



# Bronchially instilled IgY-antibodies did not decrease pulmonary *P. aeruginosa* concentration in experimental porcine pneumonia

Alexander Otterbeck<sup>1</sup>  | Paul Skorup<sup>2</sup> | Katja Hanslin<sup>1</sup> | Anders Larsson<sup>3</sup>  |  
Johan Ståhlberg<sup>3</sup> | Hans Hjelmqvist<sup>4</sup> | Miklós Lipcsey<sup>5</sup> 

<sup>1</sup>Anesthesiology and Intensive Care, Department of Surgical Sciences, Uppsala University, Uppsala, Sweden

<sup>2</sup>Section of Infectious Diseases, Department of Medical Sciences, Uppsala University, Uppsala, Sweden

<sup>3</sup>Section of Clinical Chemistry, Department of Medical Sciences, Uppsala University, Uppsala, Sweden

<sup>4</sup>Anesthesiology and Intensive Care, School of Medical Sciences, Örebro University, Örebro, Sweden

<sup>5</sup>Hedenstierna laboratory, CIRBUS, Anesthesiology and Intensive Care, Department of Surgical Sciences, Uppsala University, Uppsala, Sweden

## Correspondence

Alexander Otterbeck, Department of surgical sciences, Uppsala University Hospital, entrance 70, 1 floor, SE-751 85 Uppsala, Sweden.  
Email: alexander.otterbeck@surgsci.uu.se

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**Background:** *P. aeruginosa* possesses antibiotic resistance, making treatment difficult. Polyclonal anti-*P. aeruginosa* IgY-antibodies (*Pa*-IgY) have antibacterial effects, but have not been studied in large animal pneumonia.

**Objectives:** To test if *Pa*-IgY decreases the concentration of *P. aeruginosa* in bronchoalveolar lavage in experimental porcine pneumonia over 27 hours.

**Method:** Norwegian landrace pigs were anesthetized, mechanically ventilated, and subject to invasive monitoring. The animals were randomized to receive either *P. aeruginosa* (control,  $n = 12$ ) or *P. aeruginosa* + *Pa*-IgY antibodies with a repeated dose of *Pa*-IgY after 12 hours (intervention,  $n = 12$ ) in the right lower pulmonary lobe. Bronchoalveolar lavage (BAL) cultures and physiological measurements were obtained repeatedly for 27 hours after which the pigs were sacrificed.

**Results:** BAL bacterial concentration increased in both groups and peaked at  $10^{7.28} \pm 10^{0.21}$  CFU/mL in the intervention group vs  $10^{7.36} \pm 10^{0.50}$  CFU/mL in the control group (n.s.). BAL bacterial concentration decreased during the experiment to  $10^{5.35} \pm 10^{0.39}$  CFU/mL in the intervention group vs  $10^{5.19} \pm 10^{0.37}$  in the control group (n.s.). The intervention group had lower heart rate ( $P < .001$ ), lower cardiac index ( $P < .01$ ), and lower arterial lactate ( $P < .001$ ) compared to the control group. The core temperature was lower in the intervention group than in the control group ( $P < .001$ ).

**Conclusion:** The chosen dose of *Pa*-IgY did not decrease concentrations of *P. aeruginosa* in BAL over 27 hours. We conclude that it is unlikely that there is a large effect of this specific dose and route of administration of *Pa*-IgY in this type of model.

## 1 | INTRODUCTION

*Pseudomonas aeruginosa* is a pathogen in humans known to host several antibiotic resistance mechanisms.<sup>1,2</sup> Ventilator-associated pneumonia (VAP) caused by *P. aeruginosa* is a common intensive care unit (ICU) problem with a mortality of approximately 30%-40%.<sup>3-5</sup> Non-conventional anti-microbial therapies are thus of interest against *P. aeruginosa* infections.

IgY-antibodies are produced in hens and resemble mammalian IgG. The IgY antibodies, due to eggs being part of the human diet, pose little risk of adverse immune reactions.<sup>6</sup> The production of IgY is inexpensive. After inoculation with *P. aeruginosa* hens produce eggs containing specific polyclonal anti-*P. aeruginosa* antibodies (*Pa*-IgY).<sup>7</sup> These antibodies bind primarily to the flagella of *P. aeruginosa* augmenting the phagocytic activity of polymorphonuclear neutrophils.<sup>8</sup> Previous studies have shown increased time to airway colonization

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by *P. aeruginosa* in cystic fibrosis patients treated with *Pa*-IgY.<sup>9,10</sup> *Pa*-IgY administered in a murine pneumonia model reduced pulmonary bacterial load.<sup>11</sup> Also, we have shown that experimental tracheal colonization in pigs is counteracted with nebulized *Pa*-IgY.<sup>12</sup>

We tested the hypothesis that *Pa*-IgY decreases the concentration of *P. aeruginosa* in bronchoalveolar lavage (BAL) in experimental porcine pneumonia over 27 hours. We also studied the effect on markers of inflammation, circulatory function, respiration, and kidney function. Pigs have similarities in anatomy, immune systems, and physiological responses to humans<sup>13,14</sup> and allow for invasive monitoring and repeated blood sampling.

## 2 | MATERIALS AND METHODS

### 2.1 | Ethical approval

Ethical approval was granted by the local animal ethics committee (application C155/14) and animals were handled according to guidelines from the Animal Ethics Board (Uppsala, Sweden) and the European Union's directives for animal research. MQTiPSS recommendations have been adhered to and data are presented according to ARRIVE guidelines.<sup>15,16</sup>

### 2.2 | Study protocol

This study is a randomized placebo-controlled double-blinded experimental animal study. Twenty-four cross-bred Norwegian landrace pigs, 6–8 weeks old, were used. Pigs were randomized using sealed envelopes containing either *Pa*-IgY or placebo (NaCl) in masked test tubes and administered through masked syringes. Group allocation was concealed until after the statistical analysis. Randomization occurred pairwise to protect against unforeseen day-dependent conditions. Previous studies used a different species or a different experimental model, we considered it unreliable to extrapolate for a power calculation. Twelve pigs per group were considered a convenient sample size to detect a relevant magnitude of effect on concentration of *P. aeruginosa* in BAL. The experiments were carried out in an ICU-like setting at an animal research facility at Uppsala University with the help of experienced staff. Experiments were started at approximately 10 o'clock after 2 hours of preparation and stabilization.

Anesthesia was induced with tiletamine/zolazepam 6 mg/kg (Zoletil, Virbac) and xylazine 2.2 mg/kg (Rompun, Bayer) followed by 100 mg ketamine (Ketaminol, Intervet, Stockholm, Sweden) and 20 mg morphine (Morfin Meda, Meda) when intravenous access was established. An anesthesia maintenance solution with 1g pentobarbital (Pentobarbitalnatrium, Apoteket) and 32.5 mg morphine mixed in 1000 mL of 25 mg/mL glucose was given at 8 mL/kg/h. Subtotal muscle relaxation was achieved using rocuronium 10 mg/mL (Esmeron, MSD) infused at 2.5 mg/kg/h to prevent shivering. Ringer's Acetate (Ringer-acetat, Fresenius Kabi) was given as a bolus of 20 mL/kg and thereafter as maintenance at 2 mL/kg/h intravenously. Additional

### Editorial Comment

*Pseudomonas aeruginosa* ventilator-associated pneumonia can occur in ventilated critically ill patients. This study investigated whether externally produced antibodies introduced into the lung might be beneficial to limit growth of experimentally introduced *P. aeruginosa* in pig lungs. While the intervention did not reduce *P. aeruginosa* concentrations in lungs in the investigated doses, the idea of delivering antibodies to sites of difficult to treat infections, as in this study, still seems attractive.

morphine and ketamine were administered as needed to keep the animals anesthetized. Depth of anesthesia was monitored using clinical signs and a withdrawal reflex when pinching the pigs with forceps under the soles of the feet was used as an indication of insufficient anesthesia. This reflex is maintained at the level of muscle relaxation in our model. If the pigs show any other signs of inadequate anesthesia and respond to a bolus of ketamine, depth of anesthesia is increased. All pigs were mechanically ventilated (inspiratory: expiratory time 1:2, fraction inspired oxygen (FiO<sub>2</sub>) 0.3, tidal volume 10 mL/kg, respiratory rate 25, and positive end-expiratory pressure (PEEP) 5 cmH<sub>2</sub>O) through a tracheostomy and received a central venous catheter, a pulmonary artery catheter, an arterial catheter, and a suprapubic urinary catheter. All pigs were given 750 mg of cefuroxime peri-operatively, to which *P. aeruginosa* is naturally resistant. After preparation and 30 minutes of stabilization, the experiment started. The experiment was carried out with animals lying on their side, changing side followed by alveolar recruitment every 6 hours.

Noradrenaline 20 µg/mL (Noradrenalin, Hospira Nordic) was administered as a continuous infusion starting at 5 mL/h and increased as needed to maintain mean arterial pressure (MAP) >60 mm Hg. At cardiac output <2 L/min, a clinical decision was made to either increase the rate of noradrenaline or to give a 15 mL/kg bolus of Ringer's Acetate. Normoventilation (PaCO<sub>2</sub> 4.5–6.5 kPa) was achieved by adjusting tidal volume. Oxygenation target (PaO<sub>2</sub> 10–30 kPa) was achieved by adjusting FiO<sub>2</sub> and for repeated hypoxemia PEEP was incrementally increased. The animals were heated using heating pads, fluid warmers, and covers to maintain a body temperature between 35 and 42°C. When animals were shivering and the level of anesthesia was deemed deep enough, 50 mg of rocuronium was given as a bolus and the infusion rate increased 10%. At the end of the experiment, the pigs were sacrificed by injection of 20 mL KCl. The study protocol was established prior to the start of data collection but has not been registered beforehand.

### 2.3 | IgY-Production

The method used for production of *Pa*-IgY has been previously described.<sup>9,17,18</sup> Briefly, White Leghorn hens received an intramuscular

injection with inactivated *P. aeruginosa*, and two booster injections were administered at 4-week intervals. Afterwards the eggs were collected and the yolks were isolated. Polyethylene glycol (PEG) was added to the yolks twice to isolate the yolk proteins. Ammonium sulfate was then added to remove the PEG and further purify large yolk proteins. This achieves a *Pa*-IgY fraction with a purity of more than 90%. The activity of purified *Pa*-IgY was measured with ELISA to yield equally active batches resulting in concentrations of *Pa*-IgY at approximately 10 mg/mL as measured spectrophotometrically. *Pa*-IgY was donated by Immunsystem I. M. S. AB (Uppsala, Sweden).

## 2.4 | Intervention

The pigs were randomized to receive either *P. aeruginosa* and *Pa*-IgY (intervention,  $n = 12$ ) or *P. aeruginosa* and NaCl (control,  $n = 12$ ) intrabronchially in masked syringes. After recording baseline data and collecting baseline samples, a catheter was placed, under bronchoscopic guidance, in the main bronchus of the right lower lobe. Ten mL of 10 mg/mL *Pa*-IgY or NaCl was administered through the catheter. Directly afterwards, 20 mL of  $10^9$  CFU/mL *P. aeruginosa* (PA-103, ATC 29 260, and CCUG31589) in log-phase was administered through the catheter. The strain is a virulent clinical isolate from sputum with a type 3 secretion system and exotoxin A production. The strain has been tested for when log-phase occurs and is resistant to pig serum. Both solutions were administered in the supine position before positioning the pigs on their right side. The dose of *Pa*-IgY was chosen because it has previously produced stable growth in BAL over 27 hours in our laboratory. The dose of *Pa*-IgY was chosen for practical reasons. Our previous studies had shown effect with half the dose and we expected greater concentrations of *P. aeruginosa* in this model. Higher concentrations of *Pa*-IgY were technically unfeasible and higher volumes were expected to have unwanted respiratory effects. After 12 hours a second dose of study drug was given, this was done to compensate for a drop in antibacterial effect after 12 hours seen in our previous study.<sup>12</sup>

## 2.5 | Data collection

Physiological parameters were measured at predefined time points. Blood samples were collected every 3 hours at 0-12 hours and 24-27 hours. Samples were analyzed for complete blood counts, plasma and urine creatinine levels and plasma cytokines including interleukin 6 (IL-6, detection limit  $> 125$  pg/mL), tumor necrosis factor  $\alpha$  (TNF- $\alpha$ , detection limit  $> 30$  pg/mL), and interleukin 1 $\beta$  (IL-1 $\beta$ , detection limit  $> 62$  pg/mL). Cytokines were analyzed using commercial ELISAs for porcine IL-6, TNF- $\alpha$ , and IL-1 $\beta$  (DY686, DY690B, and DY681, R&D Systems). Plasma was analyzed for anti-*P. aeruginosa* activity using ELISA to estimate antibody activity against *P. aeruginosa* in plasma.<sup>19</sup> Blood gases were analyzed in blood gas

analyzers at the animal research facility (ABL835- Flex Radiometer and OSM3 Oximeter Radiometer). Urine was collected during three time intervals; 0-12, 12-24, and 24-27 hours for urine output and calculation of creatinine clearance. Bacterial cultures were acquired through a 10-mL NaCl BAL in the right lower lobe catheter. One-hundred  $\mu$ L of the lavage was then cultured on CLED plates to determine the *P. aeruginosa* concentration. Blood was drawn from the arterial catheter and 100  $\mu$ L was cultured on CLED plates. Cultures were also acquired from a post-mortem lower right lobe lung biopsy. All cultures were incubated at 37°C and the bacterial concentration determined with viable count technique. Briefly, the sample is serially diluted and each dilution is cultured on a CLED plate. The CLED plates are incubated overnight and visible bacterial colonies are counted and corrected for the dilution to give an estimate of colony forming units in the sample.<sup>20</sup> For a complete list of endpoints, see Table 1 in the supplemental material. Six pigs (sham group) that did not receive any study drug or *P. aeruginosa* but underwent the same experimental protocol (except for the blinding protocol) were performed in the year before and during the main experiment. These pigs are included in the same ethical application and their complete data can be found in the supplemental material. Their data are briefly presented in Table 1.

## 2.6 | Statistical analysis

Data were tested for normality and is presented accordingly as mean  $\pm$  SD or median (IQR). Differences in baseline characteristics were tested for using a two-tailed independent Student's *t*-test or two-tailed Mann-Whitney U-test according to normality. Cardiac index (CI), arterial lactate (aLactate), mean pulmonary arterial pressure (MPAP), delivery of oxygen (DO<sub>2</sub>), TNF- $\alpha$ , IL-6, IL-1 $\beta$ , creatinine, static compliance, and BAL cultures were log-normally distributed and were thus log-transformed. The anti-*P. aeruginosa* activity in plasma was normally distributed for values above cut-off and was analyzed accordingly. To test differences for repeated measurements between groups and over time, mixed linear models (ANOVA III) were used. A *P*-value  $< 0.05$  was considered statistically significant, data were analyzed using SPSS (IBM SPSS Statistics for Windows, Version 25.0. IBM Corp) and Statistica (ver 13, Dell Inc).

## 3 | Results

One pig was excluded before randomization due to a soft tissue infection and replaced immediately. Twelve pigs were randomized to each group. One pig in the control group accidentally received a dose of *Pa*-IgY at 12 hours, this pig was therefore excluded from analysis after 12 hours. This mistake resulted in researches being unblinded to the treatment of this pig before statistical analysis. All other pigs were handled according to the blinding protocol.

**TABLE 1** Data for pigs receiving anesthesia only. Pigs performed with the same experimental protocol as study pigs but receiving no study drug or *P. aeruginosa* (n = 6). Values are mean ± SD for the highest and the lowest values during the experiment. Time point indicates when during the 27 hours the value occurred

Pigs receiving anesthesia only				
	Highest value	Time point (h)	Lowest value	Time point (h)
HR (beats x min <sup>-1</sup> )	97 ± 14	0	75 ± 36	18
MAP (mmHg)	86 ± 11	6	77 ± 8	12
MPAP (mmHg)	20 ± 2	5	16 ± 1	0
Cardiac index (L x min <sup>-1</sup> x m <sup>-2</sup> )	3.91 ± 1.81	27	2.54 ± 0.37	3
SVRI (dyn x s x cm <sup>-5</sup> x m <sup>-2</sup> )	2731 ± 735	6	1071 ± 276	27
aLactate (mmol x L <sup>-1</sup> )	2.0 ± 0.6	0	0.8 ± 0.1	15
ScvO <sub>2</sub> (%)	65.4 ± 0.4	27	49.2 ± 5.1	6
WBC (10 <sup>9</sup> x L <sup>-1</sup> )	23.0 ± 5.6	9	19.5 ± 5.9	0
Core temperature (°C)	39.9 ± 0.8	21	38.3 ± 0.5	0, 24
Creatinine (µmol x L <sup>-1</sup> )	74 ± 10	12	57 ± 24	27
Static compliance (mL x cmH <sub>2</sub> O <sup>-1</sup> )	37 ± 8	0	19 ± 5	27
PaO <sub>2</sub> /FiO <sub>2</sub> (mmHg)	427 ± 98	21	326 ± 40	6

Abbreviations: HR, Heart rate; MAP, Mean arterial pressure; MPAP, Mean pulmonary arterial pressure; CI, Cardiac index; SVRI, Systemic vascular resistance index; aLactate, Arterial lactate; ScvO<sub>2</sub>, Mixed central venous oxygen saturation; WBC, White blood cell count.

### 3.1 | Baseline characteristics

The intervention group was similar to the control group at baseline (Table 2).

### 3.2 | Bacterial cultures

There was an increase in BAL concentration of *P. aeruginosa* over time ( $P < .001$ ) with no difference between groups. The bacterial concentration was the greatest after 1 hour where BAL bacterial concentration peaked at  $10^{7.28} \pm 10^{0.21}$  CFU/mL in the intervention group vs  $10^{7.36} \pm 10^{0.50}$  CFU/mL in the control group. BAL bacterial concentration decreased during the experiment to  $10^{5.19} \pm 10^{0.77}$  CFU/mL in the intervention group vs  $10^{5.35} \pm 10^{0.39}$  CFU/mL in the control group (Figure 1). Three pigs from each group had growth of *P. aeruginosa* before the start of the experiment, the range of this growth was 1-24 CFU/mL, i.e. 10<sup>6</sup> less than during the experiment. The growth of *P. aeruginosa* might represent accidental contamination of individual samples. Growth of *B. bronchiseptica* was seen at the start of the experiment for 2 pigs in the control group and 4 pigs in the intervention group, no pigs had growth of *B. bronchiseptica* after the start of the experiment, range 10 CFU/mL->3000 CFU/mL. Two of 12 pigs in the intervention group had no growth in pulmonary biopsies, while all pigs in the control groups had pulmonary biopsy growth. Pulmonary biopsy *P. aeruginosa* concentration in the control group was  $10^{3.49} \pm 10^{2.25}$  CFU/mL vs  $10^{4.26} \pm 10^{1.51}$  CFU/mL in the intervention group with no difference between groups. There was bacterial growth of *P. aeruginosa* in blood cultures in one pig in the control group at 6h and one pig in the intervention group starting at 6h until the end of the experiment with no growth at 9h. In both pigs, the growth never exceeded 500 CFU/mL. No other pigs had bacteremia. There was no growth of *P. aeruginosa* in the sham group.

**TABLE 2** Characteristics at baseline

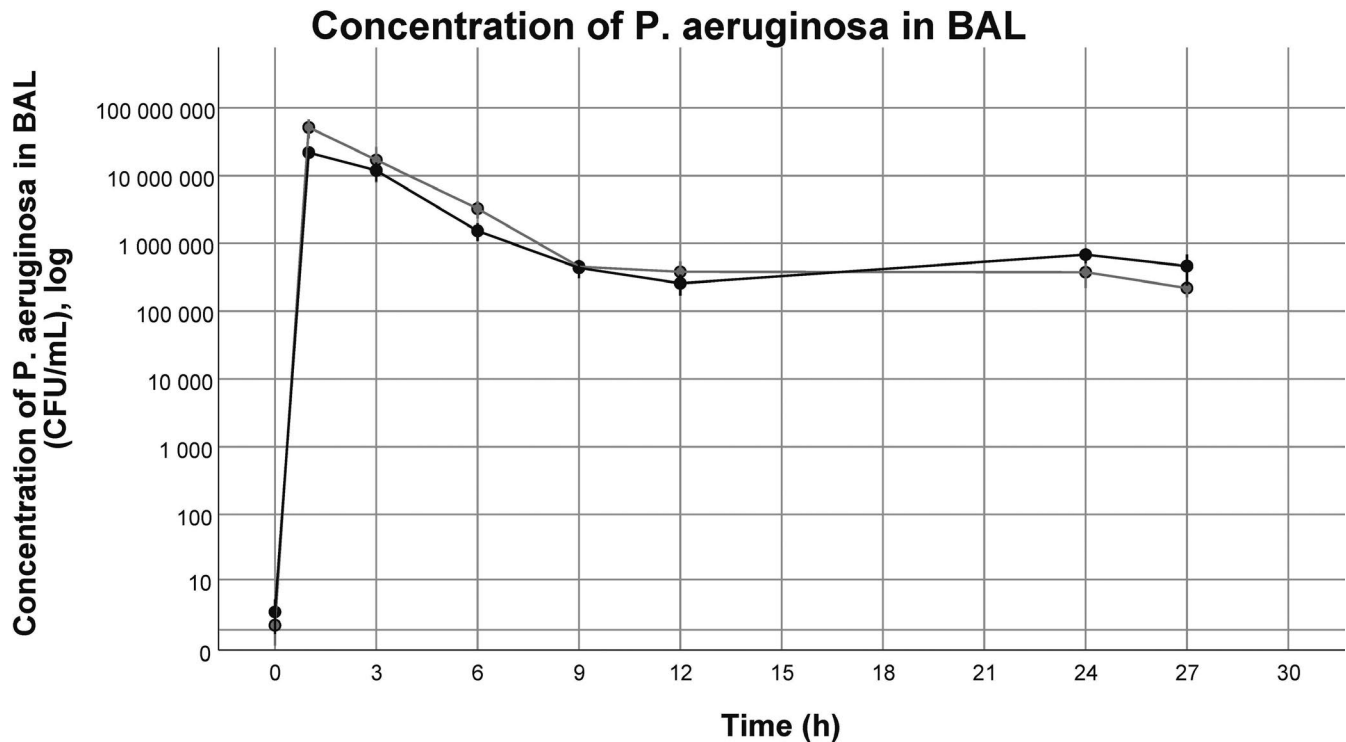
Baseline characteristics		
	Intervention	Control
Weight (kg)	28.7 ± 1.6	28.9 ± 1.4
HR (min <sup>-1</sup> )	96 ± 17	105 ± 22
MAP (mm Hg)	91 ± 14	89 ± 11
Temperature (°C)	38.8 ± 0.8	39.3 ± 1.0
aLactate (mmol x L <sup>-1</sup> )	1.7 (1.4-2.0)	1.6 (1.4-1.8)
Hemoglobin (g x L <sup>-1</sup> )	84 ± 7	88 ± 6
WBC (10 <sup>9</sup> x L <sup>-1</sup> )	13.6 (11.5-15.7)	15.4 (12.4-18.4)
Neutrophil count (10 <sup>9</sup> x L <sup>-1</sup> )	7.0 ± 2.6	7.9 ± 2.9
CI (L x min <sup>-1</sup> x m <sup>-2</sup> )	3.79 ± 0.99	3.84 ± 1.03
Static compliance (mL x cmH <sub>2</sub> O <sup>-1</sup> )	37 ± 12	34 ± 10
PaO <sub>2</sub> /FiO <sub>2</sub> ratio (mm Hg)	413 ± 72	384 ± 55

Note: Values are mean ± SD or median (IQR).

Abbreviations: HR, Heart rate; MAP, Mean arterial pressure; CI, Cardiac index; aLactate, Arterial lactate; WBC, White blood cell count; IL-6, Interleukin-6; TNF-α, Tumor necrosis factor α.

### 3.3 | Physiological parameters and laboratory analyses

Core temperature changed over time in both groups ( $P < .001$ ) with lower temperatures in the intervention group ( $P = .001$ ) (Figure 2A,B,C). White blood cell count (WBC), neutrophil granulocyte count (NGC), and IL-6 increased over time ( $P = .004$ ) with no difference between groups. There was no change over time or difference between groups for TNF-α or IL-1β. Heart rate (HR) increased over time ( $P < .001$ ) with



**FIGURE 1** Number of colonies of *P. aeruginosa* in BAL cultures. Values were log converted for analysis, note that the scale is logarithmic. Values presented are non-log converted and represent mean values, error bars denote SEM. Black line represents intervention group, gray line represents control group. BAL = Broncho alveolar lavage. T = Change in variable 0-27 hours,  $P < .05$ . Data were analyzed using mixed linear models

lower values in the intervention group ( $P < .001$ ). MAP decreased over time ( $P < .001$ ) with no difference between groups. Fifty percent of the pigs in the control group received noradrenaline at some point during the experiment and 67% in the intervention group. The median infusion rate for time points with ongoing noradrenaline infusion was 0.33 (1.04)  $\mu\text{g}/\text{kg}/\text{min}$  in the control group vs 0.06 (0.06)  $\mu\text{g}/\text{kg}/\text{min}$  in the intervention group with no difference between groups. CI increased over time, after a nadir at 2 hours, in both groups ( $P < .001$ ) with lower levels in the intervention group ( $P < .001$ ).  $\Delta$ lactate decreased over time in both groups ( $P < .001$ ) with lower levels in the intervention group ( $P < .001$ ), 11 of 24 pigs had lactate levels higher than 2 mmol/L at some point. MPAP increased over time in both groups with a nadir at 3 hours ( $P < .001$ ). There was a difference in MPAP between groups with higher values in the intervention group ( $P < .001$ ). Mixed central venous oxygen saturation ( $\text{ScvO}_2$ ) changed over time ( $P < .001$ ) with a nadir at 3 hours with no difference between groups. Systemic vascular resistance index (SVRI) decreased over time ( $P < .001$ ) with higher values in the intervention group ( $P = .041$ ).  $\text{DO}_2$  increased over time ( $P = .005$ ) with lower values in the intervention group ( $P < .001$ ). The ratio of venoarterial difference in  $\text{pCO}_2$  to the arteriovenous difference in oxygen content ( $\Delta\text{pCO}_2/\Delta\text{cO}_2$ ) changed over time in both groups ( $P = .009$ ) with higher values in the intervention group ( $P < .001$ ). Static compliance decreased over time ( $P < .001$ ) in both groups with lower values in the intervention group ( $P = .006$ ).  $\text{PaO}_2/\text{FiO}_2$  ratio changed over time in both groups ( $P < .001$ ) with no difference between groups. Hemoglobin (Hb) changed over time ( $P = .010$ ) with no difference

between groups. Creatinine clearance decreased over time ( $P = .010$ ) in both groups with no difference between groups. Creatinine levels increased over time in both groups ( $P < .001$ ) with lower values in the intervention group ( $P = .006$ ).

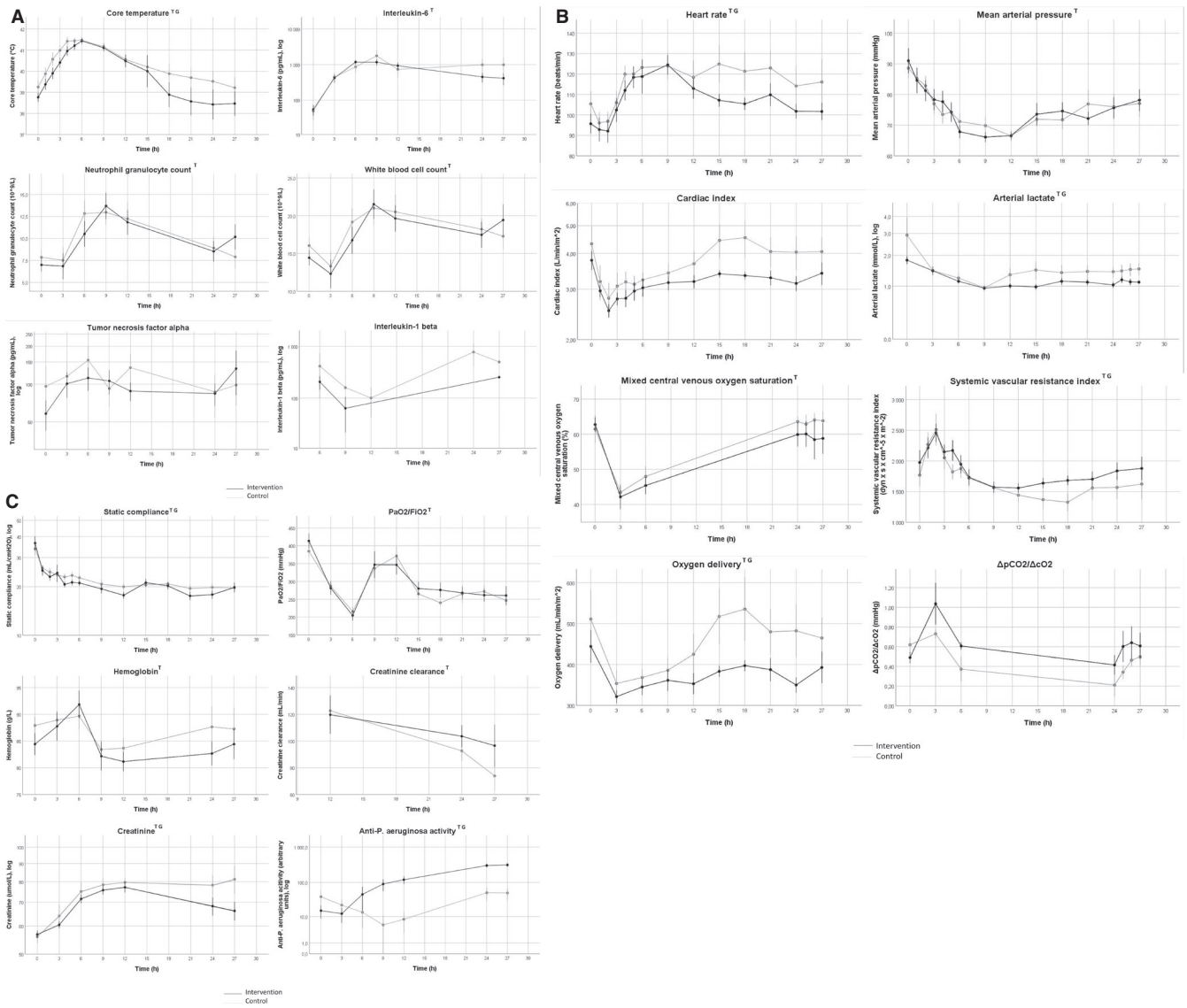
### 3.4 | Anti-*P. aeruginosa* activity

There was an increase over time ( $P < .001$ ) in anti-*P. aeruginosa* activity as measured by ELISA with a difference between groups ( $P < .001$ ) and a group-time interaction ( $P < .001$ ) (Figure 2C). There was a constant increase in the intervention group and values below the cut-off for analysis in the control group.

## 4 | DISCUSSION

Administration of *Pa*-IgY through the airways into the pulmonary lobe did not reduce BAL concentration of *P. aeruginosa* over 27 hours in this experiment.

Previous studies show an effect of *Pa*-IgY on patients with cystic fibrosis, in murine pneumonia and porcine tracheal colonization.<sup>9-12</sup> The total dose of *Pa*-IgY administered in this study was four times larger than in the previous porcine study while growth in BAL cultures in the control group was 10 000 times higher. The dose of *P. aeruginosa* is high compared to experimental studies of



**FIGURE 2** (A) Markers of inflammation over time. (B) Circulatory function over time.  $\Delta pCO_2/\Delta cO_2$  = venoarterial difference in partial pressure of carbon dioxide / arteriovenous difference in oxygen content. (C) Respiratory function, hemoglobin, kidney function, and anti-*P. aeruginosa* activity over time. Values indicate mean values, error bars denote SEM. log = Values were log converted for analysis, note that the scale is logarithmic. Values presented are non-log converted. Black line represents intervention group, gray line represents control group. PaO<sub>2</sub> = Arterial partial pressure of oxygen. FiO<sub>2</sub> = Fraction of inspired oxygen. T = Change in variable 0-27 hours,  $P < .05$ . G = Group difference after baseline to 27 hours,  $P < .05$ . Data were analyzed using mixed linear models

VAP.<sup>21</sup> We chose this dose of *P. aeruginosa* because it has previously produced reliable pneumonia and culture results in pilot experiments (unpublished data). In our BAL cultures, we had growth of  $10^{5.19} \pm 10^{0.63}$  CFU/mL which is a magnitude more than what is generally common in patients.<sup>22</sup> Future studies could be done with higher Pa-IgY:*P. aeruginosa* ratios. Also the delivery of Pa-IgY might have been hampered by atelectasis and blockage of smaller airways by exudate and purulent secretions. We found increased anti-*P. aeruginosa* activity in plasma after bronchial administration, indicating that Pa-IgY was delivered to the bloodstream via the capillary-alveolar membrane. These findings suggest that unventilated regions of the lung could be reached if antibodies were administered intravenously.

Concentrations of *P. aeruginosa* increased immediately and remained high throughout the experiment. Initial cultures most likely represent the recent instillation of bacteria into the right lower lobe. Inflammatory parameters peaked at 6 hours and P/F ratio as well as static compliance deteriorated until 6 hours, this is postulated to represent the transition from colonization to pneumonia. Manifest pneumonia is apparent when examining the lungs post mortem with widespread atelectasis, hemorrhage, and purulent secretions of the affected lobe (see supplement). In the sham pigs, circulatory variables expressed more normal hemodynamic values than pigs receiving *P. aeruginosa* (HR, MAP, and ScvO<sub>2</sub>), values for core temperature were lower and values for PaO<sub>2</sub>/FiO<sub>2</sub> were higher. This suggest that the model produces pneumonia with an inflammatory and hemodynamic

response with impairment in respiratory function. White blood cell count, static compliance, and plasma creatinine were more similar to the experimental animals, implying that anesthesia itself has an effect on some parameters.

The intervention group had lower HR, less hyperdynamic CI, lower aLactate, higher SVRI, and lower DO<sub>2</sub>. This might suggest that Pa-IgY affects aspects of the circulatory response to severe infections.  $\Delta pCO_2/\Delta cO_2$  was higher in the intervention group, suggesting increased CO<sub>2</sub> without increased anaerobiosis. This could be explained by decreased CI leading to stagnation of venous blood and therefore increased  $\Delta CO_2$ .<sup>23</sup> An inverse correlation between  $\Delta pCO_2$  and CI has previously been observed.<sup>24</sup> The lower aLactate levels in the intervention group also support that the increase in  $\Delta pCO_2/\Delta cO_2$  is flow dependent and not due to inadequate oxygen delivery. The difference in creatinine levels could be due to better hemodynamics which lessen pre-renal kidney injury or due to decreased inflammatory activity which could attenuate inflammatory kidney injury.<sup>25</sup> Although the mechanisms of the circulatory effects are not elucidated by our data, one might hypothesize that Pa-IgY binds to targets on the bacteria that normally elicit an inflammatory response. Pa-IgY are polyclonal and bind to several targets in the bacteria but it has been shown that Pa-IgY often bind to the flagella which are known to stimulate the immune system via the TLR-5 receptors.<sup>26,27</sup> This is in line with the lower core temperature seen in the intervention group. However, the lack of group difference in WBC, NGC, or in inflammatory cytokines suggests other inflammatory pathways. The TLR-5 pathway induces several inflammatory mediators, one of which is IL-8 which was not measured in this study.<sup>28</sup> Increases in pulmonary vascular resistance are a common reaction to bacteremia and sepsis in pigs.<sup>29</sup> The intervention group had higher MPAP which might imply an increased inflammatory reaction in the lungs due to Pa-IgY. This is in line with in vitro studies showing that Pa-IgY leads to activation of neutrophils.<sup>8</sup> The effects of Pa-IgY on our secondary endpoints are hypothesis generating and whether these effect are beneficial or not in the context of pneumonia cannot be answered by this study.

The strength of this study lies in its use of a mechanically ventilated large animal model with an experimental pneumonia resembling clinical VAP caused by aspiration. The model allows for complex physiological measurements and repeated blood and tracheal sampling. Risk for bias has been decreased by a randomized and blinded design. The number of animals is small but allows for observation of major effects. Culture of pulmonary biopsies showed heterogeneous growth, culturing of the entire right lower lobe might have produced more reliable cultures. We observed the pigs for 27 hours. However, longer experiments could be of interest since bacteria may increase for 72 hours in the lungs.<sup>30</sup> For a few variables (ScvO<sub>2</sub>, aLactate, and creatinine), there is a tendency toward increasing differences at the end of the experiment, it would be of interest to study these variable for a longer time.

Future research should explore different doses of Pa-IgY to treat pneumonia caused by *P. aeruginosa* and the ability of Pa-IgY to attenuate the inflammatory response. Intravenous administration may

also be considered in pneumonia to access atelectatic parts of the lung based on our data suggesting that Pa-IgY crosses the capillary-alveolar barrier.

## 5 | CONCLUSIONS

In summary, in this anesthetized and mechanically ventilated porcine model of experimental pneumonia, pulmonary instillation of the chosen dose of Pa-IgY did not decrease concentrations of *P. aeruginosa* in BAL over 27 hours. We conclude that it is unlikely that there is a large effect of this specific dose and route of administration of Pa-IgY in this type of model.

## ACKNOWLEDGEMENTS

We would like to thank the staff at the animal research facility for their help with animal handling and adherence to the experimental protocol.

## CONFLICTS OF INTEREST

A. Larsson and J. Stålberg are shareholder and employees of Immunsystem I. M. S. AB (Uppsala, Sweden), respectively. Immunsystem I. M. S. AB produced and marketed the antibodies used in this study at the time of the study. Immunsystem I. M. S. AB had no influence on the experimental protocol.

## ORCID

Alexander Otterbeck  <https://orcid.org/0000-0002-3577-4247>

Anders Larsson  <https://orcid.org/0000-0003-3161-0402>

Miklós Lipcsey  <https://orcid.org/0000-0002-1976-4129>

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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