Original article

The effect of plasma transfusion in an experimental two-hit animal model of transfusion-associated circulatory overload with heart failure

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Background - Transfusion-associated circulatory overload (TACO) is a leading cause of transfusion-related morbidity and mortality. TACO follows a two-hit pathophysiology, where comorbidities like cardiac or renal failure act as the first hit followed by blood transfusion as a second hit. Observational studies suggest that plasma transfusion is more likely to cause TACO than other blood products. We conducted a randomized animal study to gather evidence that plasma transfusion can induce TACO.

Material and methods - As a first hit a large myocardial infarction was created in male Wistar rats. Then animals were randomized to receive 4 units of solvent/detergent-treated pooled plasma (SDP), fresh frozen plasma (FFP), a colloid control (albumin 5%) or a crystalloid fluid control (Ringer's lactate) (n=10 per group). The primary outcome was the difference between pre- and post-transfusion left-ventricular end diastolic pressure (ΔLVEDP). Secondary outcomes were markers for acute lung injury; lung wet/dry weight ratio, PaO_2/FiO_2 ratio and pulmonary histological assessment.

Results - Pre-transfusion characteristics were similar between groups. ΔLVEDP increased significantly after transfusion with SDP (7.7 mmHg; 4.5-10.5) and albumin (13.0 mmHg; 6.5-15.2), but not after FFP (7.9 mmHg, 1.1; 11.3) compared to infusion with Ringer's lactate (0.6 mmHg; 0.4-2.2), p=0.007, p=0.0005 and p=0.14 respectively. There were no significant differences in ΔLVEDP between groups receiving SDP, FFP or albumin. There was no increase in acute lung injury in any group compared to other groups.

Discussion - Circulatory overload, measured as ΔLVEDP, was induced after transfusion of SDP or albumin, but not after infusion of Ringer's lactate. These results show that the effect of plasma transfusion on ΔLVEDP differs from fluid overload induced by crystalloid infusion. Colloid osmotic pressure may be an important component in the development of TACO and should be a target for future research.

Keywords: *transfusion reaction, plasma, pulmonary edema, animal models, transfusion-associated circulatory overload.*

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INTRODUCTION

Transfusion can be a life-saving therapy, however it is not without risk. Transfusion-associated circulatory overload (TACO) is a serious complication resulting in pulmonary

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edema and respiratory distress combined with signs of circulatory overload within 12 hours following a transfusion[1]. TACO is one of the leading causes of transfusion associated morbidity and mortality, with a varying incidence that was 3% in an observational study in non-cardiac surgery patients in 2011, but can go up to 11% depending on the patient population[2]. Mortality was reported to be 4.5% and 5% in two cohorts of TACO patients[3,4]. Although TACO can be a life-threatening syndrome, a large part of its pathophysiology is still unknown[5,6].

Most likely, TACO follows a two-hit model in which patient-related factors such as cardiac or renal dysfunction are major risk factors. A positive fluid balance can also contribute to the development of TACO as a first hit[3,7]. The second hit is conveyed by transfusion. Several transfusion-related factors can promote the development of TACO, one of them being the type of transfusion product[8-10]. Plasma transfusion has been suggested to be a risk factor for TACO in observational studies and results in a higher TACO incidence compared to transfusion with red blood cells or platelets[9,10]. Moreover, an increased volume of transfused plasma was found to be associated to an increased TACO incidence[11].

Plasma transfusion is a therapy used to treat massive bleeding, for example as a result of trauma, and it is used for warfarin reversal in the presence of intracranial bleeding[12]. More applications for plasma transfusion exist, like pre-procedural prophylactic coagulation correction, but evidence for these applications is low. Plasma is also frequently inappropriately transfused without an indication that is consistent with guidelines[13].

A two-hit animal model for TACO using rats was developed in our laboratory[14]. This model focuses on the increase in left ventricular end diastolic pressure (LVEDP) following transfusion, which reflects the left atrial pressure and is a surrogate marker for pulmonary capillary pressure[15]. Within this model we previously showed that LVEDP increased significantly after transfusion of red blood cells when compared to crystalloid infusion[14]. We now want to evaluate the effect of plasma transfusion in this model to gain more insight in TACO's pathophysiology and gather more evidence that plasma transfusion can induce TACO and should therefore only be given with an indication that is consistent with current guidelines or existing evidence. Our hypothesis is that LVEDP will increase after plasma transfusion compared to crystalloid infusion in this previously validated two-hit animal model with heart failure.

MATERIALS AND METHODS

General information

All animal experiments were approved by the Dutch National Commission for Animal Experiments (project license: AVD1180020017814) and executed following the ARRIVE and local guidelines. Animals were housed in an animal housing facility and were kept on a 12-hour light-dark cycle. Animals remained in the housing facility for an acclimatization period of at least 7 days. Animals were fed standard rat chow and water ad libitum. Experiments were executed between 8 am and 6 pm and animals were transported from the housing facility to the laboratory at the day of the experiment.

Animal procedures

Experiments were performed using adult male Wistar rats weighing more than 300 grams. All procedures were executed as in the validated two-hit rat model with heart failure, as previously described[14]. In brief, animals were anesthetized using 5% isoflurane with 100% oxygen, followed by an intraperitoneal injection with a mix of racemic-ketamine (9 mg/100 gr), dexmedetomidine (6.25 µg/100 gr) and atropine (0.25 µg/100 gr). Anesthetics were continued through a tail vein cannula with a mix of racemic-ketamine (50 mg/kg/hr) and dexmedetomidine (15 µg/kg/hr) for the duration of the experiment.

Animals were ventilated in a pressure-controlled mode, through a tracheotomy using a ventilator (Babylog 3000, Dräger, Lübeck, Germany). Respiratory rates were adjusted based on the arterial blood gas analyses (RapidLab 500, Siemens, Erlangen, Germany) and recruitment manouvres were performed if necessary, with a target tidal volume of 6 mL/kg. Body temperature was maintained at 37°C. The right carotid artery was cannulated and a rat pressure-volume (PV) catheter (SPR-838, Millar, Houston, TX, USA) was inserted into the left ventricle. The right jugular vein was cannulated for monitoring central venous pressure (CVP) and administration of fluids and transfusion. The left carotid artery was cannulated for monitoring mean arterial blood pressure (MAP) and blood sampling.

Isovolemic anemia was established by the exchange of arterial blood for a colloid solution (Tetraspan 6%, B. Braun,

Melsungen, Germany) with a target hematocrit of 30%±2. To induce heart failure, a left thoracotomy was performed as previously described and the left anterior descending artery (LAD) was ligated, causing a myocardial infarction[14]. The infarct was confirmed using ECG monitoring and visual inspection of the blanched myocardium. After LAD ligation, animals were allowed to stabilize for 30 minutes, before a transfusion of 4 mL (human equivalent to 4 transfusion units) was administered over 30 minutes. Animals were followed for one-hour post-transfusion before exsanguination. All animals received intravenous norepinephrine (2-8 µg/hr) to maintain a MAP >65 mmHg starting prior to the thoracotomy until the end of the experiment.

Experimental groups and donor products

Animals were randomized into one of four experimental groups after successful LAD ligation. The intervention groups received either solvent/detergent-treated pooled plasma (SDP, Omniplasma - Blood type AB, Octapharma, Laachen, Switzerland) or fresh frozen plasma (FFP, Blood type AB, Sanquin, the Netherlands), which allowed to study the possible differences of these products. The control groups received either Ringer's lactate (Baxter, Deerfield, IL, USA) to control for infused volume or 5% albumin (Albuman 20%, Sanquin, diluted 1:3) to control for colloid osmotic pressure. After randomization, ventilator settings and administration rates for medication and fluids could not be changed. Randomization was performed in a block system with blocks containing two interventions of each randomization group. There were two interventional groups and two control groups in this study. All plasma and albumin products originated from human donors. All products were aliquoted into syringes appropriate for intervention. Syringes were blinded and numbered with a unique identifier by an independent researcher. Involved researchers were blinded for transfusion product and group allocation. Blinding was maintained during experiments and data collection, but not during data analysis.

LVEDP and additional hemodynamic measurements

The primary outcome for this study was the difference between the LVEDP measured pre- and post-transfusion (ΔLVEDP). Additional secondary outcomes included other hemodynamic changes indicating volume overload, like hypertension and tachycardia that are used as supportive diagnostic criteria for TACO[1]. During the experiment continuous 30 second beat-to-beat measurements were performed at six specific timepoints. Three of these were peri-transfusion measurements; 1) pre-transfusion, 2) 15 minutes after start of transfusion, 3) post-transfusion and three measurements were taken during follow-up, 4) 15 minutes post-transfusion, 5) 30 minutes post-transfusion, 6) 60 minutes post-transfusion. Recorded data included: heart rate, MAP, CVP, left ventricular pressure and left ventricular volume. LVEDP was extracted from the pressure-volume loop data and was defined as the pressure measurement at the end of the diastolic phase. All hemodynamic data was continuously recorded using LabChart (version 6.1, AD Instruments, Oxford, UK). The volume-cuvette procedure was used to calibrate the blood conductivity using blood from four timepoints in the experiment; 1) baseline after cannulation, 2) pre-transfusion, 3) post-transfusion, 4) after termination at the end of the 60 minutes follow-up period. Parallel conductance through the myocardium was measured using hypertonic saline bolus injections before termination (NaCl 30%, 20 µL). Calibration processes were performed according to previously published protocols[16].

Sample processing and pathology

Secondary pulmonary outcomes for this study included pulmonary histology score, a lung wet/dry weight ratio and a PaO_2/FiO_2 ratio at termination. Lungs from animals that completed the experiment up to the final measurement were processed and analyzed for these secondary outcomes. After exsanguination the right lung was harvested and the lobes were separated.

The right upper lobe was fixed in 4% formalin for at least 24 hours and then processed into hematoxylin and eosin-stained slides for assessment of ALI. Slides were scored by an experienced pathologist. Scores ranged from 0-3 based on the presence and extensiveness of perivascular and intra-alveolar edema. The right lower lobe was weighed and then placed into a dehumidifying stove at 37°C for 7 days before being weighed again. A lung wet/dry weight ratio was calculated to quantify pulmonary edema. During the experiment arterial blood gas analysis was performed pre-transfusion, post-transfusion and at termination. A PaO_2/FiO_2 ratio was calculated from the results from this last arterial blood gas measurement.

Finally, to validate that all animals had a sufficient

myocardial infarct, the heart was excised and anterograde coronary perfusion with Evans blue dye was performed. Hearts were frozen, cut transversely into 2 mm slices and counterstained with triphenyltetrazolium chloride. Slices were then fixed in 4% formalin and scanned. The percentage of infarcted ventricular tissue was calculated by dividing the infarcted myocardial tissue by the total myocardial volume using image analysis software (ImageJ 1.50i, National Institute of Health, Bethesda, MD, USA)[17].

Sample size calculation

We calculated a sample size of 9 animals in each group would have 80% power to detect a difference in means of ΔLVEDP 4,15 mmHg between groups receiving SDP and Ringer's lactate, which was based on pilot data. Assuming that the common standard deviation is 2,8 using a two-group τ-test with a 0.050 two-sided significance level. One animal was added (±10%) to each group to account for post-randomization mortality. This calculation was based on pilot data (n=2 per group) and previous experimental data from our erythrocyte transfusion model[14]. To confirm an appropriate sample size, halfway into the experiment an independent researcher performed an interim analysis to assess futility and if necessary recalculate the sample size using the ΔLVEDP data from the groups receiving SDP or Ringer's lactate.

Statistical analysis

Animals that were hypotensive (MAP <65 mmHg) or died before transfusion were excluded and replaced. Animals that completed the experiment up to the post-transfusion measurement were included in all analyses for hemodynamic outcome parameters. Measurements from animals that completed the entire experiment including the follow up were used for the analysis of secondary pulmonary outcomes. The groups receiving SDP and Ringer's lactate were chosen as the primary intervention and control group before experiments started and the study was powered for the difference in ΔLVEDP between those groups. These groups were selected, because in clinical practice SDP has been the standard plasma product in the Netherlands since 2014[18]. Ringer's lactate was chosen as the primary control, because we wanted to study the differences between plasma transfusion and volume overload with an equal volume of crystalloid fluids. The primary outcome ΔLVEDP was calculated by subtracting the pre-transfusion value from the post-transfusion value. The same formula was used to calculate other delta values for hemodynamic variables. Results are expressed in medians and interquartile ranges, because normality of data could not be assumed for these group sizes. Differences in ΔLVEDP and other hemodynamic data across all randomization groups were calculated using the Kruskal Wallis test. This was also done for the secondary outcomes lung wet/dry weight ratio, PaO_2/FiO_2 ratio and histopathological score. Specific analyses between two randomization groups were performed using the Mann-Whitney U test. A two-sided p-value <0.05 was considered statistically significant. Although there are multiple outcomes between multiple groups, we chose not to adjust for multiple testing, because of the pre-clinical exploratory nature of this study. Data was analyzed using R (vers. 4.0.3, Rstudio,Boston, MA, USA) and figures were created using Graphpad Prism (vers. 9.1.0, GraphPad Software, San Diego, CA, USA).

RESULTS

Baseline characteristics were equal in all groups

Baseline characteristics were measured pre-randomization (**Table I**). Ten animals were randomized and included in each group and reached the required post-transfusion measurement point. During post-transfusion follow up there was a 40% mortality in the group that received FFP. There was no mortality observed in other randomization groups. Isovolemic anemia was adequately established in all groups with a median hematocrit of 30% and a myocardial infarction of equivalent size was achieved in all animals. There were also no significant differences in all other baseline characteristics between groups.

ΔLVEDP increased in groups receiving SDP or albumin compared to Ringer's lactate

Pre-transfusion levels of LVEDP did not differ when all four groups were compared. The median LVEDP was 5.4 mmHg (3.8; 7.1) and increased in all groups during transfusion, except for the group receiving Ringer's lactate (**Table II** and **Figure 1**). Peri-transfusion ΔLVEDP was higher in the groups receiving SDP (7.7 mmHg, 4.5; 10.5), FFP (7.9 mmHg, 1.1; 11.3) and albumin (13.0 mmHg, 6.5; 15.2) compared to the group receiving Ringer's lactate (0.6 mmHg, 0.4; 2.2) (**Figure 2**). This difference between all groups was statistically significant (p=0.011). Post-hoc

analysis, showed a significant difference between SDP and Ringer's lactate (p=0.007), albumin and Ringer's lactate (p=0.0005), but not FFP and Ringer's lactate (p=0.14). Furthermore, there were no significant differences between SDP, FFP and albumin. At the last measurement at the end of the experiment LVEDP dropped to the

Table I - *Baseline characteristics*

Characteristics	SDP (n=10)	FFP (n=10)	Ringer's lactate (n=10)	Albumin (n=10)
Weight (gram)	357 (331-382)	358 (339-362)	365 (359-370)	364 (357-384)
Fluid input (mL)*	3.2 (2.9-3.5)	3.3 (2.6-3.4)	2.9 (2.5-3.3)	2.6 (2.6-3.2)
Vent duration (hour/min)	1h50min (1h45min-2h)	1h45min (1h40min-2h05min)	1h45min (1h30min-1h50min)	1h45min (1h40min-2h)
Cardiac infarct size (%)	20 (19-22)	21 (19-22)	21 (20-23)	21 (19-22)
pH	7.39 (7.35-7.41)	7.34 (7.31-7.37)	7.37 (7.35-7.89)	7.37 (7.36-7.38)
Lactate (mmol/L)	2.7 (2.0-3.1)	3.3 (2.7-3.8)	2.7 (2.3-3.0)	2.9 (2.7-3.0)
Hematocrit (%)	36 (35-39)	36 (34-37)	37 (36-38)	37 (36-37)

Weight was measured at the start of the experiment before anesthesia, cardiac infarct size was measured after termination with a triphenyltetrazolium chloride staining, all other variables were measured before randomization. *Fluid input was defined as all intravenously administrated fluid, but did not include volume used to achieve isovolemic anemia, which was only used as volume replacement. SDP: solvent-detergent pooled plasma; FFP: fresh frozen plasma.

Table II - *Hemodynamic measurements*

Pre-transfusion	SDP	FFP	Ringer's lactate	Albumin
Heart rate (bpm)	297 (277; 334)	280 (271; 305)	285 (276; 320)	313 (299; 358)
MAP (mmHg)	77 (71; 81)	73 (66; 79)	69 (67; 80)	75 (68; 79)
CVP (mmHg)	1.8 (1.2; 3.1)	1.3 (1.1; 1.6)	0.9 (0.7; 1.3)	1.2 (0.2; 1.6)
LVEDP (mmHg)	5.1 (3.0; 6.7)	5.5 (5.3; 7.6)	5.2 (4.3; 7.2)	6.1 (3.6; 7.1)
Cardiac output (mL/min)	20 (16; 30)	19 (12; 21)	19 (17; 23)	22 (18; 24)
Post-transfusion	**SDP**	**FFP**	**Ringer's lactate**	**Albumin**
Heart rate (bpm)	346 (311-375)	306 (299-347)	285 (273-351)	328 (301-356)
MAP (mmHg)	126 (112-147)	108 (101-116)	97 (91-103)	127 (114-136)
CVP (mmHg)	1.5 (1.1-2.0)	2 (1.3-2.4)	1.3 (1-1.5)	2.3 (0.5-2.5)
LVEDP (mmHg)	13.3 (9.5-23.3)	11.7 (8.6-19.7)	6.9 (4.7-8.4)	18.2 (11.0-21.9)
Cardiac output (mL/min)	25 (16-31)	22 (15-27)	24 (16-26)	21 (15-28)

bpm: beats per minute; MAP: mean arterial pressure; CVP: central venous pressure; LVEDP: left ventricular end diastolic pressure; SDP: solvent-detergent pooled plasma; FFP: fresh frozen plasma.

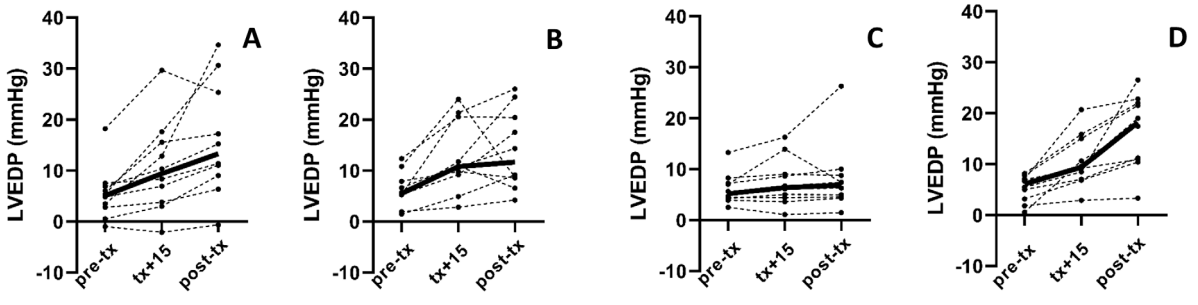

Figure 1 - LVEDP peri-transfusion LVEDP values for individual animals are displayed for all randomization groups (A=SDP; B=FFP; C=Ringer's lactate; D=albumin)
SDP: solvent-detergent pooled plasma; FFP: fresh frozen plasma.

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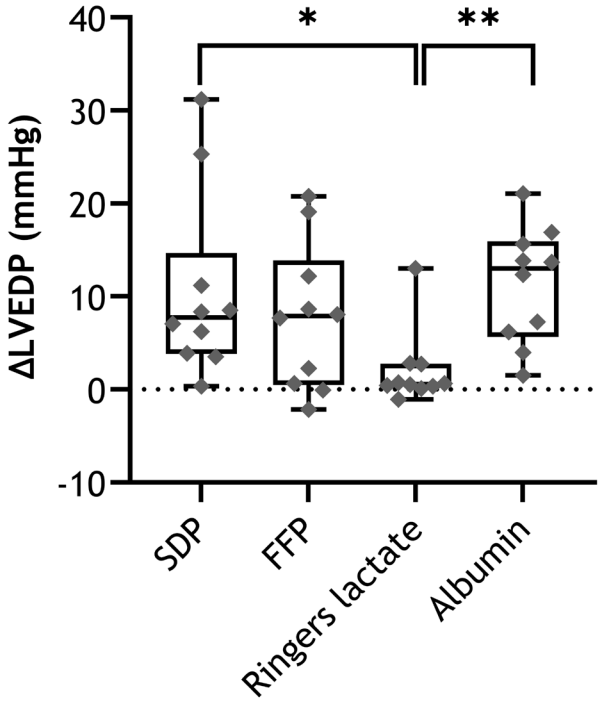

Figure 2 - ΔLVEDP is calculated as post-transfusion LVEDP – pre-transfusion LVEDP
SDP: solvent-detergent pooled plasma; FFP: fresh frozen plasma; *p: 0.007; ** p: 0.0005.

pre-transfusion level in groups receiving SDP and Ringer's lactate. LVEDP remained slightly increased (2.2 mmHg, 0.2; 3.2) in animals receiving albumin and decreased (–2.6 mmHg, –4.8; –1.9) in animals receiving FFP. ΔLVEDP levels for all follow-up timepoints are available in the *Online Supplementary Content*, **Table SI**.

Mean arterial pressure increases after transfusion with SDP or albumin

Four animals in the FFP group died after transfusion. Hemodynamic measurements from these animals were used for analysis up until post-transfusion measurement. In this experiment MAP increased after transfusion in animals receiving SDP compared to Ringer's lactate (increase of 48 *vs* 26 mmHg). MAP also increased in the albumin group and less so in the FFP group (increase of 54 and 36 mmHg respectively) (**Table III**). ΔMAP was significantly different when all four groups were compared (Kruskal-Wallis p=0.007). There were statistical significant increases in MAP after transfusion with SDP or albumin compared to Ringer's lactate (p=0.005 for both comparisons) Data on hemodynamics for all measurements is available in the *Online Supplementary Content*, **Table SI**.

Pulmonary secondary outcomes did not differ between groups

In this model a higher LVEDP did not lead to an increase in pulmonary edema. The median lung wet/dry weight ratio was 4.59 (4.41-4.75) in the group transfused with SDP compared to 4.55 (4.45-4.59) in animals that received Ringer's lactate. Albumin infusion did also not lead to a significantly increased lung wet/dry weight ratio (4.62; 4.44-4.80). There was also no significant difference in histopathological or PaO_2/FiO_2 ratio scoring at termination (**Figure 3**). Moreover the threshold for acute respiratory distress syndrome (PaO_2/FiO_2 <300 mmHg) was not reached in any of the groups[19]. Scores for individual groups are available in the *Online Supplementary Content*, **Table SII**. For these secondary pulmonary markers 4 animals in the FFP group were excluded, because of mortality during follow-up. In the Ringer's lactate group one animal had missing measurements for lung wet/dry weight ratio and pulmonary histological score, due to a lab error. There was one animal in the albumin group and three in the SDP group with missing data on PaO_2/FiO_2 ratio.

Table III - *Peri-transfusion hemodynamic differences*

Δ post-transfusion	SDP	FFP	Ringer's lactate	Albumin	p-value
Heart rate (bpm)	32 (11; 63)	10 (3; 33)	12 (−5; 19)	8 (−19; 30)	0.25
MAP (mmHg)	48 (32; 62)	36 (34; 38)	26 (18; 30)	54 (35; 64)	0.007
CVP (mmHg)	−0.1 (-1.2; 1.2)	0.8 (0.2; 0.9)	0.2 (−0.1; 0.7)	0.8 (0.1; 1.3)	0.40
LVEDP (mmHg)	7.7 (4.5; 10.5)	7.9 (1.1; 11.3)	0.6 (0.4; 2.2)	13.0 (6.5; 15.2)	0.011
Cardiac output (mL/min)	3.7 (−0.7; 6.1)	2.3 (−2.6; 14.1)	1.1 (−3.8; 7.0)	0.4 (−1.8; 5.2)	0.80

bpm: beats per minute; MAP: mean arterial pressure; CVP: central venous pressure; LVEDP: left ventricular end diastolic pressure; SDP: solvent-detergent pooled plasma; FFP: fresh frozen plasma.

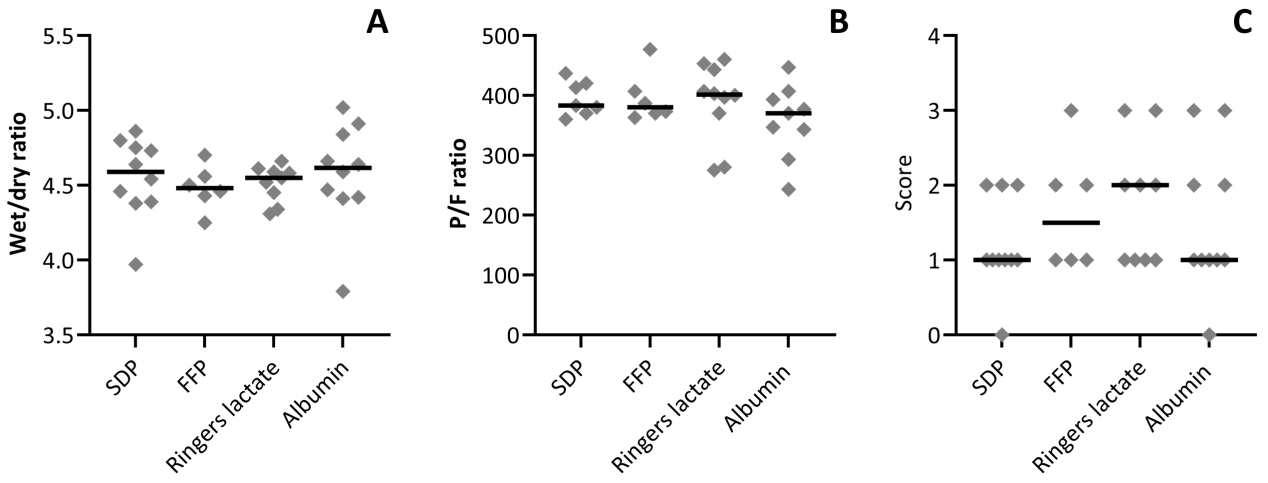

Figure 3 - Pulmonary outcomes Individual values are displayed, median values for each group are indicated with black lines (A=pulmonary wet/dry weight ratio, B=paO$_2$/FiO$_2$(P/F) ratio before termination of the experiment, C=histopathological score)
SDP: solvent-detergent pooled plasma FFP: fresh frozen plasma.

DISCUSSION

This study evaluates the effect of plasma transfusion on pulmonary capillary pressure in a validated two-hit TACO animal model. In some observational studies plasma transfusion carried a higher risk for TACO development than transfusion of other blood products[9,10]. In this randomized animal study we confirmed that plasma transfusion can lead to an increased pulmonary capillary pressure. Our results show that ΔLVEDP increased significantly after transfusion with SDP or albumin compared to infusion with Ringer's lactate. An increased mean arterial pressure, which is a hemodynamic change supporting the diagnosis of TACO, was observed in randomization groups that received SDP or albumin[1]. There were no significant differences in pulmonary secondary outcomes, so there was no increase in pulmonary edema or lung injury after plasma transfusion in this animal model.

Both plasma transfusion (SDP and FFP) and albumin infusion led to a ΔLVEDP above 4mmHg. This increase was defined as the threshold for a clinically relevant difference based on previous research[20]. There were no significant differences when effects from SDP, FFP and albumin on ΔLVEDP were compared. This observation is supported by a meta-analysis showing comparable risk differences for developing TACO after transfusion with different plasma products[21]. The current study included two control groups, the Ringer's lactate group only controlled for administered volume, the other received 5% albumin and thereby controlled for both volume and colloid osmotic pressure. When these groups were compared, there was a significant difference in ΔLVEDP. These results indicate that colloid osmotic pressure may be an important component in rising pulmonary capillary pressure after plasma transfusion. This is in line with previous observations that albumin administration can lead to circulatory overload and pulmonary edema[22].

We hypothesize that increased intravascular colloid osmotic pressure after plasma transfusion could aid in retaining intravascular volume and thereby increase hydrostatic pressure in the pulmonary capillaries. Recruitment of fluid from the extravascular space is less likely. The classical Starling model describes the net flow over the endothelium as a result of intravascular pressure, interstitial pressure and colloid osmotic pressure[23]. When it is revised to account for the endothelial glycocalyx, there is no fluid absorption from the extravascular space by colloid osmotic pressure gradients[24]. Moreover it has been shown that most plasma and albumin products have (sub)-physiological colloid osmotic pressure levels and are therefore unable to increase the intravascular colloid osmotic pressure above its normal level[25].

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This study has a number of limitations. It focuses primarily on hydrostatic pulmonary capillary pressure, which is a central feature in the TACO pathogenesis. However, other mechanisms may also be at play in TACO's pathophysiology[5,8]. Previous studies indicate that for example inflammatory processes may also contribute to the pulmonary edema formation that results in TACO[26,27]. Our model is capable of showing increased pulmonary capillary pressure, but does not display pulmonary edema as a consequence of this rising pressure. This model displays a first phase of TACO where the pressure in the lung vasculature is already increased, but pulmonary edema is not yet present. Because of this lack of pulmonary edema, it is not possible to research other possible pathophysiological pathways and their effect on edema formation.

Our experiments used a two-hit acute heart failure model, caused by ligation of the left anterior descending artery. This is different for e.g., chronic heart and kidney failure that usually precede TACO[7,9]. Another aspect in our study that differs from clinical practice is that the transfusion products used are from human origin and therefore from another species than the recipient. We chose human products, because it enabled us to study the effects of SDP and compare these to FFP. Furthermore, this human plasma is not a cell containing product and has been used in previous animal studies[28,29].

In the FFP group the ΔLVEDP resembled that in the SDP group. However, the ΔLVEDP did not differ significantly compared to Ringer's lactate, due to a large spread in the FFP group, caused by variability in ΔLVEDP between animals in this groups. There was also a 40% mortality rate in the FFP group, which was not seen in the other experimental groups. Variability in ΔLVEDP persisted when the animals that died were removed from the analysis, indicating that this phenomenon was also present in surviving animals. There were no changes in markers for lung injury in these animals. These effects are most likely due to the FFP product. A cross-species reaction caused by antibodies in a product of human origin cannot be excluded as an explanation for these observations. However, human FFP products have been successfully used in previous experiments with rats and mice[28,29]. Another cause could be that there are donor-dependent variances in this FFP unit that contribute to the observed reaction. This effect was only seen in the FFP group and not in the SDP group. This can be a result of the production process of SDP, where pooling of the plasma leads to removal of individual differences between units, dilution and reduction of possible virus loads and neutralization of present antibodies that might cause a cross-species reaction[30].

In our experimental study we proved the capability of plasma to induce circulatory overload. The clinical implication of this study is that plasma transfusion is not without risk and should only be performed if alternative products like specific coagulation factor concentrates are not sufficient. Future studies should focus on further unravelling the pathophysiology of TACO. An animal model with a phenotype of respiratory distress would be a more refined TACO model and could aid to study other processes, like inflammation, possibly contributing to TACO development. Furthermore, studies are necessary to provide evidence-based preventative and treatment strategies for TACO and specifically for TACO after plasma transfusion. Alternatives for plasma transfusion that are investigated or already available including prothrombin complex concentrates and low-volume lyophilized plasma could be examined for their potential to prevent a rising pulmonary capillary pressure and therefore prevent TACO[31-33].

CONCLUSIONS

In this two-hit animal model in rats the first hit consisted of a myocardial infarct and the second hit of a transfusion of a plasma product or control substance. In this model circulatory overload was induced after transfusion of SDP. The same effect was seen in the colloid control group receiving albumin, but not in the Ringer's lactate group. These results show that the effect of plasma transfusion on pulmonary capillary pressure differs from fluid overload induced by crystalloid infusion. Colloid osmotic pressure might be an important factor for the development of TACO.

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AUTHORSHIP CONTRIBUTIONS

EB, RK and AV designed the experiment. EB, RK and MW executed the experiments, collected the data and performed the data analysis. JR performed the pathological assessment. EB, RK and AV wrote the manuscript. All Authors critically evaluated the manuscript.

DISCLOSURE OF CONFLICTS OF INTEREST

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