Random allogeneic blood transfusion in pigs: characterisation of a novel experimental model

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Abstract:

Background: Organ cross-talk describes interactions between a primary affected organ and a secondarily injured remote organ, particularly in lung-brain interactions. A common theory is the systemic distribution of inflammatory mediators that are released by the affected organ and transferred through the bloodstream. The present study characterises the baseline immunogenic effects of a novel experimental model of random allogeneic blood transfusion in pigs designed to analyse the role of the bloodstream in organ cross-talk.

Methods: After approval of the State and Institutional Animal Care Committee 20 anesthetized pig were randomized in a donor and an acceptor (each n=8): the acceptor animals each received high-volume whole blood transfusion from the donor (35-40 ml kg^{-1}). Four animals received balanced electrolyte solution instead of blood transfusion (control group; n=4). Afterwards the animals underwent extended cardiorespiratory monitoring for eight hours. Post mortem assessment included pulmonary, cerebral and systemic mediators of early inflammatory response (IL-6, TNF-alpha, iNOS), wet to dry ratio, and lung histology.

Results: No adverse events or incompatibilities occurred during the blood transfusion procedures. No hints for systemic inflammatory response were found and the pulmonary function was not affected through blood transfusion. Lung histopathology scoring did not display relevant intergroup differences. Neither within the lung nor within the brain an up-regulation of inflammatory mediators was detected. High volume random allogeneic blood transfusion in pigs neither impaired pulmonary integrity nor induced systemic, lung, or brain inflammatory response.

Conclusion: This approach can represent a novel experimental model to characterize the blood-bound transmission in remote organ injury.

1   **Random allogeneic blood transfusion in pigs: characterisation of a novel**

2   **experimental model**

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27   secondarily injured remote organ, particularly in lung-brain interactions. A common theory is the

28   systemic distribution of inflammatory mediators that are released by the affected organ and

29   transferred through the bloodstream. The present study characterises the baseline immunogenic

30   effects of a novel experimental model of random allogeneic blood transfusion in pigs designed to

31   analyse the role of the bloodstream in organ cross-talk.

32   **Methods:** After approval of the State and Institutional Animal Care Committee 20 anesthetized

33   pigs were randomized in a donor and an acceptor group (each n=8): the acceptor animals each

34   received high-volume whole blood transfusion from the donors (35-40 ml kg^{-1}). Four animals

35   received balanced electrolyte solution instead of blood transfusion (control group; n=4).

36   Afterwards the animals underwent extended cardiorespiratory monitoring for eight hours. Post

37   mortem assessment included pulmonary, cerebral and systemic mediators of early inflammatory

38   response (IL-6, TNF-alpha, iNOS), wet to dry ratio, and lung histology. **Results:** No adverse

39   events or incompatibilities occurred during the blood transfusion procedures. Systemic cytokine

40   levels and pulmonary function were unaffected. Lung histopathology scoring did not display

41   relevant intergroup differences. Neither within the lung nor within the brain an up-regulation of

42   inflammatory mediators was detected. High volume random allogeneic blood transfusion in pigs

43   neither impaired pulmonary integrity nor induced systemic, lung, or brain inflammatory response.

44   **Conclusion:** This approach can represent a novel experimental model to characterize the blood-

45   bound transmission in remote organ injury.

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49 **Introduction:**

50 Single organ injury or failure does not exert an isolated effect on the primary affected organ

51 system. Remote organs consecutively develop secondary injuries that are not connected to the

52 initial insult. This is referred to as deleterious organ cross-talk and described particularly for lung

53 and brain interactions, where this pathomechanism is well known, but not fully understood. E.g.

54 mechanical ventilation in the intensive care unit may cause inflammatory response and induce

55 damage to distant organs. On the other hand, severe brain injury is associated with an increased

56 risk of lung failure (Mascia et al. 2007; Pelosi & Rocco 2011).

57 Different theories for this phenomenon consider neuronal response, hormone release or circulating

58 inflammatory mediators as causative (Han 2017; Mrozek et al. 2015; Pelosi & Rocco 2011). The

59 hypothesis of inflammatory mediator distribution is promising as several studies show

60 inflammatory response after a primary hit. The blood stream seems to be the distributor in remote

61 organ injury and cross-talk (Hegeman et al. 2009; Klein et al. 2016; Mrozek et al. 2015). In theory,

62 inflammatory cytokines that are released into the systemic circulation induce remote organ injury.

63 Hence, for experimental purposes this effect might be transferable from one individual to another

64 via whole blood transfusion. Porcine models, in this context, offer advantages in experimental

65 assessment of blood stream-related distribution of inflammatory mediator transmission. Firstly,

66 higher blood volumes can be transferred in comparison to common laboratory rodent species.

67 Secondly, because major inter-individual blood incompatibilities are missing (Smith et al. 2006).

68 Only few reports reveal bronchospasm, disseminated intravascular coagulation, bleeding, or

69 progressive hypotension during blood transfusion in pigs (Hunfeld et al. 1984; Sheil et al. 1972;

70   Smith et al. 2006). This porcine system is based on blood groups A0, whereas the human group B

71   is missing. Additional 16 different antigens are known to form the subgroups A-P (Smith et al.

72   2006). Unlike the human Rhesus antigen, which is highly immunogenic and prone for transfusion

73   reactions or hemolysis, the porcine Rhesus antigen is coded by a single gen without any

74   polymorphisms and less pathologic relevance (Avent & Reid 2000; Omi et al. 2003). However,

75   the distinct effects of porcine blood transfusion with focus on end-organ inflammatory response

76   are barely examined. In order to further elucidate the role of the blood stream as distributor in

77   remote organ injury the present study aims to characterize the baseline immunogenic effects of

78   random allogeneic blood transfusion in pigs. The approach may allow for experimental separation

79   of blood-bound effects on organ integrity from an already injured subject: hence, we hypothesized

80   that single, large-volume blood transfusion from a healthy donor neither exhibits inflammatory

81   response nor impairs the pulmonary function in the acceptor. As secondary hypotheses, the

82   systemic and cerebral inflammatory response is analyzed.

83

84   **Materials & Methods**

85   The prospective-randomized animal study was approved by the State and Institutional Animal

86   Care Committee (Landesuntersuchungsamt Rheinland-Pfalz, Koblenz, Germany; approval

87   number: 23 177-07/G 14-1-084). The study was conducted in accordance with the ARRIVE

88   guidelines and the international guidelines for the care and use of laboratory animals. The

89   experiments were performed from 12/2014 till 3/2015. Twenty juvenile German Landrace pigs

90   (*sus scrofa domestica*, weight 28 ± 2 kg) were examined. Eight animals served as blood donors.

91   Eight animals received allogenic blood transfusion. The control group consisted of four animals

92   that received the same amount of balanced electrolyte solution instead of blood transfusion. One

93   acceptor animal was excluded due to complicated airway management and multiple intubation

94   attempts with subsequent respiratory failure before the transfusion. Per protocol and approval, the

95   donor animals that inevitably suffered from hemorrhagic shock were subsequently included in

96   another study protocol focusing on fluid resuscitation. All experiments were conducted under

97   continuous general anesthesia. This manuscript adheres to the applicable EQUATOR guidelines.

98

99   **Anesthesia and instrumentation**

100   Anesthesia and instrumentation procedure was previously described in detail in our former studies

101   (Ziebart et al. 2015; Ziebart et al. 2018): Briefly, in each case two animals received an

102   intramuscular injection of ketamine (8mg kg^{-1}) and midazolam (0.2 mg kg^{-1}) followed by induction

103   of general anesthesia via an ear vein cannula (propofol 4 mg kg^{-1}, and fentanyl 4 µg kg^{-1},

104   atracurium 0.5 mg kg^{-1}), which was maintained by continuous infusion (propofol 8-12 mg kg^{-1} h^{-1}

105   ^{1}, fentanyl 0.1-0.2 mg h^{-1}). After orotracheal intubation the animals were ventilated in volume-

106   controlled mode (VCV; AVEA, CareFusion, USA): positive end-expiratory pressure (PEEP) 5

107   cmH_2O, tidal volume 7 ml kg^{-1}, inspiration to expiration ratio 1:2, fraction of inspired oxygen

108   (FiO_2) 0.4 and variable respiration rate to achieve an end-tidal CO_2 < 45 mmHg. In case of

109   respiratory insufficiency, mechanical ventilation parameters escalated according to an adaption of

110   the ARDS network tables. (Ziebart et al. 2014) Afterwards, the animals were randomized into an

111   acceptor and a donor animal. Ultrasound guided femoral vascular access under sterile conditions

112   included a central venous line, a pulse contour cardiac output catheter (PiCCO, Pulsion Medical,

113   Germany), and a pulmonary arterial catheter in the acceptor animals. The donor animals received

114   a central venous catheter, an arterial line, a pulse contour cardiac output system (PiCCO, Pulsion

115   Medical Systems, Germany) and a large-bore venous introducer. Extended hemodynamics,

116 transpulmonary thermodilution-derived parameters, spirometry, and gas exchange were

117 continuously monitored (Datex S/5 Monitor, GE Healthcare, Germany; PiCCO, Pulsion Medical

118 Systems, Germany). Hematological and blood gas measurements were repetitively conducted.

119 Electrical impedance tomography (EIT; Goe-MF II, CareFusion, Germany) was used to measure

120 variations of the thoracic bioimpedance that are associated with pulmonary aeration. Sixteen

121 adhesive electrodes were placed around the thorax and the ventilation distribution was measured

122 for three lung compartments (Level 1-3; non-dependent, central, dependent) (Bodenstein et al.

123 2012; Ziebart et al. 2014). Multiple breath-nitrogen washout/washin technique was applied to

124 measure the functional residual capacity (Kamuf et al. 2017). Body temperature was measured by

125 a rectal probe and a surface warming device prevented hypothermia. All animals received a

126 background infusion of 5 ml kg^{-1} h^{-1} balanced electrolyte solution (Sterofundin, B.Braun,

127 Germany).

128 After anesthesia, instrumentation, and 20-minute recovery period baseline values were measured

129 and recorded.

130

131 **Blood withdrawal procedure**

132 From the donor animals 35-40 ml kg^{-1} blood was taken for allogenic blood transfusion through the

133 arterial catheter. The whole blood was collected in specific collector bags system (Composelect,

134 Fresenius-Kabi AG, Homburg Germany). These bags comprised 63 ml CPD/100 ml SAG-M -

135 RCC (Citrate phosphate dextrose, Erythrocyte storage in hypertonic conservation medium). After

136 collection in a primary bag. The blood was drained through a leucocyte-depleting filter into a

137 second bag that contains the anticoagulants. Finally, the whole blood was collected into a bag with

138 a connector for a transfusion system. Afterwards the whole blood was stored by room temperature

139   for about ten minutes before transfusion to avoid negative effects of long-time storing or cooling.

140   Afterwards the donor animals were transferred to the fluid resuscitation protocol that was included

141   in the same approval: they were euthanized by intravenous injection of propofol (200mg) and

142   potassium chloride (40mval) within the scope of this protocol. (Ziebart et al. 2018)

143

144   **Experimental protocol:**

145   The experimental protocol is summarized in figure 1. Immediately after blood withdrawal the

146   whole blood was transfused into the recipients through the venous vascular access sheath over 15

147   minutes through a transfusion system with an integrated aggregate-filter. A control group (n=4)

148   underwent the identical instrumentation and protocol, though received an equal amount of

149   balanced electrolyte solution instead of allogeneic blood transfusion. Afterwards the animals were

150   monitored for eight hours. In case of hemodynamic deterioration (mean arterial pressure < 60

151   mmHg) continuous noradrenaline infusion was administered. After finishing the protocol, the

152   animals were killed in deep general anesthesia by injection of propofol (200 mg) and potassium

153   chloride (40 mval).

154

155   **Post mortem analysis**

156   Repetitively taken serum blood samples were used to determine the serum levels of TNF-alpha

157   and IL-6 by enzyme-linked immunosorbent assays (Porcine Quantikine ELISA Kits, R/D Systems,

158   Germany). After finishing the protocol, the lung and the brain each were removed en-bloc. Four

159   representative tissue samples (dependent periphery, dependent central, non-dependent central,

160   non-dependent periphery) from the right lung were extracted, formalin-fixed, and used for

161   histological evaluation of alveolar damage in the upper and the lower lung. After paraffin

162 embedding and hematoxylin/eosin staining the samples were analyzed in investigator-blinded

163 manner by means of standardized scoring scheme (Ziebart et al. 2015; Ziebart et al. 2014).

164 Additional lung samples were cryopreserved for mRNA expression analysis of inflammatory

165 mediators (IL-6, TNF-alpha, iNOS) using real-time polymerase chain reaction (rt-PCR;

166 Lightcycler 480 PCR System, Roche Applied Science, Germany) as described in detail by

167 Hartmann et al. (Hartmann et al. 2015). The mRNA expression was normalized to peptidylprolyl

168 isomerase A (PPIA). The same technique and markers were used to quantify the cerebral mRNA

169 expression from the right hippocampus and the right frontal cortex. Furthermore, the lung and

170 brain water content were determined through the tissue wet to dry ratio. For this procedure the

171 weight of the removed organs was determined directly and after two days of complete drying.

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173

174

175 **Statistics**

176 All parameters are presented mean and standard deviation (± SD) or displayed as plots. The

177 analysis focusses on the relevant time points baseline, 0h, 4h 8h. Additional data are added as

178 supplemental files. Group effects over time (Transfusion, Control) were compared by two-way

179 analysis of variance (ANOVA) with post-hoc Student-Newman-Keuls-Test. Mann-Whitney-U-

180 Test was used to analyze the post mortem parameters. A p-value lower than 0.05 was accepted as

181 significant. The software package SigmaPlot 12.5 (Systat Software, USA) was used.

182

183 **Results**

184 The estimated total blood volume of the pigs (74 ml kg^{-1}) was 2084±166ml (Lyhs & Wachtel

185 1966). The transfused blood volume was 48±4%. No adverse events or major incompatibilities

186   occurred during the blood transfusion procedures. Beneath a temporary increase of the mean

187   pulmonary arterial pressure following blood transfusion and a slight increase of the central venous

188   pressure the cardiopulmonary monitoring and blood gas analyses showed no relevant differences

189   between the transfusion and control animals over eight hours (table 1, 2 & 3). The lactate level

190   was in a normal range and even decreased within eight hours. Through blood transfusion

191   hemoglobin, hematocrit and erythrocyte counts significantly increased without relevant changes

192   in leucocyte amounts (figure 2). Marker of systemic inflammatory response did not differ between

193   the groups as well (figure 2 & 3). The pulmonary function as measured by blood gas analysis and

194   ventilation distribution was not affected by blood transfusion (table 3 & figure 4). Only the

195   alveolar-arterial oxygen difference increased. At baseline two animal started at a $PaO_2/FiO_2 < 400$

196   mmHg without signs of local or systemic illness. A modification of the initial respiratory setting

197   due to hypoxemia was not required in any animal. Lung histopathology scoring did not display

198   relevant intergroup differences (control vs. transfusion; 10±1 vs. 11±3; each p > 0.05). The tissue

199   wet to dry ratio as surrogate of edema formation between both groups was not increased following

200   transfusion (control vs. transfusion; lung: 4.7±0.2 vs. 4.6±0.2; brain: 3.3±0.3 vs. 3.4±0.3; each p >

201   0.05). Neither within the lung nor within the brain the mRNA expression analysis revealed an up-

202   regulation of inflammatory markers (figure 5 & 6).

203

204   **Discussion**

205   The present study establishes a novel model for characterization of remote organ injury related to

206   the distribution of inflammatory mediators through the systemic circulation. We found that one-

207   time high-volume allogeneic blood transfusion in healthy pigs neither impairs pulmonary integrity

208   nor mediates inflammatory response within the lung, brain, or systemic circulation. Accordingly,

209   random allogeneic blood transfusion in pigs is feasible, and per itself does not trigger inflammatory

210 response in the acceptor animals. Hence, this model could be an appropriate approach to identify

211 the main mediators in injurious organ interactions, i.e. by transfusion of blood from severely brain

212 or lung injured subjects to healthy ones without co-founders. Other experimental protocols with

213 comparable porcine transfusion models investigate questions like acute kidney injury or effects of

214 blood storing and cooling. Several similar but, in some points, different protocols have been

215 described that make use of autologous instead homologous blood, heavier animals to minimize the

216 need for donor animals, or previously induce a hemorrhagic shock to reduce the rate of circulatory

217 overload (Biagini et al. 2017; Masuda et al. 2018; Patel et al. 2011; Wozniak et al. 2018).

218 In principle, blood transfusion can be associated with severe side effects. Beneath

219 incompatibilities, particularly pulmonary complications like transfusion-related acute lung injury

220 (TRALI) or transfusion-associated circulatory overload (TACO) are described. Other systemic

221 side effects include hypotension, anaphylaxis, hemolytic reactions and transfusion-transmitted

222 bacterial infections (Bux & Sachs 2008). Our study reveals a significant increase of mean arterial,

223 pulmonary arterial and central venous pressure immediately following transfusion, which was

224 underlined by increase of hemoglobin and hematocrit. These symptoms comply with subclinical

225 TACO induced by supraphysiological increase of intravascular volume (Roubinian et al. 2017).

226 Transfusion of longer stored but not freshly drawn blood was shown to considerably increase

227 pulmonary arterial pressure blood, which is presumably an effect of mediators released through

228 storage. (Baron et al. 2012; Solomon et al. 2013) In our study, similar but short-termed effects

229 occurred despite blood transfusion immediately after withdrawal. Furthermore, fluid overload,

230 which inevitably also occurs in our study, is also associated with pulmonary arterial hypertension.

231 (Yilmaz et al. 2016) (Wolsk et al. 2019) As we found no coincident pathologies that allow

232   distinguishing between fluid overload and mediator-based effects, identification the exact

233   mechanism is beyond the scope of this study.

234   Relevant impairment of the pulmonary gas exchange or alterations in extravascular lung water

235   content, functional residual capacity or ventilation distribution were not observed after blood

236   transfusion. Over eight hours PaO_2/FiO_2 decreases to a certain degree going along with a raised

237   alveolar-arterial oxygen difference. In the absence of histopathologic lung injury and during low-

238   grade invasive mechanical ventilation, this is most likely a consequence of atelectasis formation

239   that inevitably occurs during general anesthesia, muscular paralysis and mechanical

240   ventilation.(Hedenstierna & Edmark 2015) Two animals had a slightly reduced PaO_2/FiO_2 ratio (<

241   400 mmHg) already during baseline measurements without any signs for pulmonary or systemic

242   illness: these two animals did neither respond differently to the transfusion nor showed signs of

243   increased histopathologic lung damage despite formally exhibiting a $PaO_2/FiO_2 < 300$ mmHg after

244   eight hours. Increase and withdrawal of intravascular fluid volume in pigs was shown to alter the

245   endexpiratory lung volume as measured by EIT, but did not affect tidal volume or ventilation

246   distribution, which is in line with prior results (Bodenstein et al. 2012). TRALI represents another

247   facet that directly affects the lung following blood transfusion. It is characterized by respiratory

248   distress, hypoxemia, and non-cardiogenic lung edema that is induced by leukocyte-antibodies and

249   neutrophil-priming substances (Bux & Sachs 2008). These interact with neutrophil granulocytes,

250   which thereby are activated. The resulting clots impair pulmonary capillary perfusion and induce

251   capillary leakage by releasing toxic enzymes and reactive oxygen species (Sachs et al. 2006; Toy

252   et al. 2005). We did not find any TRALI surrogates such as edema formation or increasing counts

253   of leucocytes or specifically neutrophil granulocytes in our porcine model. Furthermore, the

254   histopathological analysis did not reveal relevant inflammatory cell immigration. Additionally, the

255 regional tidal volume distribution was unaltered, which is described as sign of severe pulmonary

256 illnesses (Chiumello et al. 2013). Gender-specific forms of TRALI are particularly relevant in

257 multiparous women as blood donors (Palfi et al. 2001) (Schmickl et al. 2015). Hence, the exclusive

258 use of male pigs may represent an approach to minimize the TRALI risk. TACO on the other hand

259 tends to appear primarily in patients suffering from serious pre-existing illnesses. In our study

260 healthy animals with intact pulmonary vascular barrier and competent kidney function were used,

261 which enables rapid compensation of the intravascular overload (Bux & Sachs 2008; Roubinian et

262 al. 2017).

263 Single organ injury or failure can subsequently affect the integrity of distant remote organs leading

264 to multiple organ failure. In particular, deleterious interactions of the lung and the brain have been

265 described (Pelosi & Rocco 2011). Several models suggest that the lung may play a pivotal role as

266 causative but also remotely injured organ: in mechanically ventilated rats that underwent an

267 aggressive ventilator regime showed remarkable cerebral inflammatory response (Quilez et al.

268 2011). Cyclic variations of the PaO_2 caused by non-protective ventilation in lung injured pigs are

269 transmitted to the cerebral tissue and may trigger cerebral injury (Klein et al. 2013; Klein et al.

270 2016). Furthermore, also a cerebral insult is capable to induce or aggravate lung injury (Heuer et

271 al. 2011). Even mechanical ventilation by itself can represent a trigger for cerebral cytokine

272 expression. (Kamuf et al. 2018) Accordingly, we also assessed brain inflammatory response

273 measured by cortical and hippocampal mRNA expression of key inflammatory mediators, which

274 also were unaffected by the transfusion procedure. In comparison to human blood the use of

275 porcine model may offer several advantages for studies concerning remote organ injury: Unlike

276 human blood, where transfusion of incompatible blood products is deleterious, pig blood lacks the

277 human blood group B, the secondary absorption of antigens from the plasma to the blood cells and

278 the Rh polymorphism (Omi et al. 2003). Additionally human blood is polygene coded, which

279 results in a much higher incompatibility potential (Smith et al. 2006).

280 The study has several limitations. The study was not designed to assess long-term effects of blood

281 transfusion but to exclude acute side effects of allogeneic blood transfusion itself with regard to a

282 novel experimental approach to elucidate pathomechanisms of deleterious remote organ injury.

283 Logistically it was not possible to investigate different pig subspecies or breeds. Hence,

284 generalization of the reported findings lies beyond the scope of this study. Animal care reasons

285 and the need for additional blood donor animal required a strict limitation of the animal numbers,

286 whereas the chosen group numbers were in line with previous studies inflammatory acute effects

287 in pigs (Hartmann et al. 2015; Ziebart et al. 2014). Exchange of the transfused blood by

288 simultaneous blood withdrawal may be a possibility to avoid fluid overloading. However, given

289 the risk of unpredictable removal of the donor blood, hemodynamic variations, and potential

290 microcirculatory or inflammatory impairment during this complex procedure, we decided to

291 decline this option. Small volume withdrawal prior to the transfusion procedure may represent a

292 reasonable compromise, and may reduce the risk of TACO (Masuda et al. 2018). The use of plasma

293 instead of whole blood is an interesting alternative approach to optimize this setting: theoretically,

294 plasma administration will not increase the hemoglobin content, whereas full concentration of

295 potentially inflammatory mediators should be present. This approach, however, was not feasible

296 for the present study. Additionally, we did not measure cytokine contents in the transfused blood,

297 as the donor animals were healthy and the blood was transfused within minutes. These

298 measurements need to be included in studies focusing on severely injured donor animals.

299

300 **Conclusion**

301   In conclusion, high volume random allogeneic blood transfusion in pigs neither impaired

302   pulmonary integrity nor induced systemic, lung, or brain inflammatory response. No further side

303   effects beyond expectable and temporary alterations of hemodynamics and blood count related to

304   the rapid increase of blood volume were detected. Random allogeneic blood transfusion in pigs

305   may represent an experimental model to characterize the blood-bound transmission in remote

306   organ injury.

307

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310   this study are included in the doctoral thesis of Moritz Schaefer, and content of the professorial

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312   poster sessions at the German Anesthesia Congress (DAC), Nuernberg, Germany in 2017.

313

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430

Figure 1

Figure 1: Experimental flow chart

After preparation and baseline measurement the animals were observed for eight hours. The hemodynamic and spirometric parameters were recorded continuously. Blood samples were taken every two hours. EIT (electrical impedance tomography) was measured hourly. Histopathological analysis of the lung was performed after the end of the experiment. Pulmonary and cerebral mRNA expression was quantified. (Transfusion n=7; Control n=4)
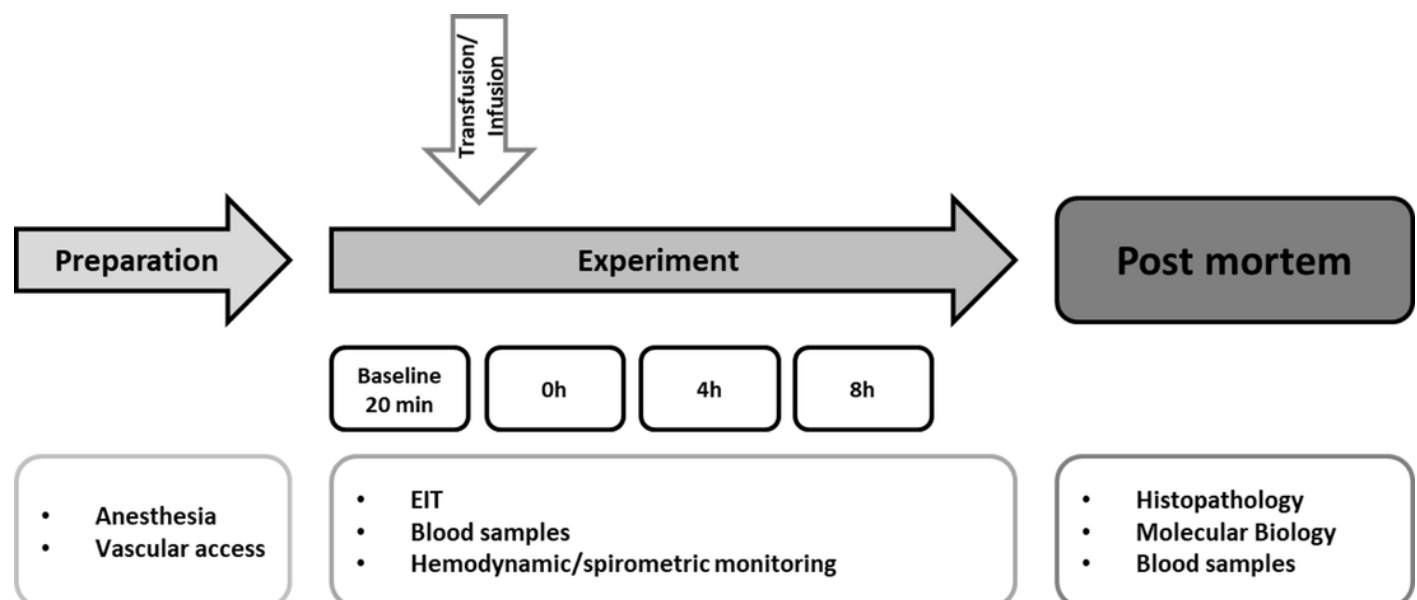
Figure 2

Figure 2: Hematological parameters.

Parameters are displayed as percentage of baseline. A significant increase of hemoglobin, hematocrit and erythrocytes was observed after transfusion. The decrease of these parameters in the control group was not significant; * = p<0.05; vs. baseline. (Transfusion n=7; Control n=4)
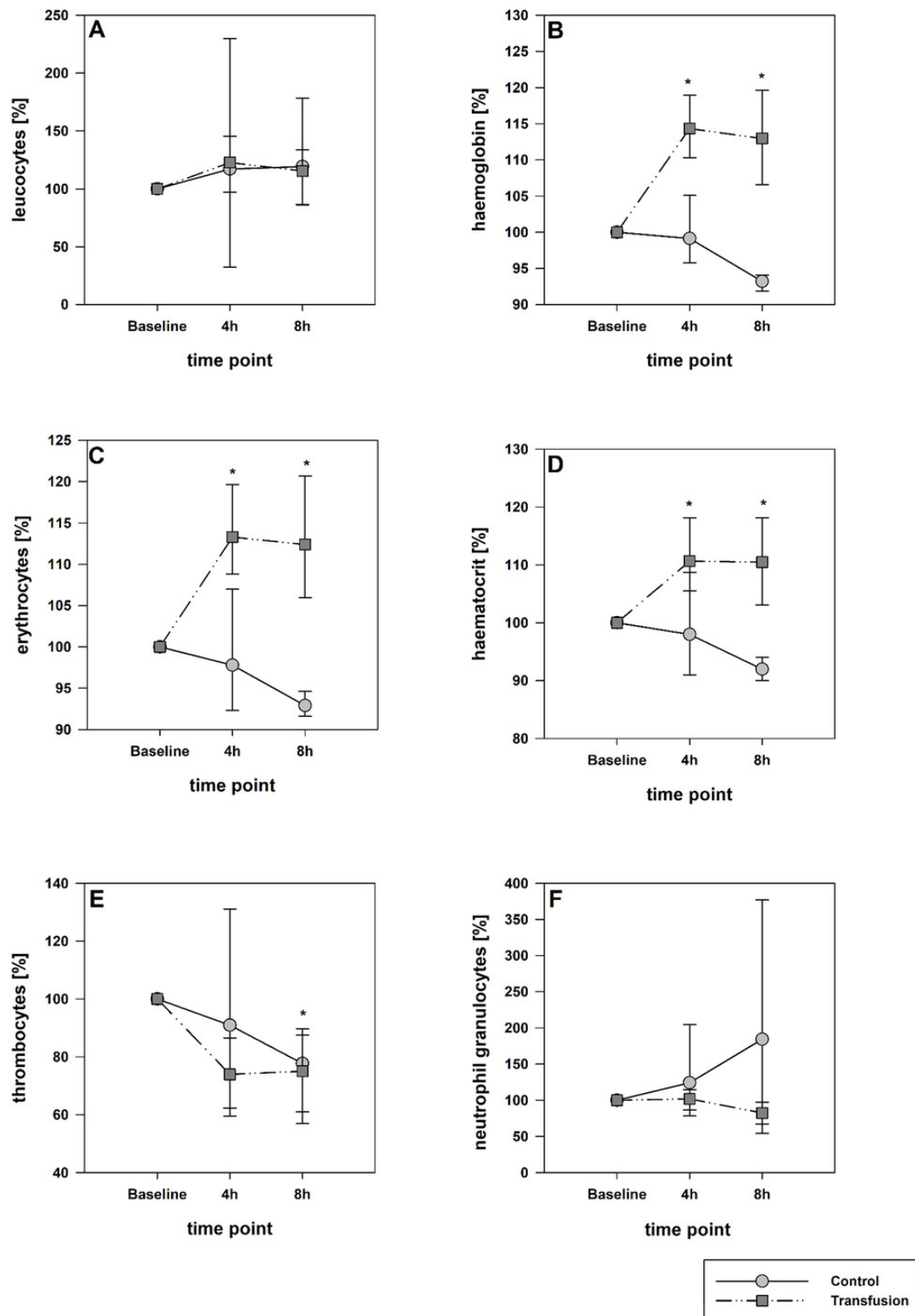
Figure 3

Figure 3: Systemic inflammatory cytokines levels.

Parameters are displayed are presented as pg/mL. The systemic inflammatory cytokines level did not differ between both study groups, but there is a significant decrease of TNF-alpha to baseline in both groups; * = p<0.05; vs. baseline. (Transfusion n=7; Control n=4)
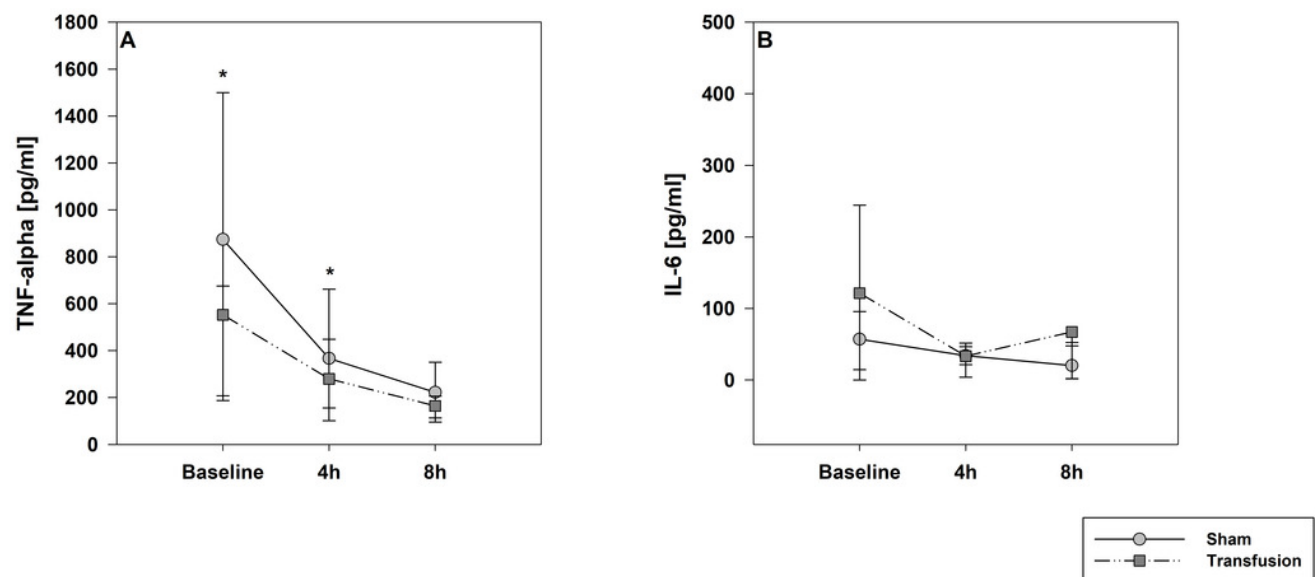
Figure 4

Figure 4: Regional distribution of the tidal volume.

Non-dependent, central and dependent lung areas (% of the global tidal amplitude). No significant differences (each p > 0.05). The regional distribution of the tidal volume did not differ between both study groups. (Transfusion n=7; Control n=4)
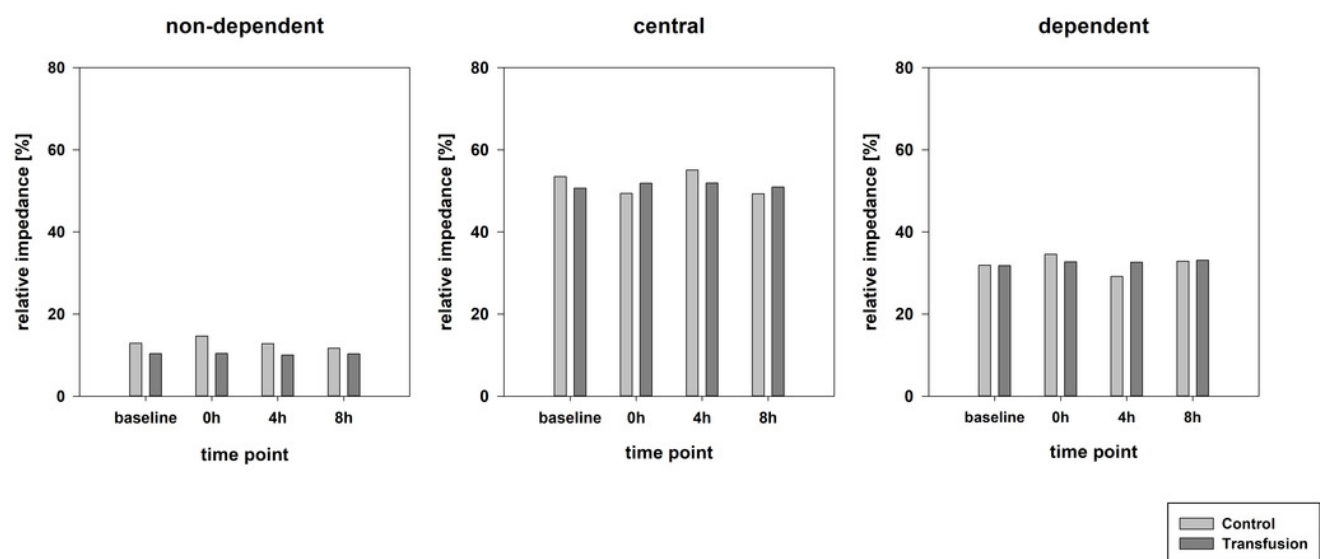
Figure 5

Figure 5: Pulmonary mRNA expression of inflammatory mediators.

Cryopreserved lung samples were collected after the experiment from upper and lower lung sections. mRNA expression of inflammatory parameters (IL-6, TNF-alpha, INOS) were analyzed via real-time polymerase chain reaction. mRNA expression is normalized to peptidylprolyl isomerase A (PPIA); no significant intergroups differences (each $p > 0.05$). (Transfusion n=7; Control n=4).

lower Lung

upper Lung

Control
Transfusion
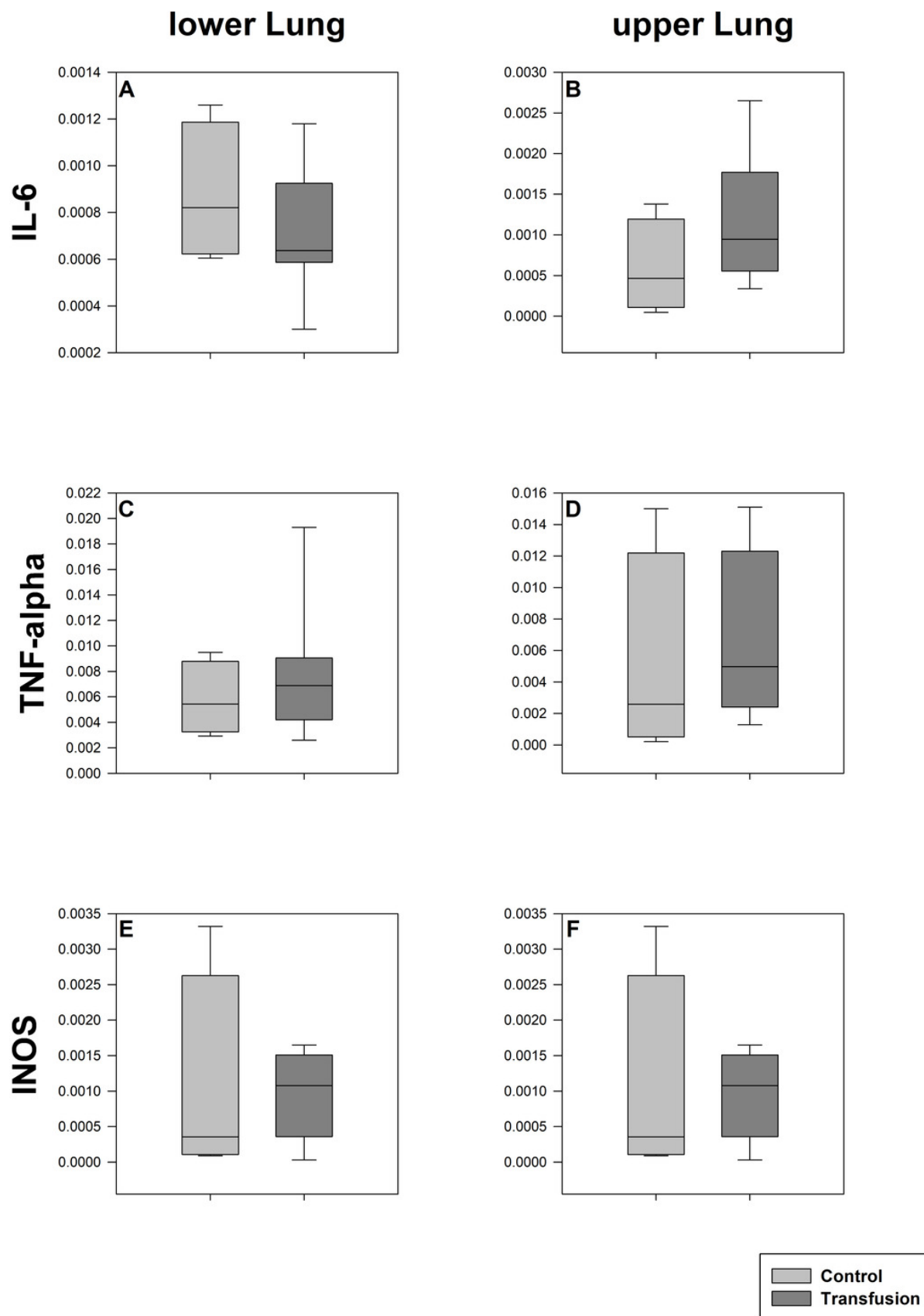
Figure 6

Figure 6: Cerebral mRNA expression of inflammatory mediators.

Cryopreserved cerebral samples were collected after the experiment from cortex and hippocampus regions. mRNA expression of inflammatory parameters (IL-6, TNF-alpha, INOS) were analyzed via real-time polymerase chain reaction. mRNA expression is normalized to peptidylprolyl isomerase A (PPIA); No significant intergroups differences (each $p > 0.05$). (Transfusion n=7; Control n=4).

Cortex

Hippocampus
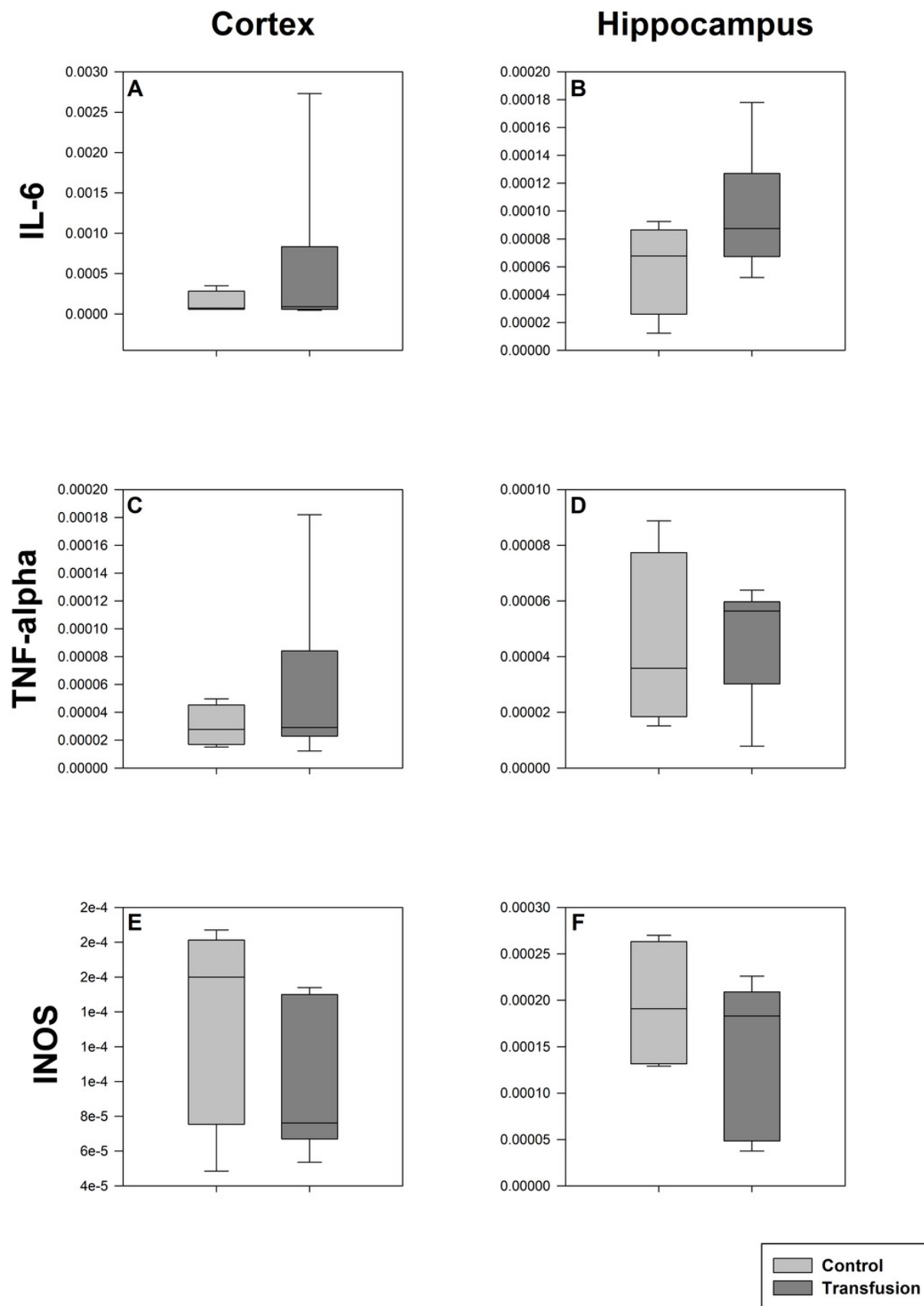
Table 1: Hemodynamic parameters

No significant intra- and intergroup changes of hemodynamic parameters were observed after blood transfusion or fluid infusion, with the exception of a temporary pulmonary arterial pressure increase and raise of the central venous and mean arterial pressure after transfusion / fluid administartion. (Transfusion n=7; Control n=4)

Parameter		BLH MEAN (SD)	0h MEAN (SD)	4h MEAN (SD)	8h MEAN (SD)
MAP [mmHg]	Transfusion	70 (13)	98* (14)	82 (9)	81 (14)
	Control	77 (12)	93* (3)	73 (7)	71 (5)
HR [min^{-1}]	Transfusion	83 (19)	83 (13)	84 (20)	85 (13)
	Control	78 (9)	78 (10)	80 (7)	73 (6)
PAP [mmHg]	Transfusion	18 (5)	27 (6)*#	20 (2)	19 (3)
	Control	16 (4)	19 (3)	17 (3)	12 (7)
P_{es} [mmHg]	Transfusion	5 (1)	7 (2)	4 (1)	3 (2)
	Control	6 (0)	6 (1)	4 (1)	4 (0)
CO [l min^{-1}]	Transfusion	3.6 (0.7)	3.7 (0.7)	3.2 (0.3)	3.2 (0.6)
	Control	3.4 (0.3)	4.3 (0.9)	3.4 (0.3)	2.9 (0.4)
GEDI [ml kg^{-1}]	Transfusion	820 (125)	850 (96)	778 (107)	812 (81)
	Control	820 (97)	916 (20)	808 (62)	803 (75)
EVLWI [ml kg^{-1}]	Transfusion	11 (2)	12 (2)	12 (2)	12 (3)
	Control	13 (5)	11 (0)	15 (3)	13 (2)
CVP [mmHg]	Transfusion	5 (2)	9 (2)*	7 (2)*	7 (2)*
	Control	7 (1)	8 (2)	7 (1)	7 (1)
S_pO_2 [%]	Transfusion	100 (0)	100 (0)	99 (1)	99 (1)
	Control	100 (0)	100 (0)	100 (0)	100 (0)

*indicates $p<0.05$ vs. Baseline value. # indicates $p< 0.05$ in intergroup comparison

MAP: mean arterial pressure; HR: heart rate;
PAP: mean arterial pulmonary pressure; P_{es}: esophageal pressure;
CO: cardiac output; GEDI: global enddiastolic volume index;
EVLWI: extravascular lung water index; CVP: central venous pressure;
S_pO_2: oxygen saturation

Table 2: Spirometric parameters

No significant intra- and intergroup changes of spirometric parameters were observed after blood transfusion or fluid infusion. (Transfusion n=7; Control n=4)

Parameter		BLH MEAN (SD)	0h MEAN (SD)	4h MEAN (SD)	8h MEAN (SD)
F_iO_2 [%]	Transfusion	39 (0)	39 (0)	39 (0)	39 (0)
	Control	39 (0)	39 (0)	39 (0)	39 (0)
MV [l/min]	Transfusion	6.2 (0.7)	6.1 (0.8)	6.2 (1)	6.6 (1.3)
	Control	6.4 (1.7)	5.8 (1.3)	6.5 (1.6)	7 (1)
TV [ml kg^{-1}]	Transfusion	8 (0)	8 (0)	8 (0)	8 (0)
	Control	8 (1)	8 (1)	8 (1)	8 (1)
P_{peak} [cm H_2O]	Transfusion	17 (2)	18 (2)	20 (2)	20 (2)
	Control	16 (2)	17 (1)	17 (3)	19 (3)
P_{mean} [cm H_2O]	Transfusion	9 (1)	9 (0)	10 (1)	10 (1)
	Control	9 (1)	8 (0)	9 (1)	10 (1)
PEEP [cm H_2O]	Transfusion	4 (0)	4 (0)	4 (0)	4 (0)
	Control	4 (0)	4 (0)	4 (0)	4 (0)
C_p [ml/cmH_2O]	Transfusion	19 (3)	17 (2)	16 (2)	16 (3)
	Control	20 (3)	20 (2)	19 (3)	17 (3)
R_{AW} [kPA/l/sec]	Transfusion	10 (1)	11 (1)	12 (1)	12 (1)
	Control	11 (1)	11 (1)	11 (1)	11 (2)
FRC [ml]	Transfusion	728 (206)	691 (260)	691 (240)	714 (235)
	Control	823 (369)	610 (87)	674 (77)	788 (351)

*indicates p<0.05 vs. Baseline value. # indicates p< 0.05 in intergroup comparison

F_iO_2: fraction of inspired oxygen; MV: minute volume; TV: tidal volume; Ppeak: peak inspiratory pressure; Pmean: mean airway pressure; PEEP: positive end-expiratory pressure C_p: pulmonary compliance; R_{AW}: airway resistance; FRC: functional residual capacity

Table 3: Blood gas parameter

No relevant intra- and intergroup changes of blood gas parameters were observed after blood transfusion or fluid infusion. Only the lactate levels decrease within the normsl range and the $AaDO_2$ increases in both groups. (Transfusion n=7; Control n=4)

Parameter		BLH	0h	4h	8h
		MEAN (SD)	MEAN (SD)	MEAN (SD)	MEAN (SD)
S_vO_2 [%]	Transfusion	60 (12)	66 (8)	58 (6)	56 (6)
	Control	63 (13)	64 (0)	63 (11)	53 (8)
pH	Transfusion	7.47 (0.028)	7.505 (0.027)	7.503 (0.017)	7.495 (0.019)
	Control	7.485 (0.02)	7.5 (0.033)	7.545 (0.054)	7.506 (0.069)
BE [mmol/ml]	Transfusion	4.5 (4)	7.3 (1.4)	8.5 (2.2)	6.6 (3.6)
	Control	5.9 (4.5)	6.1 (0)	8.9 (2.4)	6.4 (3.4)
$_{art}CO_2$ [mmHg]	Transfusion	40 (4)	40 (4)	41 (3)	40 (5)
	Control	39 (5)	41 (4)	38 (6)	38 (4)
$_{et}CO_2$ [mmHg]	Transfusion	37 (3)	38 (3)	40 (1)	39 (3)
	Control	35 (5)	35 (4)	34 (5)	35 (4)
$avCO_2$ diff [mmHg]	Transfusion	9 (3)	8 (4)	8 (2)	5 (6)
	Control	10 (5)	-8 (21)	7 (1)	8 (1)
PaO_2 [mmHg]	Transfusion	185 (34)	181 (23)	154 (33)	141 (26)
	Control	206 (43)	230 (49)	186 (44)	171 (24)
PaO_2/F_iO_2 [mmHg]	Transfusion	464 (86)	452 (58)	386 (81)	352 (64)
	Control	514 (108)	576 (122)	464 (109)	428 (60)
$AaDO_2$ [mmHg]	Transfusion	53 (30)	58 (22)	82 (32)*	97 (27)*
	Control	33 (44)	7 (46)	55 (45)	69 (27)*
Temp. [°C]	Transfusion	37.1 (0.8)	36.9 (1.2)	38 (0.8)	38.5 (0.4)
	Control	35.8 (2.9)	35.4 (3.9)	37.6 (2.2)	38.3 (0.6)
Potassium [mmol/l]	Transfusion	4 (0.3)	3.6 (0.7)	4.2 (0.7)	3.9 (0.6)
	Control	4 (0.6)	4 (0.1)	4.5 (0.3)	4.2 (0.2)
Lactate [mmol/l]	Transfusion	1.4 (0.5)	1.1 (0.4)	0.7 (0.2)*	0.6 (0.2)*
	Control	1.4 (0.4)	1.3 (0.1)	0.9 (0.2)*	0.8 (0.3)*