfunction denoted by decreased stroke work (calculated as stroke volume multiplied by LV pressure generated) and maximum decrease in left ventricular pressure during relaxation (Fig. 2B-D). Postinfarction ejection fractions, measured through electrical impedance by PV catheter, were unreliable due to conformational

TABLE 2. Application of clinical TACO criteria				
	MI model		AKI model	
Postinfusion characteristics	RL (n = 6)	RBC (n = 6)	RL (n = 5)	RBC (n = 5)
Matched TACO criteria	17	83	40	100
≥4 major criteria, (%, n)	(1/6)	(5/6)	(2/5)	(5/5)
Respiratory distress	478	467	533	504
PaO$_2$/FiO$_2$ ratio	(463–492)	(418–476)	(522–545)	(503–513)
Pulmonary edema	4.8[†]	4.6[‡]	4.8	4.8
WD ratio	(4.8–4.8)	(4.5–4.8)	(4.8–4.8)	(4.8–4.8)
Tachycardia: Δ HR (bpm)	27.6 ± 24	29.3 ± 23	−0.49 ± 12	16.3 ± 16
Hypertension	8.7	17.7	23.0	37.7
ΔMAP, mm Hg	(2.5–20.6)	(4.3–32.4)	(16.0–27.2)	(34.6–37.9)
Fluid balance	9.0	8.9	7.8	7.8
Total infused volume, mL*	(8.0–9.1)	(8.6–9.0)	(7.7–7.9)	(7.7–8.0)
Evidence supporting volume overload	−2.04	0.39[§]	−0.51	0.04
- ΔLVEDP, mm Hg	(−4.06––1.12)	(−0.27–1.27)	(−1.01––0.19)	(−0.78–0.25)
- ΔCVP, mm Hg	−0.47	−0.09	−0.45	−0.61
	(−0.65–0.15)	(−0.22–0.03)	(−0.68 – −0.37)	(−1.35 – −0.40)

The 2011 International Society of Blood Transfusion definition of TACO is used. All values are at the end of the experiment, changes in HR, MAP, LVEDP, and CVP are compared to pretransfusion values. Data is presented as either mean ± SD or median (IQR) as appropriate.

* Total infused volume includes anesthesia, maintenance fluid, vasopressors, and, respectively, transfusion or infusion product.

† WD ratio measured in 5/6 animals, one value missing.

‡ WD ratio was significantly higher compared to healthy reference value (p < 0.05).

§ RBC was significantly higher compared to RL (p < 0.01).

AKI = acute kidney injury; CVP = central venous pressure; HR = heart rate; LVEDP = left ventricular end-diastolic pressure; MAP = mean arterial pressure; MI = myocardial infarction; RL = Ringer's lactate; WD ratio = wet weight/dry weight ratio.
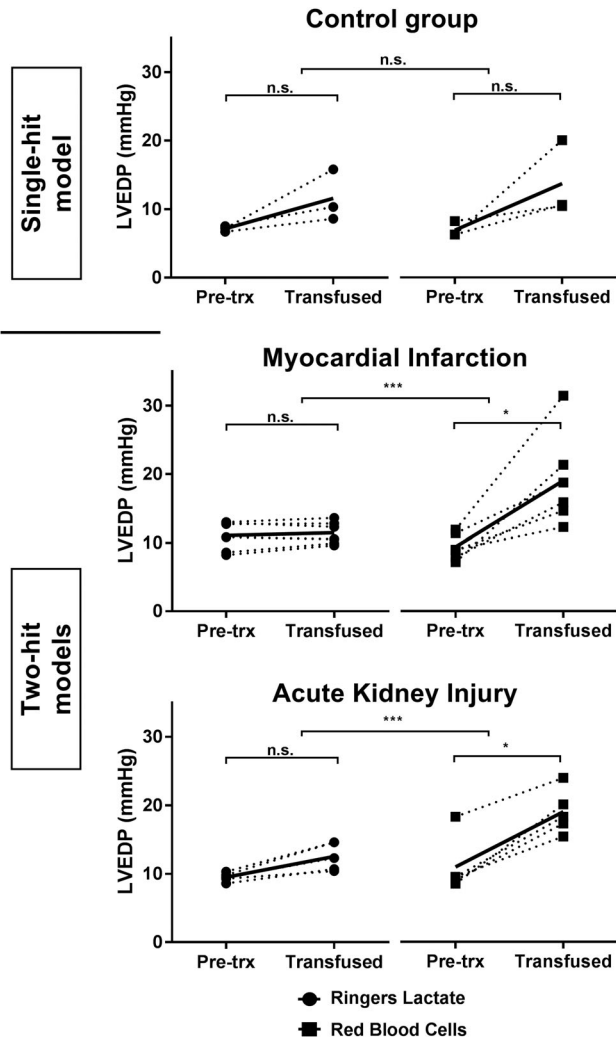
Fig. 3. Volume incompliance and transfusion result in circulatory overload. LVEDP is shown for all groups, with the single-hit model showing overall little difference between infusion arms. In both two-hit models, MI and AKI, a large increase in LVEDP is seen in the RBC transfused groups but not in the arms receiving RL. *p < 0.05; **p < 0.01; ***p < 0.001.

changes of the ventricle caused by ligation of the coronary artery. In the MI model, LVEDP increased significantly only in the RBC transfusion group: +8.0 mm Hg (IQR, 7.5–14.1; p = 0.03) versus +0.3 mm Hg after RL infusion (IQR, −0.2–1.1, ns) (Fig. 3B). A linear-regression model was fitted to estimate the LVEDP over the time points before and after transfusion, and RBC transfusion was a significant predictor (p = 0.001).

AKI animals had bilateral generalized signs of acute tubular injury on histology, with pronounced serum creatinine elevation 239 μmol/L (IQR, 96–491) (Fig. 2E-F). The AKI model showed a similar significant increase in LVEDP only after transfusion, +8.3 mm Hg (5.9–8.8; p = 0.03) versus +2.9 mm Hg (2.3–4.0, ns). RBC transfusion was a significant predictor of ΔLVEDP (p < 0.001). During the 1-hour follow-up period, LVEDP remained significantly higher in the RBC infused arms up to 60 minutes in the MI group and 30 minutes after transfusion in the AKI group (Appendix S3, available as supporting information in the online version of this paper).

Application of clinical TACO criteria

Four of five criteria are required to clinically diagnose TACO; results are shown in Table 2. In the MI experiment, 83% (five of six transfused animals) had TACO, with 100% (5/5) of the transfused animals in the AKI model. Transfused animals overall had worse clinical outcomes based on the TACO criteria. The PaO_2/FiO_2 ratio was slightly lower in transfused animals compared to RL in all experiments (controls, MI and AKI model; Fig. 4A), though not statistically significant. The WD ratio did not differ between randomization arms; specifically, there was no increase in pulmonary edema in the RBC-transfused group compared to the RL group. Apart from increasing LVEDP, transfusion resulted in a more pronounced, though not significant, rise in heart rate compared to those receiving RL: +28.2 bpm (IQR, 10.2–62.9) versus +23.8 bpm (IQR, −1.1–40.1). Blood pressure markedly increased (p = 0.02) in the transfused group compared to the fluids group, with MAP increasing by +35.1 mm Hg (IQR, 15.4–37.8) versus +14.5 mm Hg (IQR, 8.3–26.5). The final major criterion, fluid
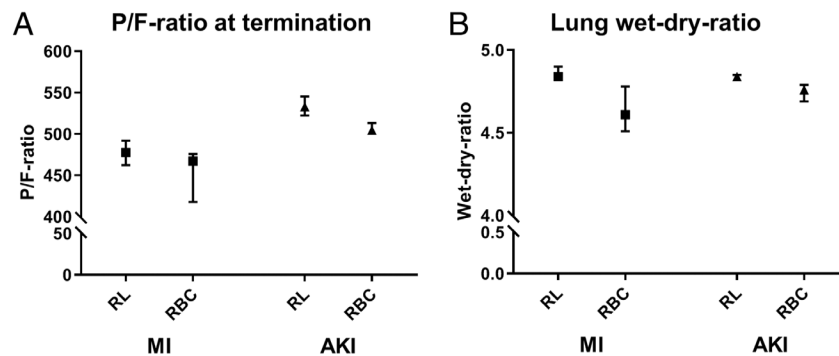
Fig. 4. Pulmonary outcomes. (A) Lung oxygenation was not statistically different between infusion groups. (B) Lung wet-dry ratios did not differ between RBC and RL arms.

balance, was positive in all models, as animals were isovolemic and received an infusion of RBCs or RL along with continuous intravenous anesthetics, maintenance fluids, and vasopressors. There was no change in CVP between pretransfusion and end of experiment.

Pulmonary outcomes

Histopathological examination of the lungs was performed using a 0- to 3-point increasing severity rating scale. In 29 of 36 (80.6%) excised lungs, there was perivascular edema present (Appendix S4A, available as supporting information in the online version of this paper); the rated edema, however, did not differ between transfused and volume-infused arms. More severe signs, such as interstitial or intra-alveolar edema, were not present. Post hoc analysis showed that peak LVEDP at the end of transfusion did not correlate to the degree of pulmonary edema (R = −0.01, ns) or WD ratio (R = −0.18, ns). The BALF was not an exudate and did not show any signs of inflammation with between randomization arms, as there was no difference in total protein concentration or inflammatory cytokine concentrations (Appendix S4B-C, available as supporting information in the online version of this paper).

DISCUSSION

TACO is currently the leading cause of transfusion-related morbidity and mortality; however, to date, no studies have investigated the pathophysiology of TACO, and no randomized studies have investigated the difference between volume overload by transfusion or crystalloids. To our knowledge, this clinically relevant animal model is the first to suggest that TACO is a distinct condition, different from fluid overload. The main findings of this study are 1) volume incompliance is essential in the development of TACO; 2) RBC transfusion can result in circulatory overload, whereas infusion of equal volumes of fluids does not; and 3) in this TACO model, pulmonary capillary pressure rapidly increases during transfusion and remains elevated during the 1-hour follow-up.

Observational studies showed that AKI and cardiac failure are risk factors for the development of TACO.[9,10,14,18,19] We confirmed that these underlying conditions result in a decreased volume compliance, which is essential for the development of TACO in our animal model. Without an underlying first hit, there is no significant effect of transfusion on pulmonary pressures. Healthy volume-compliant animals can accommodate volume challenges. In the two-hit models, there is a rapid and direct increase in pulmonary capillary pressures only after RBC transfusion. Volume incompliance likely decreases the threshold to develop circulatory overload, since a subsequent RBC transfusion leads to volume overload, whereas an equal volume of crystalloids does not.

To our knowledge, this is the first evidence of TACO being different from conventional volume overload. All animals had a positive fluid balance, and with equal amounts of fluids infused, there is a greater increase in pulmonary capillary pressure, heart rate, and blood pressure in the transfusion group. Our results confirm initial findings of increased pulmonary pressures after transfusion reported by Masuda et al.[11] Massive volume infusion of otherwise healthy swine, that is, infusion of an additional 100% of ECV, similarly increased pulmonary pressures after whole blood but not LR. The main limitation in the previous study was the use of mean pulmonary artery pressure, instead of left atrial pressure or LVEDP, as this pulmonary artery pressure can be elevated in the absence of circulatory overload. Massive transfusion of whole blood into healthy euvolemic subjects does not reflect clinical practice. To increase clinical relevance and translatability, in this model, animals were made anemic and processed RBC units were transfused. Results of our model are consistent with the only study performed in humans by Nand et al.,[34] who transfused a group of anemic patients measuring pulmonary capillary wedge pressure (PCWP), the gold standard for assessment of pulmonary capillary pressure in humans. They showed a significant rise in wedge pressure after transfusion; however, they lacked a fluid control group. Additionally, a recent large observational study in intensive care unit patients showed an increase in PCWP after transfusion,[37] as well as a cardiac surgery study showing similar results.[38] We see a similar effect of transfusion on capillary pressures; however, with our RL-infused control group, we show that this increase is specifically transfusion product related.

Concurrent analysis of the control, MI, and AKI models provides deeper insight into the development of circulatory overload, addressing the questions: why does circulatory overload only occur in volume incompliant animals, why only after transfusion, and why are the effects sustained over a longer period of time? Isolated heart failure can logically result in backward failure after a volume challenge; however, results are reproduced in an isolated kidney injury model. While kidney injury can precipitate circulatory overload, an equivalent volume of crystalloids or transfusion have different effects. This study focused on investigating whether TACO is a distinct entity. There are a number of possible mechanisms we hypothesize that are product dependent as well as recipient factors that might be involved in developing TACO. Colloid osmotic pressure (COP) increase due to transfusion is a potential mediator for TACO.[39] Blood products contain protein, which increases either plasma protein concentration or total protein levels if isooncotic, resulting in volume recruitment from the peripheral tissues. Transfusion can thereby potentially increase intravascular volume by more than the volume infused. Further research into transfusion product COP, pre and posttransfusion plasma COP, and circulating volume is required. Cell free hemoglobin is released during storage and lysis of RBCs. Being an avid scavenger of nitric oxide, it causes vasoconstriction, which combined with a volume challenge can result in circulatory overload. In healthy human volunteers, an association has been shown between transfusion and pulmonary vasoconstriction.[40]

Additionally, hemoglobin containing RBC microparticles are suggested to react similarly to free hemoglobin in scavenging nitric oxide.[41] Interestingly, both free hemoglobin and microparticles rise commensurably with increased storage duration, potentially linking RBC storage lesion to TACO.

Other potential mechanisms include direct disruption of the endothelial barrier contributing to formation of edema. A first hit with an inflammatory component or second hit of an allogeneic blood product can result in inflammation, which is known to disrupt glycocalyx and therefore endothelial barrier function.[42,43] Additionally, microparticles also confer inflammatory effects[44] and directly interact with the vascular endothelium influencing barrier function.[45] While our results do not support permeability edema or an inflammatory effect, as BALF protein levels were not elevated, TACO can take up to 6 hours to become clinically manifest, and delayed capillary leakage cannot be ruled out. Finally, blood viscosity is increased by transfusion,[46] and it reduces cardiac output.[47,48] Inversely, hemodilution and lowering of viscosity increase cardiac output in animal studies[49] and healthy volunteers.[13] Increased viscosity results in more cardiac strain, which may especially impact volume-incompliant patients prone to TACO.

Our model has several limitations. First, although animals were anemic, the hematocrit of 30% as a target before transfusion might be lower in the clinical setting. This was a practical choice, as lower targets resulted in higher mortality rates in pilot studies. An equivalent volume of four units was chosen, which was based on pilot studies and effect size. Although this volume is given in some clinical settings such as the intensive care unit, cardiac care unit, and operating room, at first sight it appears not to reflect the standard patient receiving a blood transfusion. However, rats require a large volume of fluid intake, drinking the equivalent of their own circulating volume per 24 hours. Therefore, a greater volume is required to overload the circulation and the four units translate to a much smaller volume in the human setting. This inherent difference in fluid homeostasis is a limitation of using small mammals as hemodynamic models.

Furthermore, a follow-up duration of 60 minutes was chosen to limit the amount of bias in our model, as no changes to ventilatory parameters, fluid infusion, and anesthetic and vasopressor infusion rates were made after randomization. Finally, this animal model lacks an allogeneic component to blood transfusion. Inbred Lewis rats are syngeneic; therefore, immunological sequalae of transfusion are unlikely as opposed to transfusion in the clinical setting.[50,51] It has been shown that TACO is associated with fever in up to one in three cases and has even been associated with anti-HLA Class II antibodies.[52] This model rather functions as a model for true circulatory overload on the spectrum of TACO–transfusion-related acute lung injury, resulting in hydrostatic and not permeability pulmonary edema. An immunological component to TACO is possible, and lack of allogeneicity of transfusion in this model may have limited the degree of pulmonary outcomes. Another explanation may be the application of positive pressure ventilation, which may have prevented the onset of pulmonary edema.

Future studies can use this model to further investigate the pathophysiology of TACO, identifying mechanisms through which transfusion but not conventional fluid overload result in increased hydrostatic pressure. These models should use the revised 2019 TACO criteria.[17] The current animal model is the first step to investigating the aforementioned potential mechanisms; however, due to the additional experiments are required to investigate them. Furthermore, this model is well suited to test the effect of different blood products, including plasma and platelets units, on hydrostatic pressure. Finally, prophylactic and therapeutic treatment strategies such as diuretics or infusion velocity can be tested to evaluate effect on pulmonary capillary pressure before pursuing human trials.

CONCLUSION

We have developed a two-hit animal model for TACO, characterized by an increase in pulmonary hydrostatic pressure only in volume-incompliant animals following RBC transfusion and not after crystalloid infusion. These results indicate that TACO is different from conventional volume overload. This animal model is the first step to identifying pathways in the development of TACO and is a step up toward testing of therapeutic and prophylactic strategies for this life-threatening syndrome.

CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

Appendix S1. Supplementary Appendix.
Appendix S2. Analysis of statistical outlier and drop-out animals.
Appendix S3. LVEDP remains elevated post-transfusion.
Appendix S4. Secondary pulmonary outcomes.