Lung–brain 'cross-talk': Systemic propagation of cytokines in the ARDS via the bloodstream using a blood transfusion model does not influence cerebral inflammatory response in pigs

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Background: Interorgan cross-talk describes the phenomenon in which a primarily injured organ causes secondary damage to a distant organ. This cross-talk is well known between the lung and brain. One theory suggests that the release and systemic distribution of cytokines via the bloodstream from the primarily affected organ sets in motion proinflammatory cascades in distant organs. In this study, we analysed the role of the systemic distribution of cytokines via the bloodstream in a porcine ARDS model for organ cross-talk and possible inflammatory changes in the brain.

Methods: After approval of the State and Institutional Animal Care Committee, ARDS induction with oleic acid injection was performed in seven animals. Eight hours after ARDS induction, blood (35–40 ml kg-1) was taken from these seven 'ARDS donor' pigs. The collected 'ARDS donor' blood was transfused into seven healthy 'ARDS-recipient' pigs. Three animals served as a control group, and blood from these animals was transfused into three healthy pigs after an appropriate ventilation period. All animals were monitored for eight hours using advanced cardiorespiratory monitoring. Postmortem assessment included cerebral (hippocampal and cortex) mediators of early inflammatory response (IL-6, TNF-alpha, iNOS, sLCN-2), wet-to-dry ratio and lung histology. TNF-alpha serum concentration was measured in all groups.

Results: ARDS was successfully induced in the 'ARDS donor' group, and serum TNF-alpha levels were elevated compared with the 'ARDS-recipient' group. In the 'ARDS-recipient' group, neither significant ARDS alterations nor upregulation of inflammatory mediators in the brain tissue were detected after high-volume random allogenic 'ARDS-blood' transfusion. The role of the systemic distribution of inflammatory cytokines from one affected organ to another could not be confirmed in this study.

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29 Abstract

30 **Background:**

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32 secondary damage to a distant organ. This cross-talk is well known between the lung and brain.

33   One theory suggests that the release and systemic distribution of cytokines via the bloodstream

34   from the primarily affected organ sets in motion proinflammatory cascades in distant organs. In

35   this study, we analysed the role of the systemic distribution of cytokines via the bloodstream in a

36   porcine acute respiratory distress syndrome (ARDS) model for organ cross-talk and possible

37   inflammatory changes in the brain.

38   **Methods:**

39   After approval of the State and Institutional Animal Care Committee, ARDS induction with oleic

40   acid injection was performed in seven animals. Eight hours after ARDS induction, blood (35–40

41   ml kg^{-1}) was taken from these seven 'ARDS donor' pigs. The collected 'ARDS donor' blood was

42   transfused into seven healthy 'ARDS-recipient' pigs. Three animals served as a control group,

43   and blood from these animals was transfused into three healthy pigs after an appropriate

44   ventilation period. All animals were monitored for eight hours using advanced cardiorespiratory

45   monitoring. Postmortem assessment included cerebral (hippocampal and cortex) mediators of

46   early inflammatory response (IL-6, TNF-alpha, iNOS, sLCN-2), wet-to-dry ratio and lung

47   histology. TNF-alpha serum concentration was measured in all groups.

48   **Results:**

49   ARDS was successfully induced in the 'ARDS donor' group, and serum TNF-alpha levels were

50   elevated compared with the 'ARDS-recipient' group. In the 'ARDS-recipient' group, neither

51   significant ARDS alterations nor upregulation of inflammatory mediators in the brain tissue were

52   detected after high-volume random allogenic 'ARDS-blood' transfusion. The role of the

53   systemic distribution of inflammatory cytokines from one affected organ to another could not be

54   confirmed in this study.

55

Introduction

57   Brain and lung injuries are some of the most common causes of patient admission to the

58   intensive care unit.[1,2] The management of these patients is complex, and high rates of morbidity

59   and mortality are associated with these illnesses. Injuries to the brain or lung result in

60   inflammatory responses, which frequently lead to secondary injuries in remote organs. This

61   phenomenon is referred to as organ cross-talk, and it describes the interactions between a

62   primary affected organ and a secondarily injured remote organ. Interorgan signalling networks

63   are not only described for brain–lung cross-talk, but for other organ pairs as well. The

64 dysregulations of these interorgan communication networks are also known in renal and
65 metabolic diseases.[3,4] The remote organ is primed and more sensitive to other stimuli that can
66 cause organ damage (e.g., infections). The progression of brain injury to acute lung injury
67 because of the excessive release of cytokines and other neurohumoral factors is often explained
68 as a 'double hit'.[5] Patients who have survived ARDS often develop some degree of cognitive
69 deterioration, which has long-term effects on the brain because of the intensive therapy
70 required.[6] A common theory is that there is a systemic distribution of inflammatory mediators
71 released from the affected organ and transmitted via the bloodstream to other organs, such as the
72 brain[5,7–9]. Three transmission pathways of lung–brain cross-talk have been described: a
73 humoural, a cellular and a neuronal pathway.[10] Furthermore, dysregulations of the hypothalamo-
74 pituitary-adrenal axis contribute to changes in the stress and inflammatory response after lung
75 injury.[11] At the very least, alterations in blood brain barrier's (BBB) function contribute to a
76 local inflammatory response in the central nervous system (CNS).[12] In the past, little attention
77 has been paid to brain–lung cross-talk.[1] Hence, the present study investigates the role of the
78 systemic distribution of cytokines via the bloodstream in a porcine ARDS model for organ cross-
79 talk and possible inflammatory changes in the brain.

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89 **Materials & Methods**
90 *Anaesthesia and instrumentation:*
91 This prospective randomised animal study was conducted after approval by the State and
92 Institutional Animal Care Committee (Landesuntersuchungsamt Rheinland-Pfalz, Mainzer
93 Straße 112, 56068 Koblenz, Germany; reference number: 23 177-07/G 14-1-084), here in
94 accordance with the ARRIVE guidelines.[13]
95 The anaesthesia and instrumentation are highly standardized in our group and were previously
96 reported.[7,14] To minimise stress, the animals (Sus scrofa domestica) stayed in their familiar

97 environment for as long as possible. A local breeder took care of their general condition. After

98 intramuscular injection of ketamine (8 mg kg^{-1}) and midazolam (0.2 mg kg^{-1}) the sedated animals

99 were delivered to the laboratory. After supine positioning, fentanyl (4 µg kg^{-1}) and propofol (4

100 mg kg^{-1}) were injected intravenously to induce general anaesthesia. Throughout the entire

101 experiment, anaesthesia was maintained through the continuous infusion of fentanyl (0.1–0.2 µg

102 kg^{-1} h^{-1}) and propofol (8–12 mg kg^{-1} h^{-1}). A single dose of atracurium (0.5 mg kg^{-1}) was

103 administered to facilitate endotracheal intubation. The animals were ventilated in volume-

104 controlled mode (AVEA, CareFusion, USA): tidal volume 8 ml kg^{-1}; positive end-expiratory

105 pressure (PEEP) 5 cmH$_2$O, fraction of inspired oxygen (FiO$_2$) 0.6, inspiration to expiration ratio

106 1:2 and variable respiration rate to achieve an end-tidal P$_{CO2}$ < 6 kPa. Under ultrasound

107 guidance, four femoral vascular catheters were placed as follows: central venous line (drug

108 administration); pulse contour cardiac output system (PiCCO; Pulsion Medical Systems,

109 Germany); arterial line (blood withdrawal); and large-bore venous introducer (fluid

110 resuscitation). The haemodynamic and spirometric parameters were permanently measured and

111 recorded (S5, GE Healthcare, USA). The transpulmonary thermodilution technique with single

112 indicators was used to measure cardiac output, global end-diastolic volume and extravascular

113 lung water. To analyse the regional ventilation distribution, we used an electrical impedance

114 tomography device (EIT; Goe-MF II, CareFusion, San Diego, CA, USA), which records thoracic

115 bioimpedance variations associated with tidal ventilation. Electrodes were placed on a transverse

116 lung section just below the axilla. Regional ventilation distribution was examined for the

117 nondependent, central and dependent lung areas (Levels L1–L3) as a percentage of the global

118 tidal amplitude.

119

120 *Experimental design:*

121 The experimental protocol is displayed in Figure 1. After instrumentation and a 30 min

122 consolidation, baseline measurements were documented, and the ARDS was induced with oleic

123 acid injection (0.1 ml kg^{-1}, Applichem GmbH, Germany) until the partial pressure of

124 oxygen/fraction of inspired oxygen ratio (PaO$_2$/FiO$_2$) was below 200. This is a common ARDS

125 model in pigs, as reported by Kamuf et al.[14] Blood samples, EIT measurements and spirometric

126 parameters were taken at baseline and after ARDS induction at 4 and 8 hours (h). After 8 h,

127 blood sampling was performed (35–40 ml kg^{-1}) in seven animals (Group 1, 'ARDS donor', n =

128   7). Blood was collected from the donor animals via the arterial catheter for allogeneic blood

129   transfusion. Whole blood was collected in a specific collector bag system (Composelect,

130   Fresenius-Kabi AG, Homburg Germany). These bags contained 63 ml CPD 100 ml^{-1} SAG-M -

131   RCC (Citrate phosphate dextrose, erythrocyte storage in hypertonic conservation medium). After

132   collection in a primary bag, the blood was drained through a leucocyte-depleting filter into a

133   second bag containing the anticoagulants. Finally, the whole blood was collected into a bag with

134   a port for a transfusion system. The collected blood was stored cool and protected from light at

135   4°C for 12 h. At the end of observation, the animals were euthanised during deep general

136   anaesthesia by central venous administration of propofol and potassium chloride. The organs

137   were removed from the dead animal for further examination. The following day, the recipient

138   animal was prepared as described above, and the collected stored blood was transfused after 30

139   min of baseline time at 0 h. Blood samples, EIT measurements and spirometric parameters were

140   also taken at baseline, after blood transfusion, after 4 h and after 8 h. These animals were also

141   euthanised during deep general anaesthesia by central venous administration of propofol and

142   potassium chloride. The organs were removed from the dead animal for further examination. The

143   animals in Group 2 (donor (s) = donor-sham, common blood transfusion, n = 3) were prepared as

144   the animals in the group without the induction of an ARDS with oleic acid. After 8 h of the

145   experiment, blood withdrawal was performed, as described above, and transfused on the

146   following day (receiver (s) = receiver-sham, n = 3).

147

148   *Postmortem analysis*

149   Repeatedly collected serum blood samples were used to determine the serum levels of TNF-

150   alpha enzyme-linked immunosorbent assays (Porcine Quantikine ELISA Kits; R/D Systems,

151   Wiesbaden, Germany). At the end of the protocol, the lung and brain were each removed en-

152   bloc. Cortex and hippocampus samples were cryopreserved for an mRNA expression analysis of

153   inflammatory mediators like interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-alpha),

154   inducible nitric oxide synthase (iNOS) and soluble lipocalin-2 (sLCN-2) by real-time polymerase

155   chain reaction (rt-PCR; Lightcycler 480 PCR System; Roche Applied Science, Penzberg,

156   Germany), as described in detail by Ziebart et al.[7] mRNA expression was used to better compare

157   the results with previous studies and normalised to peptidylprolyl isomerase A (PPIA).[7] To

158   determine the lung damage, we used a standardised scoring system, as described in detail from

159   our group previously.[15] Furthermore, the lung water content was determined through the tissue

160   wet-to-dry ratio. For this procedure, the weight of the removed left lung tissue sample was

161   determined directly and after 2 d of complete drying.

162   *Statistics:*

163   Group size was adjusted comparably with recently published studies.[7,16] The values are displayed

164   as the mean and standard deviation (SD) or violin plots (median and interquartile range). To

165   analyse the effect between the three groups over time, we performed a two-way analysis of

166   variance (ANOVA) with pairwise multiple comparison correction using the Holm-Sidak method.

167   The Mann–Whitney U-test was used to analyse the postmortem parameters. A p-value less than

168   0.05 was accepted as significant. Statistical analysis was performed with the SigmaPlot 12.5

169   software package (Systat Software, USA) and GraphPad Prism 9.3.1 (GraphPad Software, USA).

170

171

172   **Results**

173   All animals survived the 8 h experimental period (donor n = 7; receiver n = 7; donor-sham n = 3;

174   receiver-sham n = 3). ARDS induction with oleic acid infusion was successfully performed in all

175   animals (donors), with no observed adverse events. The PaO_2/FiO_2-ratio was significantly

176   reduced after ARDS induction (donor group 0 h vs. baseline healthy (BLH): 436 ± 35 vs. 180±

177   19; p < 0.001). Furthermore, a significant intergroup difference was observed between the

178   PaO_2/FiO_2 ratio at 0 h (donor vs. donor-sham/receiver/receiver-sham: 180±19 vs. 455±

179   35/423±90/410±119; p < 0.001). Figure 2 illustrates the PaO_2/FiO_2 ratio. In the donor and

180   receiver groups, the mean pulmonary arterial pressure increased after ARDS induction and blood

181   transfusion without being significant. No further significant alterations in the other groups were

182   measured. The key hemodynamic data set is summarized in Table 1 and documents comparable

183   conditions in all groups throughout the trial. The end-diastolic lung water index (ELWI)

184   increased significantly in the donor group after ARDS induction (0 h vs. BLH: 12±3 vs. 14±3; p

185   < 0.035). Furthermore, a demonstrably increased ELWI was measured in the receiver group (8 h

186   vs. BLH: 15±4 vs. 11±2; p < 0.001). As summarized in Table 2, spirometry data showed no

187   intergroup differences. The distribution of regional ventilation showed no intergroup differences.

188   Leukocyte count was significantly increased in the donor group after ARDS induction (4 h/8 h

189   vs. BLH: 18.5±8.9/18.1±5.9 vs. 10.6±3.4; p < 0.001). After the blood transfusion, significantly

190   higher haemoglobin and haematocrit counts were measured in the receiver group (a.e.

191   haemoglobin 4 h vs. BLH: 11.1±0.9 vs. 9.2±0.5; $p < 0.05$). Further, a significant higher

192   intergroup difference between the receiver and donor group was detected for haemoglobin (4

193   h/8h: 11.1±0.9/10.5±0.8 vs. 10.2±0.7/9.5±0.7; $p < 0.001$). Table 3 highlights all the

194   haematological parameters. After ARDS induction, increased serum levels of TNF-alpha were

195   measured for the donor group without being significant (Fig. 3). Lactate levels between the

196   donor and receiver groups were elevated (0 h: 3.0±1.6 vs. 1.3±0.2; $p < 0.001$). Furthermore,

197   significant differences in lactate levels over time were observed in both groups ($p < 0.001$ donor

198   group 0 h vs. BLH/4 h/8 h; $p < 0.05$ receiver group 8 h vs. 0 h/BLH; Table 4). Table 4

199   summarizes the blood gas analysis. The diffuse alveolar damage (DAD) score showed

200   significantly higher levels in the ARDS donor group compared with all other groups ($p < 0.001$

201   donor group 29.35±6.38 vs. receiver 19.50±5.79/donor-sham 18.66±4.75/receiver sham

202   18.91±5.12). The lung wet-to-dry ratio showed no intergroup differences. Hippocampal mRNA

203   expression for inflammatory response showed minimal increased levels for IL-6, iNOS and

204   sLCN-2 in the receiver group compared to the donor group without being significant (IL-6:

205   1,77e-05±6,69e-06 vs. 2,89e-06±1,09e-06; iNOS: 2,28e-04±8,61e-05 vs. 1,91e-04±7,20e-05;

206   sLCN-2: 2,27e-02±2,41e-02 vs. 1,49e-02±1,01e-02; $p > 0.05$; Fig. 4). No increased receiver

207   hippocampal mRNA expression for TNF-alpha was measured compared to the donor group.

208   Higher mRNA expression of sLCN-2 in the cortex was measured without being significant

209   (receiver vs. donor: 6,00e-03±2,27e-03 vs. 3,97e-02±1,50e-02; $p > 0.05$; Fig.5). The other

210   inflammatory markers in the cortex (IL-6, TNF-alpha, iNOS) showed no increased mRNA

211   expression in the receiver group compared to the donor group (Fig. 5). There were no significant

212   changes in the associated sham groups.

Discussion

214   The present study investigated the roll of cytokine distribution after ARDS induction and the

215   influence of the cerebral inflammatory response in a presumed interorgan cross-talk.[1,5] We found

216   that blood sampling from ARDS donors in healthy pigs did not affect lung function or induce an

217   inflammatory response in the brain or systemic circulation.

218   ARDS induction with oleic acid is a common model for the induction of pulmonary

219   inflammatory response with consecutive pulmonary dysfunction. This model is highly

220 standardised in our group. Kamuf et al. described the protocol and main advantages and
221 disadvantages of ARDS induction with oleic acid.[14] In the present study, ARDS was induced in
222 the donor group in a sustained manner (Fig. 2). A minimal increase in TNF-alpha was measured
223 as an early cytokine response marker of ARDS.[17,18] As a result of the inflammatory response, the
224 alveolocapillary unit lost its integrity. First, the extra lung water index in the donor group
225 increased significantly over time. Second, the postmortem analysis of the lung tissue confirmed
226 lung damage in the ARDS donor group. These changes are known to occur early in the
227 pathophysiological ARDS cascade and underscore the practicality of the ARDS model with oleic
228 acid.[18,19] The 'transfusion of ARDS' to the receiver group was not as severe as might be
229 expected and was not comparable to the extent seen in the donor group. At the postmortem
230 analysis, no tissue changes were noted in the presumed ARDS blood receiver group.

231 Blood transfusions can be associated with serious consequences and, in the worst case, can lead
232 to patient death.[20,21] The main causes behind this are the transfusion of incompatible blood,
233 allergenic reaction, transfusion-related acute lung injury (TRALI) or transfusion-associated
234 circulatory overload (TACO). Compared with human blood, porcine blood offers several
235 advantages.[7,22] Ziebart et al. demonstrated that random allogenic blood transfusion in pigs was
236 not associated with adverse effects of incompatibility, upregulation of systemic and local
237 (brain/lung) inflammatory markers and did not affect lung function.[7] Comparable to previous
238 studies, there were no other differences in additionally measured parameters.[7,23] Allogenic blood
239 transfusion in pigs is a safe approach to investigate the blood-bound transmission in remote
240 organ injury, as claimed in our study.

241

242 The communication between the lung and brain is a complex structure. Three communication
243 pathways have been described in the literature: a humoural, a cellular and a neural pathway.[10]
244 However, the descriptions of these pathways are not unambiguous. The activation of vagus nerve
245 afferents in the neural pathway acts rapidly; other communication pathways are more time-
246 consuming in this regard. Anti- and pro-inflammatory effects in the brain have been
247 described.[24,25] This is also known as 'inflammatory reflex'. A major stimulus is the activation of
248 pulmonary mechanoreceptors during mechanical ventilation.[10,26] In the present study, this
249 influence can be reduced by observing lung protective ventilation settings across the experiment.
250 The cellular pathway is a medium-acting pathway. In particular, TNF-alpha increases monocyte

251 chemotactic protein-1 (MCP-1) expression in the brain via the activation of TNF receptor 1 at
252 the surface of microglia. The crossing of immune cells across the blood-brain barrier (BBB) is
253 facilitated and multiplies the immune response in the brain.[27] The BBB plays a key role in the
254 slow-acting humoural signalling pathway. The lung and brain share the same inflammatory
255 markers as TNF-alpha. Once released by monocytes and macrophages in the primary affected
256 organ (i.e., lung), they can reach the brain and vice versa. As mentioned above, borderline
257 elevated TNF-alpha levels were ~~also~~ observed in the ARDS donor group in our study.
258 Unfortunately, we did not see any significant cerebral inflammatory changes. This could be due
259 the short duration of the experiment. At first, the BBB, as a protective firewall to maintain
260 homeostasis for optimal brain function, is impermeable for many molecules, such as
261 inflammatory cytokines and immune cells.[28,29] The complex structure of this neurovascular unit
262 enables unique functions: low exposure to systemic toxins and reduced traffic of inflammatory
263 cells minimise local neuronal inflammatory damage.[12]
264 However, systemic inflammation causes changes in the BBB. A lipopolysaccharide (LPS) model
265 of inflammation demonstrates that tight junction modification, glycocalyx degradation and
266 astrocyte modulations cause BBB loss of function.[30] High blood TNF-alpha levels have also
267 been shown to reduce tight junction expression and contribute to BBB anatomic changes.[31,32]
268 This CNS effect occurs 12 h after Streptococcus pneumonia is injected into the bloodstream.
269 Interestingly, this contrasts with the results of another LPS model. Intraperitoneally injected LPS
270 causes a significant upregulation of IL-1ß and TNF-alpha in the hippocampus 3 h after
271 injection.[33] Slow transmission through the circumventricular organs and choroid plexus has been
272 described and is one way to bypass the BBB.[34,35] The initiated upregulation of cortical and
273 hippocampal inflammatory markers early in the process in this study could be interpreted as the
274 nonsignificant minimally elevated levels of iNOS, IL-6 and sLCN-2. This upregulation of
275 inflammatory markers contributes to the neurocognitive deficits observed in critically ill patients.
276 After ARDS, patients have been found to have short-term confusion syndromes and long-term
277 cognitive and memory impairments.[6,26,36] A crucial key role is played by hypoxic and
278 inflammatory changes in the area of the hippocampus.[37]
279 Lipocalin-2 (LCN-2) is a protein that plays a role in various diseases and inflammatory cascades.
280 LCN-2 is involved in the acute stress response, with multiple innate immune functions in a
281 retinal degeneration model.[38] The protein initiates pro-survival pathways in the retina.

282 Furthermore, LCN-2 is secreted by astrocytes under inflammatory conditions and acts as a
283 chemokine inducer and promoter of the classical pro-inflammatory cascade.[39] Upregulated
284 expression of LCN-2 is also observed in mice with obesity and is responsible for neurological
285 degeneration. [40] Similar results have been reported in a rodent model. Increasing LCN-2
286 production is associated with decreased cognitive performance.[41] In LPS-induced acute lung
287 injury in mice, one of the most overexpressed genes in a serial gene analysis is LCN-2.[42]
288 Comparable results have been reported for sepsis-related ARDS in humans. LCN-2 is a key
289 mediator of the initial neutrophil response to the infection.[43] Therefore, our chosen study design
290 should be able to mimic similar pathological stages and induce overexpression of LCN-2. One
291 possible explanation why this is not the case could be that the role of LCN-2 during RBC
292 transfusion has not yet been studied. LCN-2 plays an important role in iron homeostasis and as
293 an antioxidant agent.[44,45] Further studies that investigate the proposed pro- and anti-inflammatory
294 effects of LCN-2 during transfusion are needed.

295 The present study has several limitations. First, the changes in cytokine activity and blood
296 concentration in cold-stored red blood cells over 24 h remain unclear. There are no studies
297 addressing this issue. It could be possible that the plasma concentration of donor cytokines
298 shows fluctuations and inhibits the systemic distribution of ARDS in the recipient group. Due to
299 local regulations, we couldn't do two attempts at one day, so the blood had to be stored. Second,
300 as reported above for sepsis-related neurological changes after bacterial injection, the
301 observation time in the present study may have been too short. CNS changes occur 12 h after the
302 injection of Streptococcus pneumonia injections. We probably observed the first hours of the
303 inflammatory cascade with the initiation of inflammatory gene expression. At the end of the
304 experiment, we observed pulmonary deteriorations in the recipient group. This could be
305 interpreted as a sufficient initiation of the proposed inflammatory cascade with signs of an acute
306 lung dysfunction because of the 'ARDS transplantation' from the donor group. CNS changes
307 were not seen at this time because of the shortness of the observation period.
308

309 Conclusions

310 In conclusion, the chosen model of random allogenic red blood cell transfusion in pigs is feasible
311 and reproducible without serious adverse events, as reported by Ziebart et al.[7] ARDS induction
312 with oleic acid is a well-known method and was performed in the present study with success.

313   Unfortunately, no ARDS-like alterations were seen in the recipient group, and CNS
314   inflammation could not be detected. The role of the systemic distribution of inflammatory
315   cytokines from one affected organ to another could not be confirmed beyond a doubt in the
316   current study. Nevertheless, the circulation of cytokines in the bloodstream plays a major role in
317   the process of organ cross-talk. In subsequent investigations, the observation period needs to be
318   expanded. Furthermore, the role of cytokine transmission over the BBB and the other reported
319   routes as a key mediator of CNS inflammation must be more clearly addressed.

320

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429
430

Figure 1

Timeline.

h: hours; EIT: electrical impedance tomography; ARDS: acute respiratory distress syndrome.

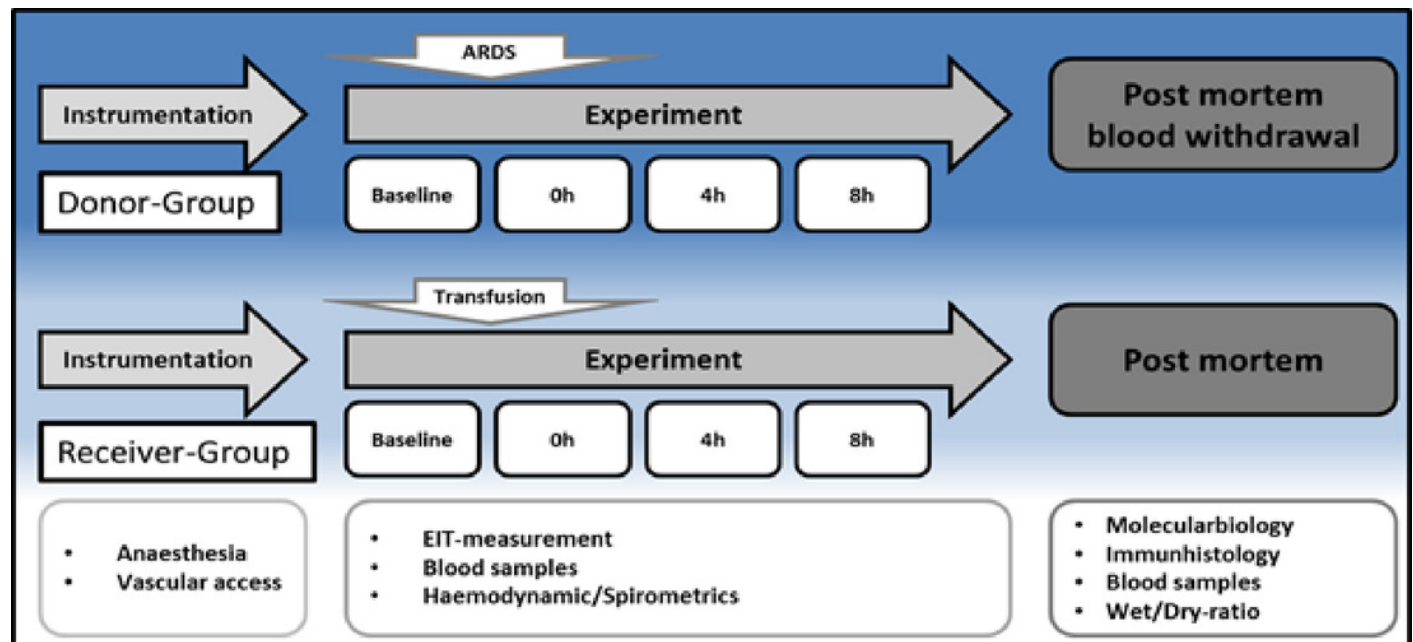



Figure 2

PaO$_2$/FiO$_2$-ratio.

+ p < 0.001 donor group 0 h vs. BLH, * p < 0.001 donor vs. recipient/donor (S)/recipient (S) at 0 h. h: hours; BLH: baseline healthy; (S): sham.

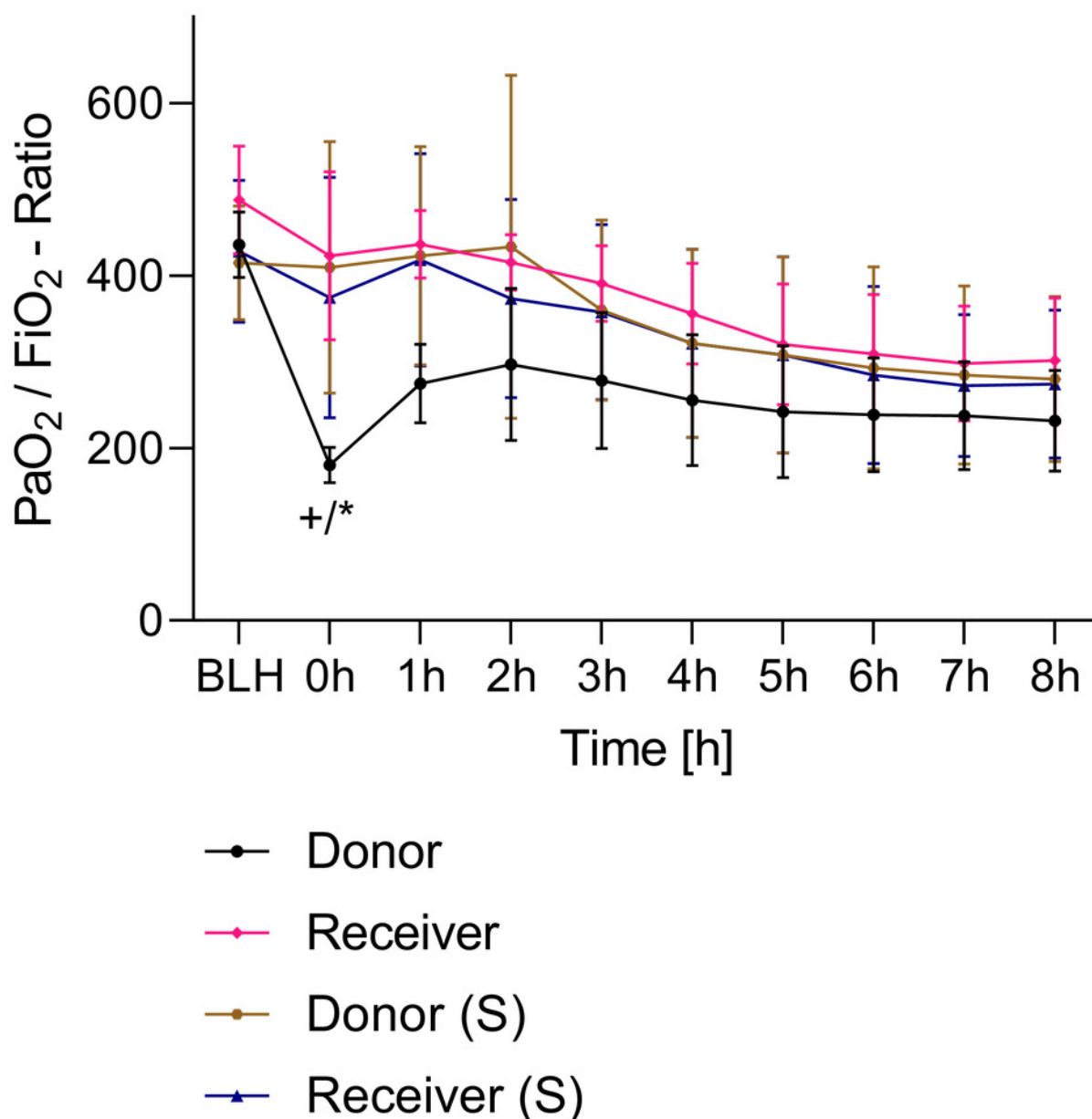



Figure 3

TNF-alpha serum concentration.

BLH: baseline healthy; h: hours; (S): sham.

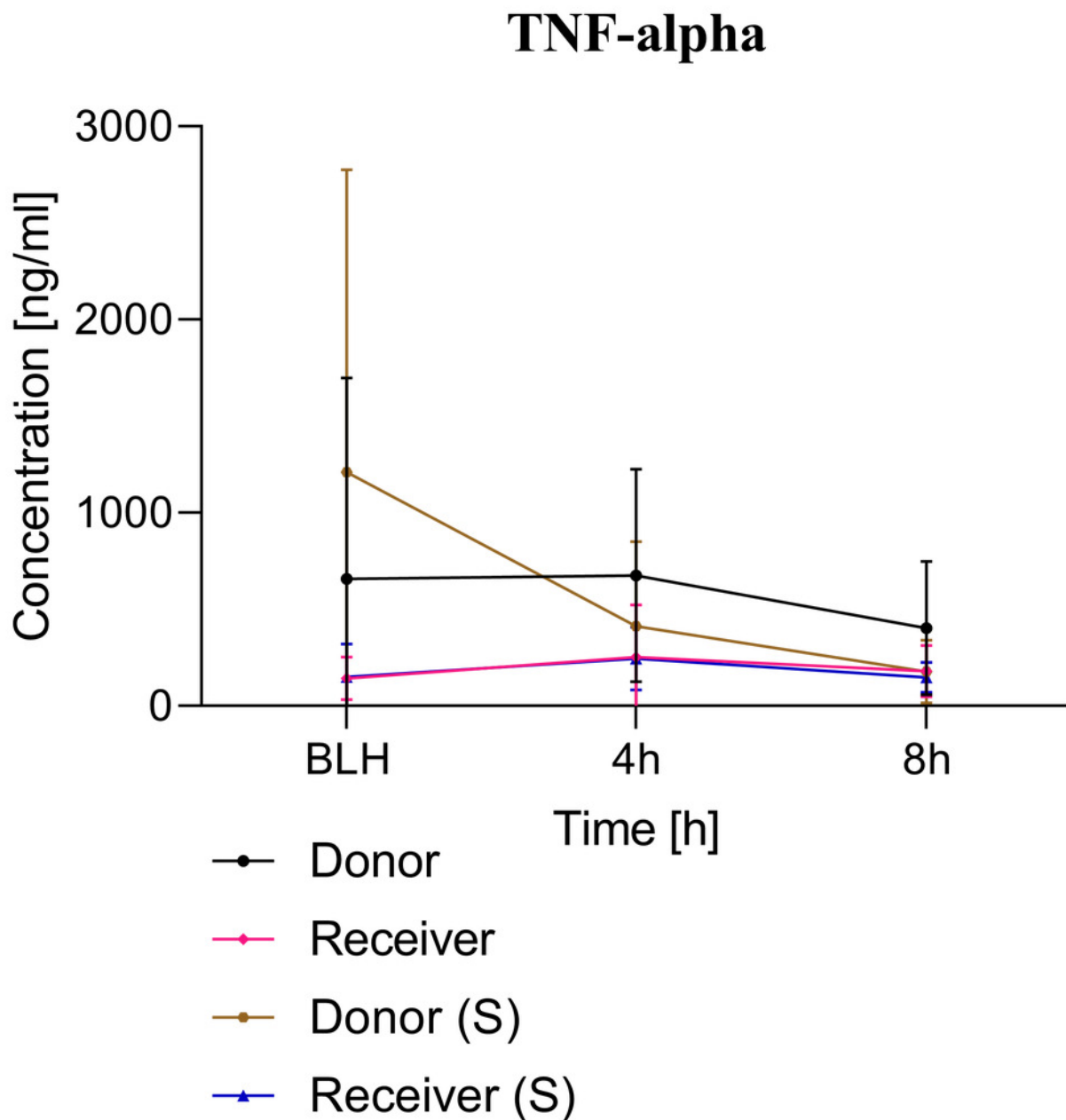



Figure 4

Tissue sample analyses hippocampus.

Inflammatory marker expressions of interleukin-6 (IL-6), tumour necrosis factor alpha (TNF-alpha), inducible nitric oxide synthase (iNOS) and soluble lipocalin-2 (sLCN-2) in hippocampal tissue relative to PPIA expression. (S): sham.

Hippocampus

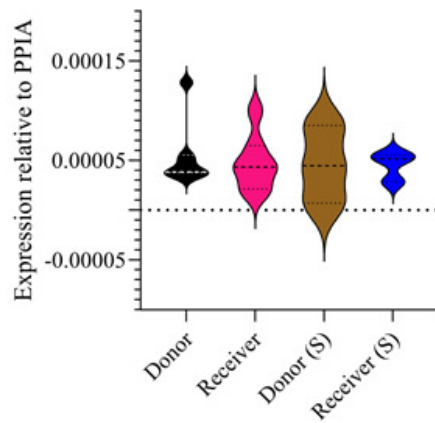
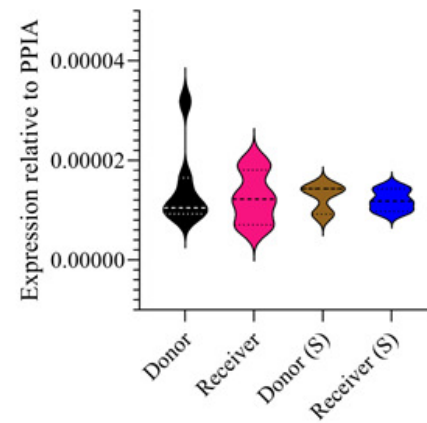
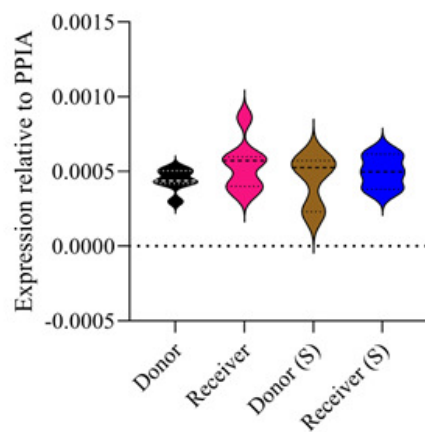
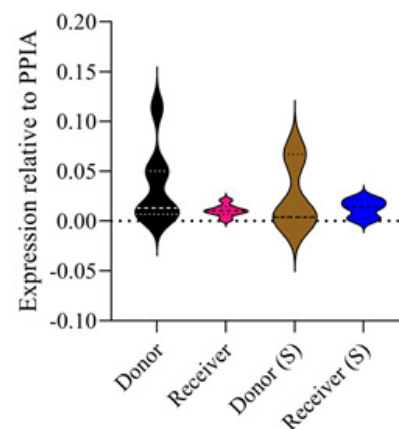



Figure 5

Tissue sample analyses cortex.

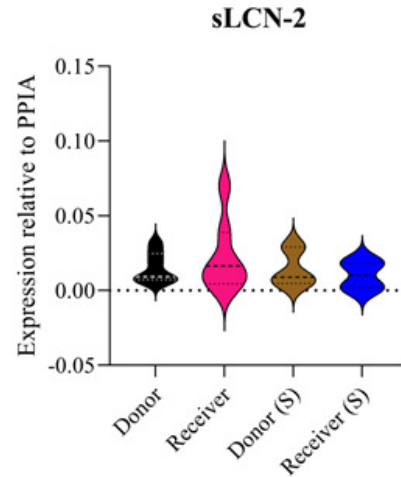
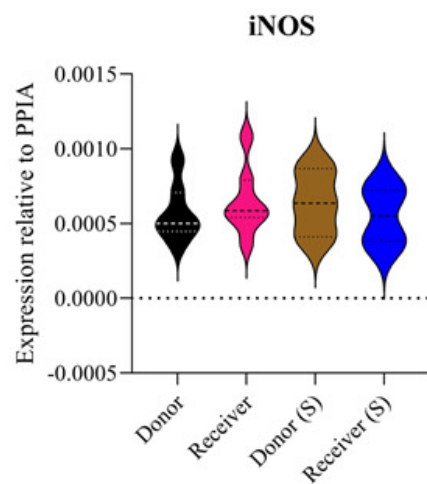
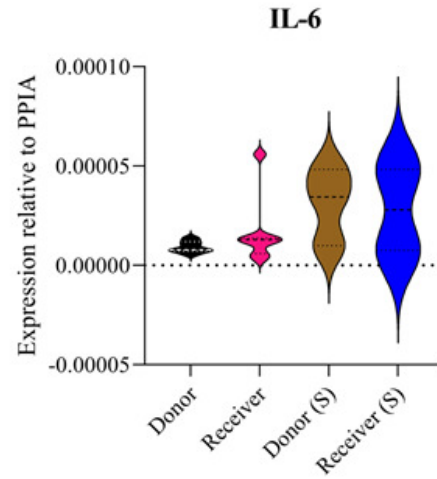
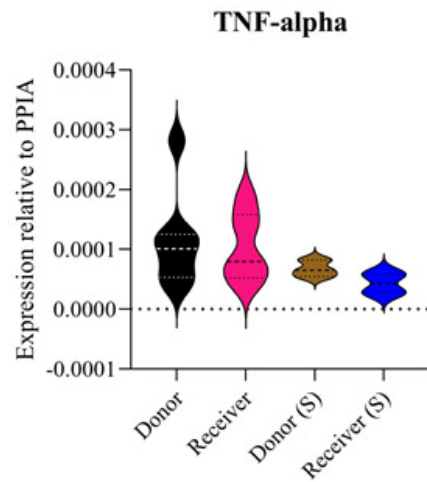
Inflammatory marker expressions of interleukin-6 (IL-6), tumour necrosis factor alpha (TNF-alpha), inducible nitric oxide synthase (iNOS) and soluble lipocalin-2 (sLCN-2) in cortical tissue relative to PPIA expression. (S): sham.

Cortex

TNF-alpha

IL-6

iNOS

sLCN-2

Hemodynamic parameters.

MAP: mean arterial pressure; HR: heart rate; PAP: mean arterial pulmonary pressure; CO: cardiac output; GEDI: global endiastolic volumen index; ELWI: entdiastolic lung water index; CVP: central venous pressure; SpO_2: oxygen saturation; (S): sham; BLH: baseline healthy; h: hours; (S): sham.

Parameter	Group	BLH MEAN (SD)	0h MEAN (SD)	4h MEAN (SD)	8h MEAN (SD)
MAP [mmHg]	Donor	71 (10)	73 (7)	66 (6)	64 (3)
	Donor (S)	61 (3)	61 (3)	66 (2)	65 (5)
	Receiver	64 (5)	97 (13)	80 (12)	74 (14)
	Receiver (S)	64 (5)	97 (13)	80 (6)	74 (6)
HR [min^{-1}]	Donor	79 (12)	137 (38)	169 (43)	135 (28)
	Donor (S)	82 (18)	82 (18)	105 (22)	98 (15)
	Receiver	76 (10)	87 (14)	91 (7)	87 (12)
	Receiver (S)	80 (16)	96 (11)	87 (11)	88 (15)
PAP [mmHg]	Donor	17 (2)	32 (7)	20 (5)	16 (6)
	Donor (S)	14 (3)	14 (3)	17 (2)	16 (3)
	Receiver	12 (4)	27 (11)	16 (4)	15 (4)
	Receiver (S)	19 (7)	38 (10)	26 (8)	24 (7)
CO [l min^{-1}]	Donor	3 (0)	4 (1)	4 (1)	5 (1)
	Donor (S)	3 (0)	3 (0)	3 (0)	4 (1)
	Receiver	3 (1)	3 (1)	3 (1)	3 (1)
	Receiver (S)	3 (0)	3 (1)	3 (0)	2 (0)
GEDI [ml kg^{-1}]	Donor	468 (78)	459 (95)	435 (84)	458 (85)
	Donor (S)	396 (85)	396 (85)	386 (82)	378 (66)
	Receiver	466 (72)	497 (93)	472 (72)	464 (84)
	Receiver (S)	380 (55)	370 (37)	488 (117)	373 (76)
ELWI [ml kg^{-1}]	Donor	12 (3)	14 (3)*	14 (2)	14 (2)
	Donor (S)	10 (1)	10 (1)	10 (1)	12 (2)
	Receiver	11 (2)	10 (2)	13 (1)	15 (4)*
	Receiver (S)	15 (8)	15 (8)	17 (10)	18 (9)
CVP [mmHg]	Donor	36 (66)	6 (1)	6 (2)	19 (28)
	Donor (S)	6 (2)	6 (2)	6 (2)	6 (2)
	Receiver	7 (2)	13 (4)	12 (8)	8 (1)
	Receiver (S)	8 (2)	15 (1)	10 (1)	10 (1)
S_pO_2 [%]	Donor	99 (1)	93 (7)	97 (2)	97 (3)
	Donor (S)	98 (1)	98 (1)	99 (1)	99 (1)
	Receiver	99 (1)	98 (2)	99 (1)	98 (2)
	Receiver (S)	98 (3)	98 (1)	99 (1)	99 (1)

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

Spirometric parameters.

SpO$_2$: oxygen saturation; ex.CO$_2$: expiratory carbon dioxide; FiO$_2$: fraction of inspired oxygen; PaO$_2$/FiO$_2$: oxygen index; Cp: pulmonary compliance; RAW: airway resistance; FRC: fraction of inspired oxygen; MV: minute volume; TV: tidal volume; Ppeak: peak inspiratory pressure; Pmean: mean airway pressure; PEEP: positive end-expiratory pressure; BLH: baseline healthy; h: hours; (S): sham.

Parameter	Group	BLH	0h	4h	8h
		MEAN (SD)	MEAN (SD)	MEAN (SD)	MEAN (SD)
$_{et}CO_2$ [mmHg]	Donor	40 (3)	43 (3)	40 (3)	38 (2)
	Donor (S)	39 (3)	39 (3)	39 (2)	39 (1)
	Receiver	40 (3)	39 (2)	41 (2)	38 (2)
	Receiver (S)	42 (1)	44 (1)	38 (1)	36 (1)
F_iO_2 [%]	Donor	41 (1)	41 (3)	41 (4)	42 (3)
	Donor (S)	40 (0)	40 (0)	40 (0)	40 (0)
	Receiver	40 (0)	40 (1)	40 (0)	40 (0)
	Receiver (S)	40 (0)	40 (0)	40 (0)	41 (1)
PaO_2/F_iO_2	Donor	436 (35)	180 (19)*/#	256 (70)	232 (54)
	Donor (S)	451 (30)	455 (35)	453 (24)	358 (46)
	Receiver	488 (58)	423 (90)	356 (54)	302 (67)
	Receiver (S)	415 (54)	410 (119)	321 (89)	281 (78)
C_p [ml cmH$_2$O^{-1}]	Donor	18 (2)	14 (1)	15 (1)	15 (1)
	Donor (S)	20 (1)	20 (1)	17 (1)	16 (1)
	Receiver	19 (2)	15 (3)	15 (2)	14 (2)
	Receiver (S)	19 (1)	16 (3)	15 (2)	15 (2)
R_{AW} [kPA l^{-1} sec^{-1}]	Donor	14 (2)	15 (2)	14 (1)	14 (1)
	Donor (S)	15 (2)	15 (2)	15 (3)	15 (3)
	Receiver	12 (1)	13 (1)	13 (1)	13 (1)
	Receiver (S)	14 (1)	15 (3)	14 (2)	15 (2)
FRC [ml]	Donor	726 (115)	622 (93)	662 (123)	649 (83)
	Donor (S)	716 (368)	716 (368)	636 (314)	670 (346)
	Receiver	753 (215)	641 (188)	588 (180)	613 (186)
	Receiver (S)	920 (156)	807 (35)	579 (211)	571 (154)
MV [l min^{-1}]	Donor	7 (1)	8 (1)	8 (1)	8 (2)
	Donor (S)	7 (1)	7 (1)	8 (1)	8 (1)
	Receiver	7 (1)	7 (1)	7 (1)	7 (1)
	Receiver (S)	7 (1)	8 (0)	8 (1)	8 (1)
TV [ml kg^{-1}]	Donor	30 (14)	41 (9)	40 (8)	28 (12)
	Donor (S)	37 (7)	37 (7)	40 (10)	41 (12)
	Receiver	34 (9)	18 (8)	22 (8)	28 (6)
	Receiver (S)	30 (7)	15 (1)	24 (2)	24 (3)

P$_{peak}$ [cm H$_2$O]	Donor	18 (1)	24 (3)	22 (1)	23 (2)
	Donor (S)	17 (2)	17 (2)	19 (2)	20 (2)
	Receiver	17 (1)	20 (4)	20 (2)	22 (2)
	Receiver (S)	18 (1)	20 (2)	20 (1)	21 (2)
P$_{mean}$ [cm H$_2$O]	Donor	9 (0)	12 (2)	11 (1)	11 (2)
	Donor (S)	9 (1)	9 (1)	9 (0)	10 (0)
	Receiver	9 (1)	10 (1)	10 (1)	10 (1)
	Receiver (S)	9 (0)	10 (1)	10 (0)	10 (0)
Peep [cm H$_2$O]	Donor	4 (0)	5 (2)	5 (1)	5 (2)
	Donor (S)	4 (0)	4 (0)	4 (0)	4 (0)
	Receiver	4 (0)	4 (0)	4 (0)	4 (0)
	Receiver (S)	4 (0)	4 (0)	4 (0)	4 (0)

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

Haematological parameters.

BLH: baseline healthy; h: hours; (S): sham.

Parameter	Group	BLH MEAN (SD)	4h MEAN (SD)	8h MEAN (SD)
Leukocytes [µl⁻¹]	Donor	10.6 (3.4)	18.5 (8.9)*	18.1 (5.9)*
	Donor (S)	13.8 (5.9)	14.3 (5.8)	13.8 (4.2)
	Receiver	17.5 (4.6)	15.2 (5.5)	14.2 (3.9)
	Receiver (S)	17.7 (4.3)	15.8 (1.3)	13.7 (0.6)
Hemoglobine [mg dl⁻¹]	Donor	9.5 (0.6)	10.2 (0.7)*	9.5 (0.7)
	Donor (S)	9.7 (0.4)	9.3 (0.4)	9.0 (0.6)
	Receiver	9.2 (0.5)	11.1 (0.9)*	10.5 (0.8)
	Receiver (S)	9.1 (0.6)	10.7 (0.7)	10.2 (0.7)
Hematocrit [%]	Donor	29.6 (2.1)	31.6 (2.4)	29.4 (2.1)
	Donor (S)	30.7 (1.1)	29.1 (1.3)	28.1 (2.3)
	Receiver	28.8 (1.7)	33.6 (2.6)*	32.5 (2.6)*
	Receiver (S)	28.5 (1.6)	32.7 (0.7)	31.1 (1.9)
Thrombocytes [µl⁻¹]	Donor	319 (58)	230 (76)	224 (82)
	Donor (S)	388 (32)	333 (39)	318 (28)
	Receiver	388 (55)	268 (57)	261 (52)
	Receiver (S)	432 (51)	337 (32)	316 (17)

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

Blood gas analysis.

SvO_2: central venous oxygen saturation; BE: base excess; art.CO_2: arterial carbon dioxide;

PaO2: arterial oxygen; (S): sham; BLH: baseline healthy; h: hours.

Parameter	Group	BLH MEAN (SD)	0h MEAN (SD)	4h MEAN (SD)	8h MEAN (SD)
Potasium [mmol l^{-1}]	Donor	4 (0,2)	4,4 (0,2)	4,9 (0,6)	5 (0,7)
	Donor (S)	3,9 (0,2)	3,9 (0,2)	5,1 (0,5)	4,3 (0,2)
	Receiver	3,9 (0,2)	3,9 (0,2)	4,4 (0,2)	3,8 (0,2)
	Receiver (S)	4,1 (0,5)	4,1 (0,4)	4,6 (0,1)	4,3 (0,1)
Lactat [mmol l^{-1}]	Donor	0,9 (0,3)	3 (1,6) */#/+	1,6 (1)	1,1 (0,6)
	Donor (S)	1 (0,4)	1 (0,4)	0,7 (0,2)	0,6 (0,2)
	Receiver	1,3 (0,3)	1,3 (0,2)	0,6 (0,1)	0,4 (0,1)*
	Receiver (S)	1,1 (0,3)	2,6 (1)	0,8 (0,1)	0,5 (0)
$_{art}CO_2$ [mmHg]	Donor	41 (3)	50 (7)	43 (6)	42 (4)
	Donor (S)	41 (2)	41 (2)	37 (1)	37 (2)
	Receiver	42 (3)	39 (6)	40 (3)	39 (2)
	Receiver (S)	43 (3)	47 (4)	37 (2)	37 (1)
PaO_2 [mmHg]	Donor	174 (14)	75 (8)	105 (26)	95 (19)
	Donor (S)	177 (9)	177 (9)	179 (10)	141 (17)
	Receiver	195 (23)	169 (36)	142 (22)	121 (27)
	Receiver (S)	171 (27)	150 (46)	129 (36)	110 (28)
pH	Donor	7,47 (0)	7,35 (0)	7,46 (0)	7,47 (0)
	Donor (S)	7,48 (0)	7,48 (0)	7,54 (0)	7,53 (0)
	Receiver	7,47 (0)	7,43 (0)	7,52 (0)	7,52 (0)
	Receiver (S)	7,48 (0)	7,4 (0)	7,57 (0)	7,55 (0)
BE [mmol l^{-1}]	Donor	4.5 (6.1)	1.9 (3.2)	6.1 (3.1)	6.5 (3.0)
	Donor (S)	6.3 (2.0)	6.3 (2.1)	8.6 (1.9)	7.5 (1.5)
	Receiver	6.2 (1.7)	4.2 (2.8)	9.1 (1.9)	7.9 (1.6)
	Receiver (S)	7.7 (0.6)	4.5 (2.1)	10.6 (0.5)	9.8 (0.7)
S_vO_2 [%]	Donor	57 (5)	50 (20)	56 (14)	59 (4)
	Donor (S)	58 (10)	58 (10)	54 (10)	53 (8)
	Receiver	57 (7)	65 (7)	59 (8)	62 (4)
	Receiver (S)	60 (15)	65 (14)	63 (11)	63 (9)

*indicates $p<0.05$ vs. baseline value; +indicates $p<0.05$ vs. 4/8h

\# indicates $p< 0.05$ in intergroup comparison