Extracorporeal cytokine adsorption reduces systemic cytokine storm and improves graft function in lung transplantation

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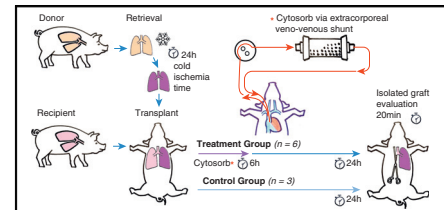
ABSTRACT

Objectives: Ischemia–reperfusion injury often coincides with a cytokine storm, which can result in primary graft dysfunction following lung transplantation. Our previous research has demonstrated allograft improvement by cytokine adsorption during ex vivo lung perfusion. The aim of this study was to investigate the effect of in vivo extracorporeal cytokine adsorption in a large animal model.

Materials and Methods: Pig left lung transplantation was performed following 24 hours of cold ischemic storage. Observation period after transplantation was 24 hours. In the treatment group (n = 6), extracorporeal CytoSorb adsorption was started 30 minutes before reperfusion and continued for 6 hours. A control group (n = 3) did not receive adsorber treatment.

Results: During adsorption, we consistently noticed a significant decrease in plasma proinflammatory interleukin (IL)-2, trends of less proinflammatory, tumor necrosis factor-α, IL-1α, and granulocyte-macrophage colony-stimulating factor as well as significantly reduced systemic neutrophils. In addition, a significantly lower peak airway pressure was detected during the 6 hours of adsorption. After 24 hours of observation, when evaluating the left lung allograft independently, we observed significantly improved CO_2 removal, partial pressure of oxygen/inspired oxygen fraction ratio, and less acidosis in the treatment group. At autopsy, bronchoalveolar lavage results exhibited significantly lower recruitment of cells and less pro-inflammatory IL-1α, IL-1β, IL-6, and IL-8 in the treatment group. Histologically, the treatment group had a strong trend, indicating less neutrophil invasion into the alveolar space.

Conclusions: Based on our findings, cytokine adsorption during and after reperfusion is a viable approach to reducing posttransplant inflammation following lung transplantation. CytoSorb may increase the acceptance of extended criteria donor lungs, which are more susceptible to ischemia–reperfusion injury. (JTCVS Open 2023;15:497-507)

Extracorporeal cytokine adsorption during lung transplantation improves graft function.

CENTRAL MESSAGE

Extracorporeal adsorption of inflammatory mediators during and after reperfusion improves immediate posttransplant graft function by decreasing inflammatory response to reperfusion in pigs.

PERSPECTIVE

The cytokine storm triggered by extended donor lungs, prolonged ischemia, and damage during reperfusion is a major factor promoting primary graft dysfunction, morbidity, and mortality after lung transplantation. A broad-spectrum adsorber may reduce the chance of this ischemia-reperfusion injury and increase the acceptance of extended criteria donor lungs.

Lung transplantation has emerged as the final therapeutic option for individuals with end-stage lung disease, with more than 4000 transplants performed annually worldwide. However, organ scarcity remains the primary challenge in lung transplantation,[1,2] as many donor organs are rejected for transplantation because of perceived damage due to prolonged ischemia time, edema, or pneumonia. Their heightened level of intrinsic tissue inflammation, combined with damages incurred during reperfusion, initiates an ischemia–reperfusion injury (IRI) and a vigorous cytokine and chemokine storm. This leads to the activation of alveolar macrophages and lymphocytes, invasion of

neutrophils, increased microvascular permeability, and cell death.[3,4] The localized and systemic inflammation causes increased pulmonary vascular resistance, pulmonary edema, and impaired oxygenation in the donor lung and frequently results in primary graft dysfunction (PGD).[5] Currently, PGD is the leading cause of early mortality following transplantation and is a major contributing factor to the onset of chronic lung allograft dysfunction.[6,7]

In our previous study, using a large animal model, we demonstrated that the removal of cytokines using CytoSorb (CytoSorbents, Inc), a size-selective hemoadsorption device, improves lung function and metabolism in ischemic lungs during ex vivo lung perfusion (EVLP).[8] This technique also reduces IRI when lungs treated with EVLP were subsequently transplanted.[9]

In a recent pilot study, CytoSorb was tested in patients who underwent lung transplantation who were still on extracorporeal membrane oxygenation (ECMO).[10] During a 24-hour period of extracorporeal adsorption, no adverse events were reported, and there was a promising systemic reduction of neutrophil activation. In a pig left lung transplant study with lipopolysaccharide-injured donor lungs, a significant improvement of lung function and reduction of PGD was reported following a 12-hour period of extracorporeal hemoadsorption.[11] However, another study did not find a beneficial effect after 6 hours of posttransplant hemoadsorption in a pig model.[12]

This study assessed the feasibility and impact of cytokine adsorption, using CytoSorb, in a well-established pig left lung transplant model.[13] We hypothesized that 6 hours of hemoadsorption with CytoSorb following transplantation of ischemic lungs would reduce the systemic cytokine storm and improve, or even allow reconditioning of, the short-term graft function.

METHODS
Animals
For this study, outbred female domestic pigs ranging from 48 to 59 kg (mean, 53 kg) were used. The study was approved by the Kanton Zurich Veterinarian Committee (ZH 047/2020). All animals were treated according to "Principles of Laboratory Animal Care" guidelines, formulated by the National Society for Medical Research, and "Guide for the Care and Use of Laboratory Animals" guidelines.

Experimental Design
Figure 1 illustrates the experimental design. The experiments involved randomly selecting a donor and recipient animal for 2 groups: a treatment group (n = 6) and control group (n = 3). In both groups, the donor lungs were preserved at 4 °C for 24 hours. Subsequently, a left lung transplantation was performed. Thirty minutes before reperfusion of the transplanted lung, extracorporeal cytokine adsorption was initiated for 6 hours using the CytoSorb device via a precannulated venovenous circuit placed in the superior vena cava using a single site 2-lumen setup. After the 6-hour period, the cytokine adsorption was stopped, and the animal was observed under deep anesthesia for a total of 24 hours. In contrast, the control group did not receive cannulation and was only observed for 24 hours. After the 24-hour period, the right pulmonary artery and right main bronchus were occluded in both groups to assess the allograft's function only.

Surgical Procedures
The donor lung was retrieved according to our established protocol.[13] First, heparin (300 IU/kg) was administered intravenously. Then, the pulmonary artery was cannulated, and the superior and inferior vena cava were ligated. The appendage of left atrium was cut, and the pulmonary artery flushed with 50 mL/kg cold (4 °C) Perfadex (Vitrolife). The trachea was clamped to maintain inflated lungs at an airway pressure of 15 cmH₂O. The heart–lung block was placed in 2 bags, containing 500 mL of Perfadex, and stored at 4 °C for 24 hours. The subsequent orthotopic left lung transplantation was performed as previously described by our group.[13] For the rest of the experiment, the thoracotomy site was loosely covered with a towel, without placement of a chest tube.

Anesthesia and Medication
All donor and recipient animals were sedated with intramuscular injection of ketamine (Ketasol-100; 15 mg/kg), azaperone (Stresnil; Elanco, 2 mg/kg), and atropine (Atropinsulfat 0.1%; Kantonsapotheke, 0.05 mg/kg). Anesthesia was induced by intravenous (i.v.) propofol (Propofol-Lipuro 1%; Braun, 1-2 mg/kg) and maintained with isoflurane (1.5%-3%) and a constant rate of propofol (2-5 mg/kg/h i.v.). Muscle relaxation was controlled with repeated administrations of rocuronium bromide (Esmeron, 0.5 mg/kg i.v.). Analgesic buprenorphine (Temgesic; Indivior, 0.01 mg/kg) was administered every 5 hours. A positive pressure ventilation with an inspired oxygen fraction (FiO₂) of 100%, tidal volume of 6-8 mL/kg, a frequency of 18 breaths/min, and a positive end expiratory pressure of 5 cmH₂O was maintained by an anesthesia ventilator. A prophylactic antibiotic dose of 500 mg of meropenem (Meronem; Pfizer) was administered intravenously.

For basic immunosuppression in recipients, methylprednisolone (500 mg, i.v.) was administered before thoracotomy and repeated 12 hours' posttransplantation. Recipients were also administered cyclosporin A (Sandimmune; Novartis) (50 mg i.v.) 15 minutes before starting reperfusion and 12 hours' posttransplantation.

Throughout the entire anesthesia period, i.v. fluids (Ringerfundin, 5 mL/kg/h) were administered and adjusted based on the recipients' blood gas analysis. Corrective vasoactive therapy was provided with dobutamine in case of mean arterial pressure below 65 mm Hg. No blood transfusions were administered during the experiment. At the end of the experiment, the animals were humanely killed, while under deep general anesthesia, by exsanguination.

Cytokine Adsorption
Upon induction of anesthesia in the recipients of the treatment-group, a percutaneous placement of a 2-lumen venous catheter (HighFlow Dolphin Catheter, 13F; Baxter International) was performed through the right internal jugular vein into the upper vena cava. Correct placement was verified by fluoroscopy to avoid right atrial cannulation. The catheter was blocked with heparinized saline solution (5000 IE/L) and connected to an extracorporeal perfusion system comprising of a centrifugal pump (Stöckert,
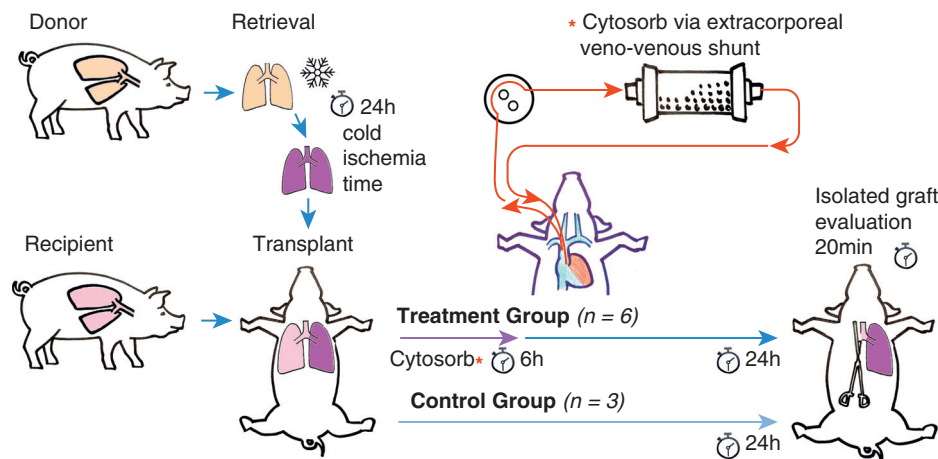
FIGURE 1. Experiment setup.

SCPC) along with a cytokine adsorber (CytoSorb; CytoSorbents, Inc). Thirty minutes before reperfusion of the transplanted allograft, the extracorporeal circuit was initiated and maintained with a flow rate of 200 mL/min. Steady blood flow was measured using transonic flow probes (Transonic Systems Inc), which used patented ultrasound transit-time technology. Over the course of 6 hours, a total of 72 L of blood were purified through this system. The heparinization level was monitored hourly using activated clotting time measurements targeting 140 to 150 seconds.

Physiologic Assessment

At the start of the recipient surgery, an introducer sheath (Avanti +Introducer, 6F; Cordis) was inserted into the left femoral artery to measure systemic arterial blood pressure. To monitor pulmonary artery pressure, a pressure catheter (DLP, 3F; Medtronic) was placed in the common pulmonary artery after transplantation of the allograft.

At baseline, and every hour up to 24 hours, hemodynamic measurements including blood gases from the femoral artery and pulmonary artery, FiO_2, dynamic lung compliance, and peak airway pressure were measured. Oxygenation was measured by means of partial pressure of oxygen, arterial/FiO_2 ratio.

To evaluate the function and mechanics of the transplanted lung after 24 hours' posttransplantation, a procedure was conducted to isolate the graft. This involved excluding the right lung from perfusion and ventilation by occluding the peritransplant encircled right pulmonary artery over 10 minutes, followed by an additional clamping of the pre-encircled right main bronchus over 10 minutes.

Plasma, Blood Cell, and Cytokine Assessment

The whole-blood samples were collected hourly in ethylenediaminetetraacetic acid–treated tubes (BD Vacutainer cat. no. 367525; Becton Dickinson). The Epoc blood analysis system (Epocal, Inc) measured perfusate, blood gases, and biochemistry. Centrifugation of blood samples for 10 minutes at 4 °C and 1339 g separated blood cells from plasma. The supernatant was collected, transferred into a clean polypropylene tube, and centrifuged for 10 minutes at 4 °C and 4000 rpm. The supernatant was stored at –80 °C for biochemical measurements. Then, 50 μL was assayed for levels of cytokines using the 13-plex Discovery assay (porcine Cytokine Array/Chemokine Array 13-Plex Panel; cat. no.: PD13; Eve Technologies). Blood samples were also analyzed for absolute cell counts and percentages for different blood cells using a VetScan HM5 benchtop hematology analyzer (Abaxis, Inc).

Bronchoalveolar Lavage

At the end of the experiment, BAL was obtained by instillation of 2 × 20 mL of saline from the left lower lobe of the allograft. Recovered

BAL was filtered through 4 layers of surgical gauze and centrifuged at 1500g for 3 minutes at 4 °C to pellet cells. The recovered wash fluid was subjected to cytologic assessment at the hospital's core laboratories, using May–Gruenwald–Giemsa-stained cytologic specimens, and subsequently processed as previously described[14] for further analysis. The number of neutrophils in the BAL was expressed as a percentage relative to the total number of cells recovered from the sample. The supernatant was stored at –80 °C for biochemical measurements. Then, 50 μL of BAL was assayed for levels of cytokines using the 13-plex Discovery assay (porcine Cytokine Array/Chemokine Array 13-Plex Panel; cat. no. PD13; Eve Technologies).

Histologic Assessment

At the end of the experiment, tissue samples taken from the left upper and lower lobes of the allograft were fixed in 6% formaldehyde, embedded in paraffin, and stained with hematoxylin–eosin. These samples were scored by a pathologist, who was blinded for the experimental design and group allocation, according to an established lung injury scoring system.[15] The following parameters were assigned 0 to 2 points, where 0 indicated no or minimal injury and 2 indicated more severe injury: (a) neutrophils in the alveolar space, (b) neutrophils in the interstitial space, (c) hyaline membranes, (d) proteinaceous debris filling the airspaces, and (e) alveolar septal thickening. To calculate the final score, the following formula was used: score = [(20*a) + (14*b) + (7*c) + (7*d) + (2*e)]/ (number of fields*100).

Pharmacokinetics of Cyclosporin A

To check for a potential adsorption of cyclosporin A, we analyzed plasma samples 2 hours after application, at 2 hours and 14 hours posttransplantation for peak levels, and at 12 hours and 24 hours' posttransplantation for trough levels. The concentration was assessed at the hospital's core laboratories using high-performance liquid chromatography technology.[16] Quantification was performed using Xcalibur Quan Browser software (Thermo Fisher Scientific).

Statistical Analyses

Results are expressed as the standard error of the mean. Noncontinuous data were compared by unpaired 2-tailed t-test when data were normally distributed, and Mann–Whitney U test when not normally distributed. Values between both study groups were analyzed by 2-way analysis of variance for repeated measures. Statistical analyses were performed with GraphPad PRISM, Version 9.1.2. (GraphPad Software, Inc).
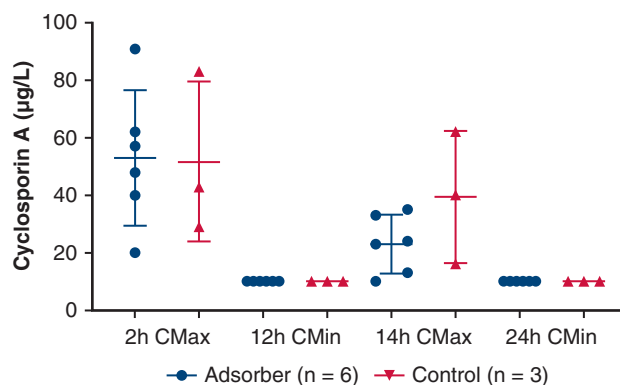
FIGURE 2. Cyclosporin A levels (mean values ± standard deviation) of the adsorbent group (n = 6) in comparison with the control group (n = 3) at peaks (CMax) at 2 and 14 hours, and at troughs (CMin) at 12 and 24 hours after transplantation.

RESULTS

Adsorptive Function

The systemic plasma concentration of the immunosuppressant Cyclosporin A was comparable between the treatment and control group (Figure 2), measured by peak levels at 2 and 14 hours ($P = .942$, $P = .167$, respectively).

Physiology

As illustrated in Figure 3, the treatment group exhibited a significantly lower peak airway pressure during the 6 hour of adsorption period compared with the control group ($P = .02$). This difference was maintained throughout the observation period. Dynamic compliance and mean pulmonary artery pressure showed no significant differences between the treatment and control groups over the 24 hours ($P = .332$, $P = .598$, respectively). Regarding decarboxylation (partial
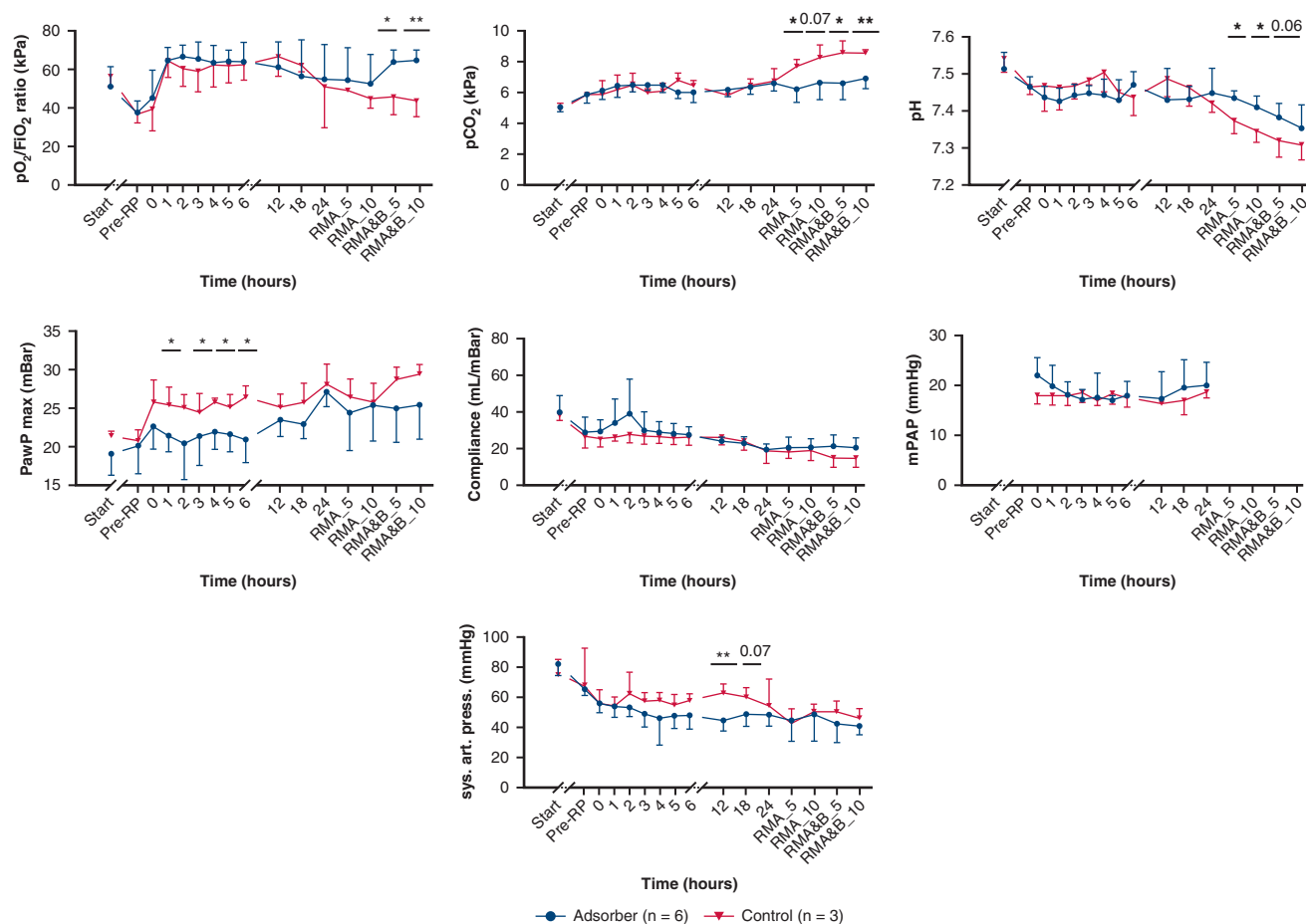
FIGURE 3. Physiological settings (mean values ± standard deviation) of the adsorbent group (n = 6) in comparison with the control group (n = 3) over the observation period. The *asterisks* are significant *P* values vs the control. *$P \leq .05$; **$P \leq .01$. *Start*, Presurgery; *Pre-RP*, prerecipient pneumonectomy; *RMA_5*, 5 minutes after closing right main artery; *RMA_10*, 10 minutes after closing right main artery; *RMA&B_5*, 5 minutes after closing right main bronchus; *RMA&B_10*, 10 minutes after closing right main bronchus; *PawP max*, peak airway pressure; *mPAP*, mean pulmonary artery pressure; *syst. art. press.*, systemic arterial pressure.

pressure of carbon dioxide) and oxygenation (partial pressure of oxygen/FiO_2 ratio), there were no significant differences observed during the adsorption period and thereafter. However, when evaluating the isolated lung allograft at the end of the 24-hour period, the treatment group exhibited significantly better decarboxylation and oxygenation compared with the control group ($P = .006$, $P = .003$, respectively). Furthermore, there was a trend of more stable pH in the treatment group during the final isolated observation period, with significantly less acidosis observed after blocking the right pulmonary artery ($P = .020$). Although systemic arterial pressure was comparable between the 2 groups during the 6 hours of adsorption, it dropped significantly in the treatment group thereafter ($P = .007$).

Blood Cells

Figure 4, *A*, presents the dynamic changes in blood cell populations over the 24-hour posttransplant period. Systemic inflammation, measured by the percentage of neutrophils, was initially increased at the start of adsorber use but significantly decreased toward the 6-hour mark of adsorption compared with the control group ($P = .023$). Lymphocytes showed a transient and significant trend of being greater in the treatment group at 6 hours ($P = .022$). There were no significant differences in monocyte levels between the groups over time ($P = .532$). During the 6 hours of adsorption, activated clotting time in the treatment group was higher than the targeted level. A significant reduction of blood platelets was observed towards the end of the experiment in the treatment group compared to the control group ($P = .024$). There was also a nonsignificant decrease in hemoglobin and red blood cell count in the treatment group ($P = .069$, $P = .154$, respectively).

Electrolytes

As illustrated in Figure 4, *B*, during the first 6 hours after transplantation, the control group exhibit a significant transient hyponatremia ($P = .015$) and hypochloremia ($P = .033$), while the treatment group remained relatively stable. In addition, significant hypocalcemia was observed around the 5-hour mark of adsorption ($P = .023$), which remained significant for most of the experiment in the treatment group, possibly due to a more activated coagulation cascade.[17] Furthermore, there was a significant increase in potassium levels toward the end of the experiment in the treatment group ($P = .03$), accompanied by a corresponding significant shift to hypoglycemia ($P = .038$). Lactate and creatinine levels showed a non-significant trend of increase during isolated allograft ventilation in the treatment group ($P = .114$, $P = .083$, respectively).

Plasma Cytokines

Figure 5 illustrates the measurements of systemic cytokines tracked over the 24-hour observation period. In the treatment-group, there was a significant trend of lower levels of proinflammatory cytokine interleukin (IL)-2 during 6-hour adsorption compared to the control group ($P = .01$). In addition, there was a nonsignificant trend towards lower proinflammatory cytokines tumor necrosis factor-α, IL-1α, and granulocyte-macrophage colony-stimulating factor in the adsorber group during the 6-hour treatment period ($P = .172$, $P = .186$, $P = .130$, respectively). Proinflammatory cytokines IL-1β, IL-6, IL-8, IL-12, IL-18, and interferon-γ showed comparable levels in both groups throughout the entire observation period ($P = .986$, $P = .462$, $P = .507$, $P = .366$, $P = .494$, $P = .281$, respectively). The anti-inflammatory cytokine IL1RA reached significantly lower levels during the 6 hours of adsorption period ($P = .005$), but peaked significantly greater shortly after ($P = .039$). Anti-inflammatory cytokines IL-4 and IL-10 showed comparable levels between the 2 groups ($P = .893$, $P = .731$, respectively).

Bronchoalveolar Lavage

BAL of the allograft at end of experiment (Figure 6) revealed significantly less pro-inflammatory IL-1α, IL-1β, IL-6, and IL-8 in the treatment-group ($P = .008$, $P = .024$, $P = .009$, $P = .0003$, respectively). There was also a trend towards lower levels of proinflammatory cytokines granulocyte-macrophage colony-stimulating factor, IL-2, and IL-18 in the treatment group, although these trends were nonsignificant ($P = .131$, $P = .155$, $P = .130$, respectively). However, the treatment group showed significantly lower levels of anti-inflammatory cytokines IL-10 and IL-1RA, and a strong trend toward lower IL-4 ($P = .026$, $P = .048$, .085, respectively). No significant differences were observed in the levels of pro-inflammatory cytokines interferon-γ, tumor necrosis factor-α, and IL-12 between the groups ($P = .714$, $P = .350$, $P = .262$, respectively). Furthermore, the treatment group also exhibited significantly lower recruitment of cells in the BAL ($P = .037$). Specifically, there was a weak trend toward a lower percentage of lymphocytes in the BAL of treatment group ($P = .2888$), but no significant differences were found in the percentage counts of neutrophils and monocytes ($P = .776$, $P = .664$, respectively).

Histology

In Figure 7, the histologic evaluation of the upper and lower lobe of the allografts at autopsy is depicted. A strong trend of less neutrophils in the alveolar space in the treatment group ($P = .055$) was detected, indicating a less intense inflammatory response. However, no significant differences were observed between the groups for neutrophils in the interstitial space ($P = .729$), hyaline membranes ($P = .529$), proteinaceous debris filling the airspaces
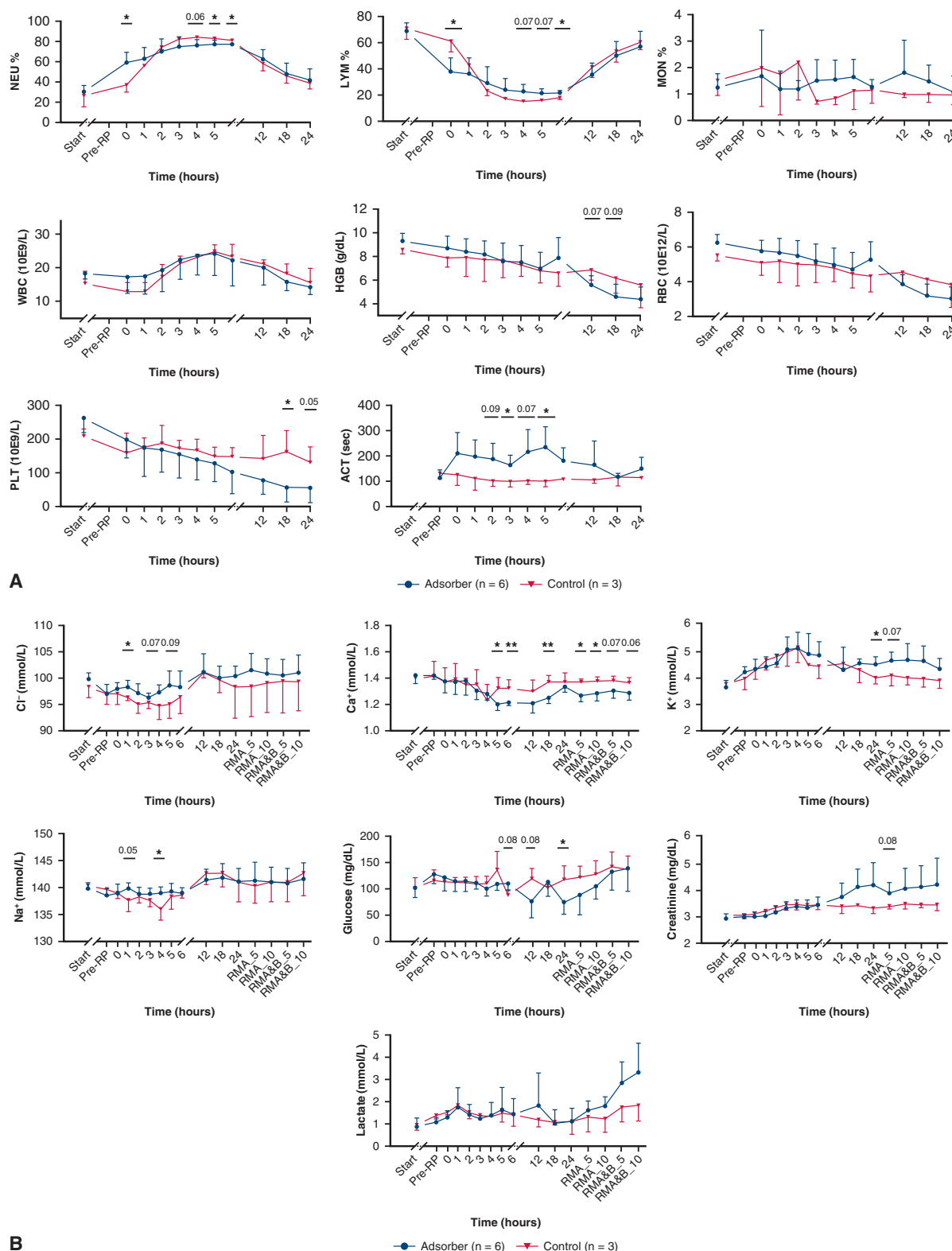
FIGURE 4. A, Blood cells and (B) plasma electrolytes of the adsorbent group (n = 6) in comparison with the control group (n = 3) over the observation period as mean values ± standard deviation. The *asterisks* are significant P values versus the control. *P ≤ .05; **P ≤ .01. *NEU*, Neutrophils; *Start*, presurgery; *Pre-RP*, prerecipient pneumonectomy; *LYM*, lymphocytes; *MON*, monocytes; *WBC*, white blood cells; *HGB*, hemoglobin; *RBC*, red blood cells; *PTL*, platelets; *ACT*, activated clotting time; *RMA_5*, 5 minutes after closing right main artery; *RMA_10*, 10 minutes after closing right main artery; *RMA&B_5*, 5 minutes after closing right main bronchus; *RMA&B_10*, 10 minutes after closing right main bronchus.
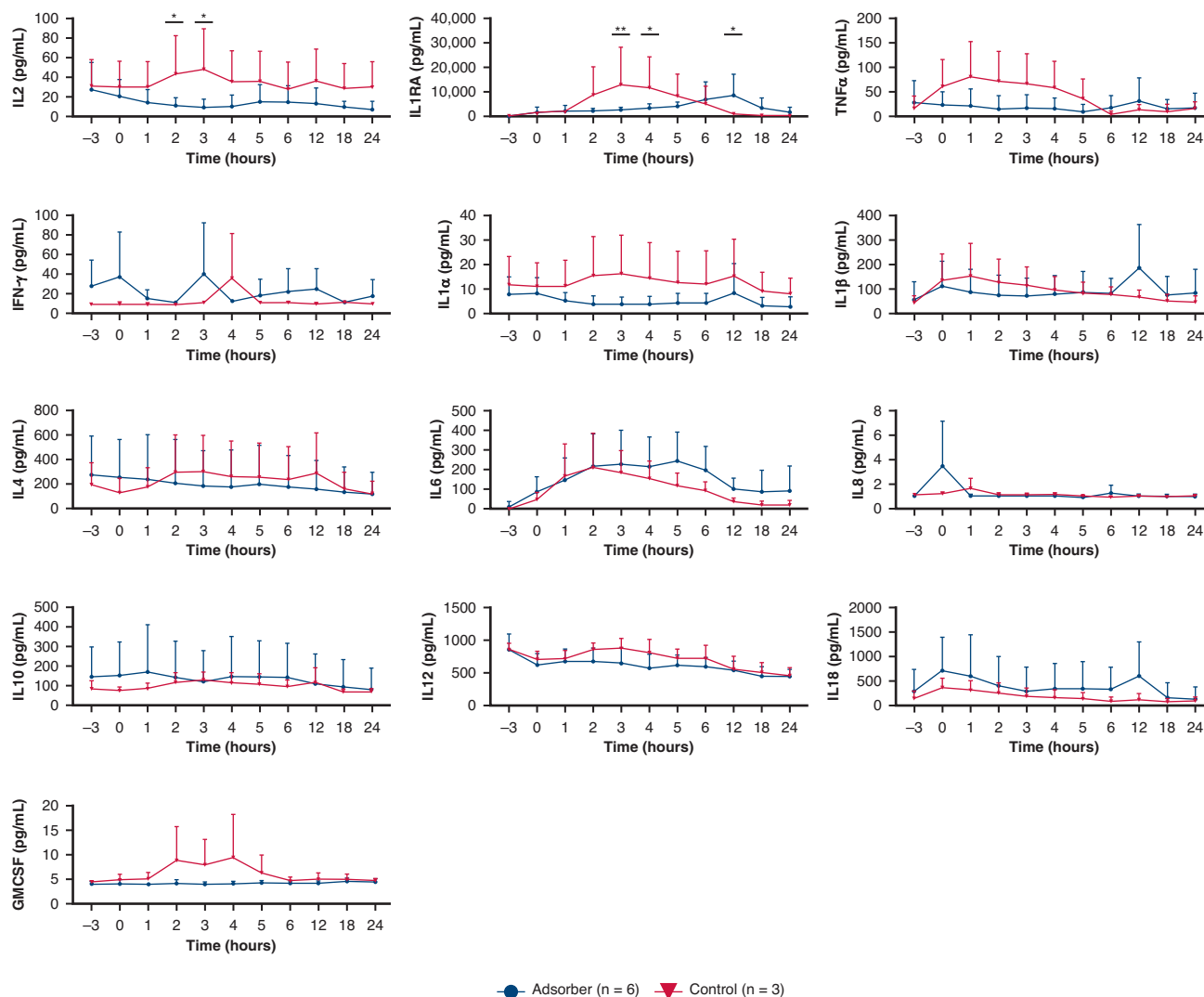
FIGURE 5. Plasma cytokines (mean values ± standard deviation) of the adsorbent group (n = 6) in comparison with the control group (n = 3) over the observation period. The *asterisks* are significant *P* values vs the control. *$P \leq .05$; **$P \leq .01$. *−3*, 3 hours' presurgery; *0*, reperfusion.

($P = .529$) alveolar septal thickening (0.284), and the overall lung injury score[15] ($P = .631$).

DISCUSSION

In this study, it was demonstrated that donor lungs subjected to prolonged cold ischemia following left lung transplantation, along with a 6-hour period of continuous venovenous extracorporeal cytokine adsorption, exhibited improvements in gas exchange (Figure 8). In addition, these lungs demonstrated reduced peak airway pressure and a significantly lower inflammatory response in the BAL.

We have demonstrated in previous studies the beneficial effects of CytoSorb on ischemic pig donor lungs during EVLP.[8,9] These preconditioned lungs showed improved outcomes in terms of inflammation and lung physiology after transplantation.[9] However, in the current in vivo study, we did not observe the same remarkable elimination of cytokines

in the plasma as seen during EVLP. Only IL-2 showed a transient significant reduction. Nevertheless, the overall effect of extracorporeal cytokine removal was still sufficient to significantly reduce neutrophil recruitment during the treatment period and ultimately create an anti-inflammatory environment in the allograft. It is possible that the complex in vivo setting masked the true net effect of cytokine adsorption, as systemic intravascular levels of cytokines remained relatively unchanged due to redistribution from the tissues. Upon the final evaluation and at autopsy, the allografts of our study were comparable with those transplanted allografts in our previous EVLP cytokine removal study. Both approaches using the CytoSorb adsorber demonstrated improved gas exchange, reduced inflammation in the bronchioalveolar lavage (pro-inflammatory IL-1β, IL-6, and IL-8) and a similar trend of decreased neutrophil invasion in the alveolar space observed in histology.
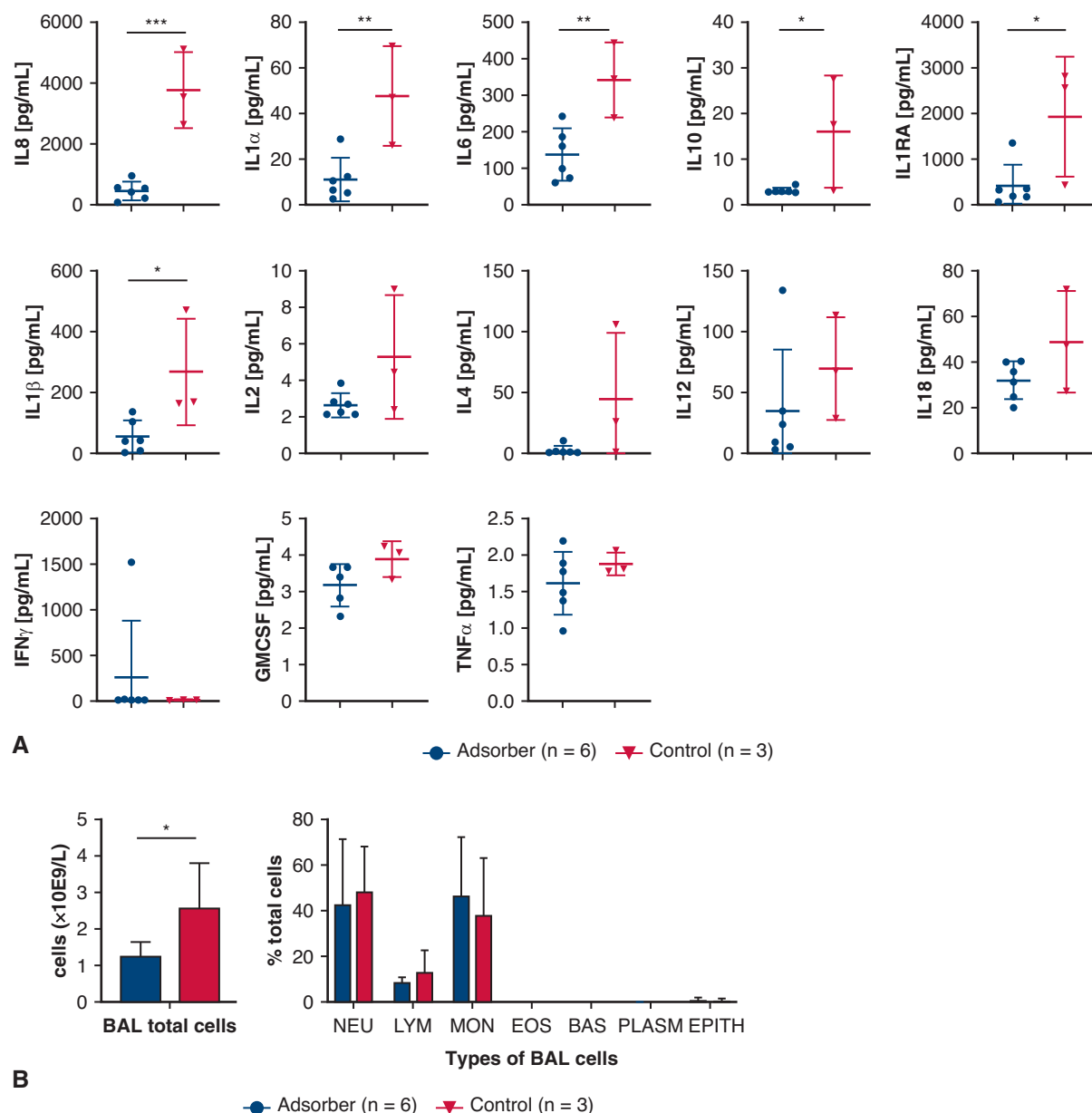
FIGURE 6. A, BAL cytokines (mean values ± standard deviation) and (B) BAL cells of the adsorbent group (n = 6) in comparison with the control group (n = 3) at autopsy. The *asterisks* are significant P values vs the control. *$P \leq .05$; **$P \leq .01$. *IL*, Interleukin; *IFN*, interferon; *GMCF*, granulocyte-macrophage colony-stimulating factor; *TNF*, tumor necrosis factor; *NEU*, neutrophils; *LYM*, lymphocytes; *MON*, monocytes; *EOS*, eosinophiles; *BAS*, basophils; *PLASM*, plasma cells; *EPITH*, epithelial cells; *BAL*, bronchoalveolar lavage.

Consistent with our current findings, a study conducted by the University of Lund using lipopolysaccharide-injured donor grafts in pig left lung transplantation also demonstrated trends of cytokine reduction in the plasma during 12 hours of extracorporeal adsorption.[11] Similarly, in a pilot study involving humans, adsorption during the initial 24 hours after lung transplantation did not yield any significant differences in plasma IL-6 and IL-8 levels.[10]

During the 6-hour period of CytoSorb adsorption, we observed a significant decrease in peak airway pressure,

indicating a protective effect. However, parameters such as compliance, mean pulmonary pressure, and gas exchange remained comparable. Similarly, the Lund group also did not find a difference in gas exchange during the treatment period.[11] However, when evaluating isolated graft function at 24 hours of reperfusion, we observed a significantly improved gas exchange in the treatment group, in accordance with Lund group.

We observed a significant reduction of inflammation in the BAL of the treatment group. Histologically, we found
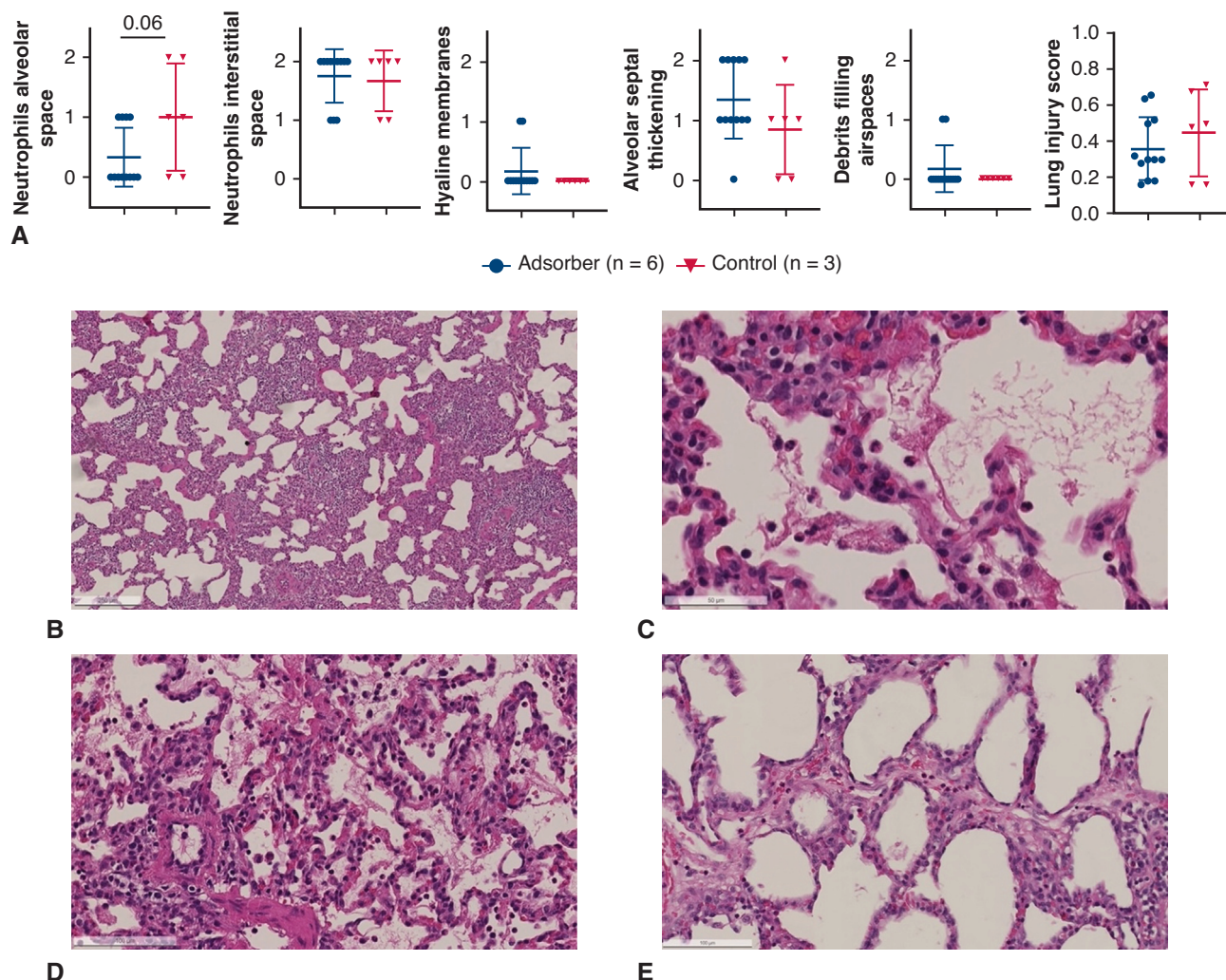
FIGURE 7. Allograft histology at autopsy. A, Mean values ± standard deviation of score[15] parameters of allograft injury of the adsorbent group (n = 6) in comparison with the control group (n = 3). Histologic examples of (B) most severe observed alveolar septal thickening; (C) hyaline membranes; (D) neutrophils and proteinaceous debris in the alveolar space; and (E) neutrophils in the interstitial space.

less neutrophil invasion into the alveolar space, indicating a less intense inflammatory response. However, we did not observe an overall difference in lung injury between the groups. It is possible that the study observation period was too short to detect a significant difference in lung injury between the treatment and control groups.

The findings from the Leuven group[12] in a similar model showed contrasting results compared with our study and the Lund group. They observed a significant increase in mean pulmonary artery pressure and pulmonary vascular resistance, along with elevated partial pressure of carbon dioxide and high cytokine levels in the plasma. They also reported a significantly greater incidence edematous grafts and increased neutrophil presence in the treatment group. They mention a significant reduction of cardiac output during the 6 hours of adsorption, suggesting potential technical issues such as catheter malpositioning, excessive flow

through the extracorporeal circuit leading to cardiac preload issues, bleeding, or instrumentation problems. In contrast, the Lund group[11] reported that their treatment group was hemodynamically more stable and required less inotropic support, which they attributed to reduced inflammation and sepsis. In addition, during human heart transplantation, reduced vasopressor demand was reported when using the cytokine adsorber.[18] Moreover, a recent pilot study in 6 humans demonstrated the safety of CytoSorb when used within the first 24 hour after lung transplantation in an ECMO setting, with no adverse events reported except for one cartridge clotting at 12 hours.[10] A recent review of 170 clinical studies also concluded positively on the safety of the adsorber.[19]

We observed a trend of lower hematocrit and significantly lower thrombocytes toward the end of the experiment in the CytoSorb group. We attribute this finding, beside of
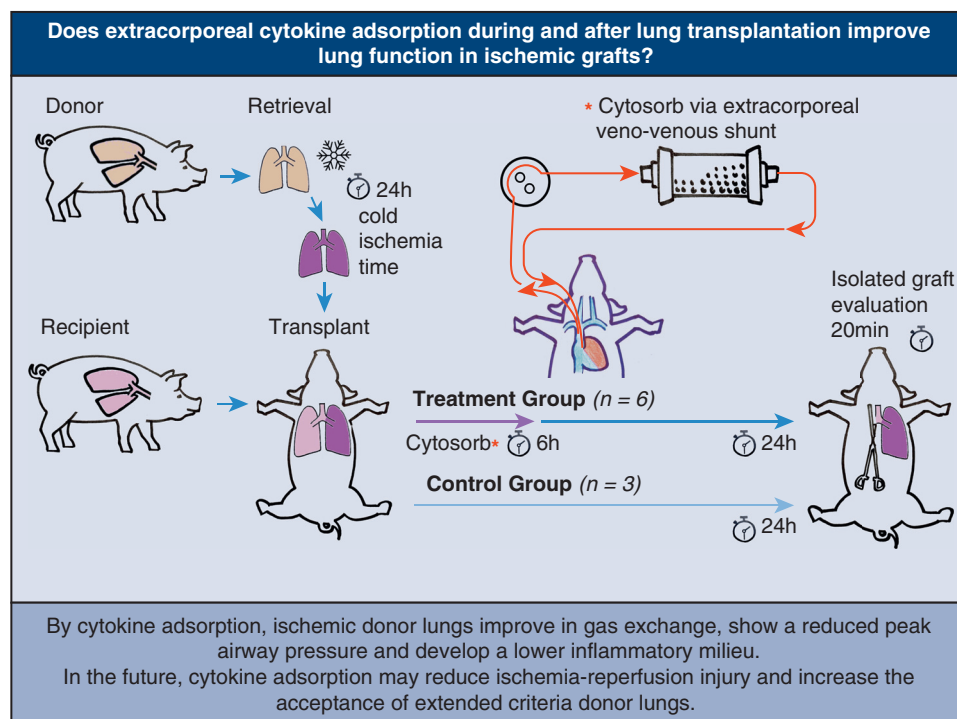
FIGURE 8. Graphical abstract.

some remaining blood in the adsorber after disconnecting the system, to the therapeutic anticoagulation that was required during the treatment period. However, anticoagulation was unexpectedly overdosed in our experiment, leading to blood loss through the operation wound. Simultaneously, we observed a slight decrease in systemic arterial pressure and electrolyte disturbances. We believe that in a more realistic clinical setting, where the thorax is closed and coagulation problems are corrected, these issues can be mitigated or at least significantly reduced. In severe cases, transfusion of platelets or red blood cells may be necessary to address these complications. No severe complications or device-related adverse events were observed in our study.

It is important to consider that the CytoSorb is nonselective, primarily adsorbing substances based on their hydrophobicity and molecular weight within the range of up to 60 kDa, and this adsorption is concentration dependent.[20,21] Therefore, the adsorber has the potential to remove not only pro-inflammatory cytokines and damage- and pathogen-associated molecular patterns but also unintended targets such as anti-inflammatory cytokines, metabolites, hormones, proteins, as well as drugs relevant to transplantation including antibiotics, antifungals and immunosuppressants.[22] In the previous study conducted in an ELVP setting, we have shown certain albumin removal effects,[8] as well as

effects on meropenem[9] and methylprednisolone.[9] However, in the case of Cyclosporin A, we did not observe a significant impact on its reduction based on our current in vivo findings. Nonetheless, due to the lack of comprehensive in vivo data on other immunosuppressants, dose adjustments are advised to maintain therapeutic drug levels during treatment with CytoSorb.

Based on our promising findings, the extracorporeal CytoSorb treatment has the potential to be used in the clinical settings in the future for the protection of lung allografts against the adverse effects of PGD. In addition, it could contribute to making organs available for transplantation that are currently considered to be unsuitable due to the extended criteria. In case of ischemic grafts, the reconditioning effect achieved through adsorption therapy could allow for longer transportation times and facilitate the scheduling of transplant operations. For the use of CytoSorb as in our study setting, the installation of a central venous circuit is required. In cases of preinstalled circuits of ECMO, heart–lung or hemodialysis machines, CytoSorb can easily be integrated as a low-flow bypass with a maximum flow of 700 mL/min. Alternatively, as previously demonstrated by our research group, CytoSorb could be a valuable tool in reconditioning donor lungs during EVLP.[9,11]

This study acknowledges several limitations. First, it was conducted with the least-possible number of animals to

reach a conclusion which may have affected the statistical power of the findings. In addition, the severity of donor lung damage and the duration of the observation period were limited by the local intensive care requirements. It is possible that more severe lung damage and a longer observation period could have revealed more significant findings. Due to the restrictions, the study was also unable to assess the potential effects of CytoSorb on acute or chronic rejection and the study acknowledges that other underlying graft damages may respond differently to CytoSorb treatment.

In conclusion, extracorporeal posttransplant cytokine adsorption is a good option in attenuating IRI after lung transplantation. CytoSorb may increase the acceptance of donor organs more prone to IRI.

Data Availability Statement

The data sets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest Statement

The authors reported no conflicts of interest.

The *Journal* policy requires editors and reviewers to disclose conflicts of interest and to decline handling or reviewing manuscripts for which they may have a conflict of interest. The editors and reviewers of this article have no conflicts of interest.

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