

Signaling Molecules for Early Detection of Adverse Interactions during Mechanical Ventilation in Animal Models

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Abstract. *Aim: The early identification of adverse interactions during mechanical ventilation, investigated by multiplexed immunoanalysis. Materials and Methods: Twenty piglets (average age 7 weeks, weight 23 kg) were intubated and divided into groups: A, spontaneously breathing; B, protectively ventilated; C, ventilated with injurious strategy; D, ventilated with lung disability. At the 1st hour (time-1) and 12th hour (time-2) of the study, brain natriuretic peptide (BNP), intercellular cell adhesion molecules (ICAM-1), vascular cell adhesion molecules (VCAM-1), tumor necrosis factor-alpha (TNF-alpha), and interleukin-6 (IL-6) were analyzed in the blood. Results: The injurious ventilated group C exhibited an increase in both cell adhesion molecules ($p<0.01$), TNF-alpha and BNP ($p<0.05$) at time-1, and at time-2 further increases ($p<0.05$). In group D, an increase in ICAM-1 and BNP ($p<0.05$) at time-1, and increases in IL-6 and ICAM-1 ($p<0.05$) at time-2, with notable decreases in urine output were observed. Overall, the lung damage correlated with TNF-alpha ($r=0.904$), IL-6 ($r=0.740$), and ICAM-1 ($r=0.756$) levels. Conclusion: All five monitored molecules quickly and reliably signaled adverse interactions.*

The study was aimed at the requirements of everyday clinical practice to establish a simple and effective means for the early detection of the development of adverse organ dysfunction.

The secondary aim of the study was to assess the extent of such changes as a result of direct lung damage, incorrect ventilation strategy and the duration of artificial lung ventilation. The early detection of functional disorders could lead to the implementation of effective counter measures, which could support organ function or temporarily avoid or prevent irreversible damage of vital systems. Evidence that the systemic inflammatory response contributes to the development of disorders in the function of vital organs (1-3) was taken as the starting point. Bearing this in mind, a number of proinflammatory cytokines, some cell adhesion molecules and natriuretic peptide, seemed to be possible potential heralds of this pathological process.

A pig model was utilized because pigs have previously been recognized as having a comparative physiology with humans, including the molecular physiology of the respiratory tract, the heart and blood circulation, the liver and the kidney (4). In a previous experimental study, we examined the effect of a high tidal volume on the development of ventilator induced lung injury (5). This model of alveolar hyperinflation and direct lung damage was used to identify early laboratory signs of the development of adverse organ interactions.

The study was based on the assumption that higher tidal volume during mechanical ventilation and direct lung damage affects the function of a number of organs (1). Changes in organ function related to mechanical lung ventilation or pulmonary disability were compared using fully instrumented and relevant experimental animal models. In all groups of animals, lung function, liver, kidneys and global performance of both heart ventricles was assessed.

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Key Words: Mechanical ventilation, adverse interactions, signaling molecules, animal models.

Table I. Organ function parameters at time-1 in the ventilated groups.

Variable	Group D Median±SD	Group B Median±SD	D vs. B P-value	Group C Median±SD	D vs. C P-value
Bilirubin	5.00±0.49	3.40±0.43	<0.01	3.60±0.08	<0.05
Fibrinogen	2.37±0.77	1.33±0.49	<0.05	2.05±0.14	NS
CrCl	0.29±0.03	0.23±0.02	<0.05	0.19±0.07	<0.001
Cfw	-1.82±0.53	-0.78±0.31	<0.01	0.31±0.04	<0.001

B, Mechanical ventilation V_T 6 ml/kg; C, mechanical ventilation V_T 10 ml/kg; D, pulmonary disability with mechanical ventilation V_T 6 ml/kg; Bilirubin, serum levels (micromol/l); fibrinogen, plasma levels (g/l); CrCl, creatinine clearance (ml/min); Cfw, free water clearance (no units). P-values calculated by paired *t*-test; NS, not significant.

Materials and Methods

The study was approved by the Multidisciplinary Ethical Committee of the Faculty of Medicine in Pilsen (Czech Republic) according to the valid regulations of the Czech Republic and European Union (Helsinki Declaration 2004), and was carried out at an accredited experimental laboratory of the Faculty of Medicine in Pilsen.

The study included a total of 20 piglets (a sex ratio 2:3 in favor of females), aged 7 weeks with an average weight of 23 kg (range 19–27 kg), and of the stained black pig breed.

Setting of animals. The animals were premedicated intramuscularly with atropine 0.07 mg/kg (Atropin; Hoechst-Biotika, Slovak Republic) and azaperon 5.0 mg/kg (Stresnil; Janssen Pharmaceutica N.V., Belgium). Thiopental 10.0 mg/kg (Thiopental, VUAB Pharma, Czech Republic) was administered the venous route for the induction of anesthesia and tracheal intubation (internal diameter 5.5 mm, Kendall; GPS Prague, Ltd., Czech Republic) (6). Thiopental 2.0 mg/kg/h was continuously venously administered and bolus doses of fentanyl 5.0 µg/kg (Fentanyl; Hexal AG, Germany) were used for analgesation. A central venous catheter (Certofix Paed 5F; B. Braun, Germany) was inserted into the internal jugular vein and an arterial line (Arrow 22G; International CR, Germany) was surgically inserted into the femoral artery. Urinary drainage was accomplished by surgical cystostomy using a permanent urinary catheter (Arrow Cystofix 10F; International CR, Germany). All the animals were given Ringer's venous infusion 2.0 ml/kg/h (Ringer, Infusia, Czech Republic) during the study.

The animals were divided into four groups that differed in pulmonary disability or strategy of ventilation. Group A, the control group, was composed of tracheally intubated and spontaneously ventilating piglets ($n=5$), connected to a ventilator for 1 hour to assess lung mechanics. Group B ($n=5$) and group C ($n=5$) were made up of animals that were tracheally intubated without primary pulmonary disability in a supine position and mechanically ventilated for 12 hours with different tidal volumes (group B 6 ml/kg and group C 10 ml/kg). The animals in group D ($n=5$), with an experimental lung injury induced by the instillation of 50 ml 0.9% sodium chloride solution into a tracheal cannula, were subsequently mechanically ventilated with a tidal volume of 6 ml/kg.

The mechanical ventilation was carried out by a pressure-controlled regime (Servo Elema 900C; Siemens, Germany) with a constant setting at a rate of 26 breaths/min, a positive end-expiratory pressure of 6 cm H_2O and a fraction of inspired oxygen of 0.21. The functions of the extrapulmonary organs were not purposely influenced pharmacologically.

Measurements and calculations. All the animals were continuously monitored (Life Scope 9; Nihon Kohden, Japan) for the following: ECG, heart rate (HR; beat/min), pulse oxymetry (SpO_2 ; %), end-tidal carbon dioxide ($etCO_2$; kPa), central venous pressure (CVP; mmHg), systolic blood pressure (BP_{syst} ; mmHg), mean arterial blood pressure (MAP; mmHg) and diastolic blood pressure (BP_{diast} ; mmHg). Urine output (UO; ml/kg/h) and the body core temperature ($^{\circ}C$) were recorded using a permanent urinary catheter. Also recorded were: the values of peak inspiratory pressure (PIP; cm H_2O), mean airway pressure (Paw; cm H_2O), breathing rate (BR; breath/min), end-expiratory pressure (PEEP; cm H_2O), expiratory tidal volume (V_{Texp} ; ml/kg), minute respiratory ventilation (V_E ; l/min), and fraction of inspired oxygen (FiO_2). The following indices were calculated: alveolar-arterial oxygen tension difference ($AaDO_2$; kPa), arterial-alveolar oxygen tension difference (a/ADO_2 ; kPa), oxygenation index (OI), hypoxemic index (PaO_2/FiO_2 ; torr) and dead space to tidal volume ratio (V_D/V_T ; %), ventilation index (VI), dynamic compliance of lung (C_{dyn} ; ml/cm H_2O /kg), and dynamic airway resistance (Raw; cm H_2O /l/s) (7).

The following parameters were measured concurrently in the urine and serum: urea (mmol/l), creatinine (µmol/l), sodium (mmol/l), potassium (mmol/l), chloride (mmol/l), osmolality (mmol/kg). Alanine aminotransferase (ALT; microkat/l), aspartate-aminotransferase (AST; microkat/l), total bilirubin (microkat/l), glucose (mmol/l) and fibrinogen (g/l) were measured in serum and plasma. Blood gases and acid-base balance analysis were obtained from arterial blood. The creatinine clearance ($CrCl=[urinary(u)-creatinine \times urine\ output]/serum(s)-creatinine$; ml/min), glomerular filtration index ($GFI=u-Na/[u-creatinine/s-creatinine]$; without units), fractional excretion of sodium ($FeNa=[u-Na/s-Na]/[u-creatinine/s-creatinine]$; without units), and free water clearance ($Cfw=urine\ output-[urine\ output \times u-osmolality]/s-osmolality$; without units) were all calculated (8).

Echocardiographic examination was performed repeatedly (probe 5.0 Hz; Sono Ace 9900; Multibeam 3D Ultrasound

Table II. Signaling molecules in groups of ventilated animals at time-1.

Group	TNF-alpha		IL-6		BNP		VCAM		ICAM	
	Median±SD	1 st Q–3 rd Q	Median±SD	1 st Q–3 rd Q	Median±SD	1 st Q–3 rd Q	Median±SD	1 st Q–3 rd Q	Median±SD	1 st Q–3 rd Q
A	72.96±7.08	68.45–73.31	28.94±7.75	28.94–28.44	1.08±0.56	0.42–1.65	25.78±1.09	25.44–26.62	8.02±2.27	4.51–8.68
B ₁	74.8±13.72	41.66–75.36	54.00±3.75	52.05–55.01	1.01±0.27	0.89–1.23	10.5±1.02	7.47–12.18	57.4±7.33	39.77–81.56
C ₁	72.61±9.22	62.76–75.09	39.73±22.27	28.88–68.07	1.48±0.55	1.09–1.79	38.26±0.42	36.76–38.06	28.86±1.34	27.77–31.75
D ₁	31.78±14.07	25.08–45.46	28.94±2.27	27.94–32.58	1.49±0.47	1.18–1.48	40.13±0.93	39.56–40.39	66.88±2.27	66.36–67.96
Comparison	P-value	R	P-value	R	P-value	R	P-value	R	P-value	R
B ₁ vs. A	NS	–0.752	NS	–0.333	NS	0.524	NS	–0.912	<0.05	0.136
C ₁ vs. A	NS	–0.279	NS	0.999	NS	0.964	<0.001	0.931	<0.001	–0.010
D ₁ vs. A	<0.01	–0.465	NS	–0.333	NS	0.085	<0.001	0.155	<0.001	0.158
C ₁ vs. B ₁	NS	0.181	NS	–0.351	NS	0.673	NS	–0.761	<0.05	0.304
D ₁ vs. B ₁	NS	–0.118	NS	–0.333	NS	–0.801	NS	–0.331	NS	–0.552
D ₁ vs. C ₁	NS	–0.349	NS	–0.351	NS	–0.109	<0.05	–0.211	<0.001	0.941

A, Spontaneously breathing; B, mechanical ventilation V_T 6 ml/kg; C, mechanical ventilation V_T 10 ml/kg; D, pulmonary disability with mechanical ventilation V_T 6 ml/kg; B₁/C₁/D₁, time-1; TNF-alpha, tumor necrosis factor alpha (pg/ml); IL-6, interleukin 6 (pg/ml); BNP, brain natriuretic peptide (ng/ml); VCAM, vascular cell adhesion molecule-1 (ng/ml); ICAM, intercellular adhesion molecule-1 (ng/ml); 1stQ–3rdQ, 1st to 3rd quartile range. *P*-values calculated by paired *t*-test; NS, not significant; *R*, Spearman's correlation coefficient.

System; Medison Co. Ltd., Korea) and the following indices were recorded: left ventricular shortening fraction (SF), Tei-index of myocardial performance of the right (RIMP) and left (LIMP) heart ventricles (IMP= [isovolumic relaxation time+isovolumic contraction time]/ ejection time). The Tei-index evaluates the global systolic and diastolic function of each ventricle. An increase in the index value represents myocardial functional impairment (9).

The following allogeneic immunoanalyses were performed for the serum and plasma: interleukin-6 (IL-6; pg/ml; RD-ELISA), tumor necrosis factor-alpha (TNF-alpha; pg/ml; RD-ELISA), intercellular cell adhesion molecule-1 (ICAM-1; ng/ml; Bender-ELISA), vascular cell adhesion molecule-1 (VCAM-1; ng/ml; Bender-ELISA) and brain natriuretic peptide (BNP; ng/ml; NTProBNP Bachem-EIA).

Study protocol. A recovery interval after complete tracheal intubation and insertion of 60 minutes (time-0) was allowed. The clinical assessment and respiratory and circulatory parameters were recorded. Blood samples were obtained after the recovery interval, one hour after instrumentation (time-1) in all the animal groups (A-D) and after twelve hours (time-2) in the mechanically ventilated groups (B-D). The blood samples were collected from the arterial line and urine from the urinary catheter. The samples were sent for laboratory analysis. Before the end of the study while each animal was under general anesthesia, tissue samples were taken from the heart, lungs, liver and kidney for morphological examination.

All the animals were euthanized at the end of the study by intravenous administration of a cardioplegic bolus dose solution at

15 ml/kg (Infuse Thomas cum procain; Ardapharma, Czech Republic). The corpses were treated according to the current regulations of the Czech Republic and European Union.

Statistical analysis. All the measured parameters and laboratory data were presented as median±standard deviation (median±SD) and 1st to 3rd quartile range (1stQ–3rdQ) and the differences between groups by median and SD with a 95% confidence interval (95% CI). For qualitative analysis of the accuracy of the variables reference interval dispersion (Wilcoxon-Shapiro), the linearity, reproducibility agreement was used. The difference between treatments was compared using two sample paired *t*-test and a bias analysis was performed according to the statistical approach described by Bland and Altman (10). Correlation was tested using non-parametric Spearman's correlation coefficient. A *p*-value less than 0.05 was considered to be significant. All the data were analyzed using statistical PC software (Analyze-it 211 Software Ltd., version 2008).

Results

All the measured values, laboratory analysis results and calculated parameters from the spontaneously breathing animals in group A were used as control data. Overall, the data obtained showed no significant differences between the groups of ventilated animals (groups B, C and D) and the control group A (*p*=3.591). No damage to the function of the monitored organs was found at time-1.

Differences between the organ function parameters in the groups of ventilated animals at time-1 are listed in Table I.

Table III. Organ function parameters at time-2 (B_{12} , C_{12}) and comparison with time-1 (B_1 , C_1) in groups B and C.

Variable	Group B_{12} Median \pm SD	Group B_1 Median \pm SD	B_{12} vs. B_1 P-value	Group C_{12} Median \pm SD	Group C_1 Median \pm SD	C_{12} vs. C_1 P-value
Bilirubin	11.60 \pm 13.88	3.40 \pm 0.43	<0.05	3.50 \pm 0.06	3.60 \pm 0.08	NS
Fibrinogen	1.22 \pm 0.39	1.33 \pm 0.49	<0.05	1.94 \pm 0.25	2.05 \pm 0.14	NS
CrCl	0.12 \pm 1.10	0.23 \pm 0.02	<0.01	0.20 \pm 0.51	0.19 \pm 0.07	NS
Cfw	0.14 \pm 1.53	-0.78 \pm 0.31	<0.01	0.29 \pm 0.04	0.31 \pm 0.04	NS
RIMP	0.31 \pm 0.03	0.24 \pm 0.01	<0.05	0.30 \pm 0.03	0.25 \pm 0.02	<0.05
LIMP	0.30 \pm 0.01	0.29 \pm 0.02	NS	0.38 \pm 0.05	0.30 \pm 0.04	<0.05
OI	2.80 \pm 1.13	3.08 \pm 1.05	NS	4.33 \pm 2.44	2.10 \pm 1.23	<0.05
UO	2.00 \pm 0.31	2.03 \pm 0.35	NS	0.96 \pm 0.32	1.70 \pm 0.20	<0.01
SF	0.44 \pm 0.07	0.45 \pm 0.05	NS	0.43 \pm 0.03	0.43 \pm 0.04	<0.05

B, Mechanical ventilation V_T 6 ml/kg; C, mechanical ventilation V_T 10 ml/kg. Serum bilirubin (μ mol/l); plasma fibrinogen (g/l); CrCl, creatinine clearance (ml/min); Cfw, free water clearance (without units); RIMP, LIMP, index of myocardial performance of the right or left heart ventricle; OI, oxygenation index; UO, urine output (ml/kg/h); SF, shortening fraction of the left heart ventricle. P-values calculated by paired *t*-test; NS, not significant.

Table IV. Signaling molecules in groups of ventilated animals at time-2 (B_{12} , C_{12} , D_{12}) and comparison with time-1 (B_1 , C_1 , D_1).

Group	TNF-alpha Median \pm SD 1 st Q–3 rd Q		IL-6 Median \pm SD 1 st Q–3 rd Q		BNP Median \pm SD 1 st Q–3 rd Q		VCAM Median \pm SD 1 st Q–3 rd Q		ICAM Median \pm SD 1 st Q–3 rd Q	
B_{12}	75.93 \pm 13.72 57.01–82.10		28.94 \pm 6.75 26.54–30.92		1.53 \pm 0.22 1.32–1.92		37.44 \pm 1.02 37.30–38.91		25.40 \pm 0.24 24.84–25.55	
C_{12}	85.74 \pm 21.62 60.25–98.74		28.94 \pm 1.12 26.74–29.87		1.83 \pm 0.42 1.72–2.01		38.90 \pm 0.92 37.62–39.23		41.12 \pm 1.37 40.90–42.33	
D_{12}	23.41 \pm 11.33 22.23–38.07		322.06 \pm 224.72 137.42–730.64		0.92 \pm 0.17 0.81–1.00		8.74 \pm 5.38 5.99–14.90		53.06 \pm 5.48 34.65–58.60	
Comparison	P-value	R	P-value	R	P-value	R	P-value	R	P-value	R
B_{12} vs. B_1	NS	0.919	NS	-0.285	<0.05	-0.038	NS	-0.571	<0.05	0.593
C_{12} vs. C_1	NS	-0.171	NS	-0.117	NS	0.279	<0.01	0.840	<0.001	-0.526
D_{12} vs. D_1	<0.05	0.250	<0.05	-0.085	<0.01	0.117	NS	-0.486	NS	-0.882
C_{12} vs. B_{12}	NS	0.567	NS	0.994	<0.01	-0.796	NS	-0.193	<0.001	0.708
D_{12} vs. B_{12}	<0.05	-0.257	<0.05	-0.214	<0.01	-0.232	NS	-0.292	NS	0.355
D_{12} vs. C_{12}	<0.05	-0.337	<0.05	-0.143	<0.01	0.497	NS	0.095	NS	-0.152

B, Mechanical ventilation V_T 6 ml/kg; C, mechanical ventilation V_T 10 ml/kg; D, pulmonary disability with mechanical ventilation V_T 6 ml/kg. TNF-alpha, tumor necrosis factor alpha (pg/ml); IL-6, interleukin 6 (pg/ml); BNP, brain natriuretic peptide (ng/ml); VCAM, vascular cell adhesion molecule-1 (ng/ml); ICAM, intercellular adhesion molecule-1(ng/ml). 1stQ–3rdQ, 1st to 3rd quartile range. P-values calculated by paired *t*-test; NS, not significant; R, Spearman's correlation coefficient of the data obtained.

Differences in the signaling molecules were already apparent between all four groups of animals at time-1 ($p < 0.01$), and are summarized in Table II.

According to the summary of the total dataset of all the ventilated animals, no damage to the function of the monitored organs was found even at time-2 and the organ function parameters in groups B and C are listed in Table III. Scattering of the data was minimal ($p = 4.187$).

The signaling molecules produced in group B at time-2 and in comparison with time-1 are summarized in Table IV.

The correlations, level of reliability and confidence intervals of targeted dependent and independent variables are summarized in Table V. The pro-inflammatory cytokines which correlated with the data expressing the function of the vital organs are listed in Table VI.

Correlations were not significant between the other signaling molecules and the organ, function or between TNF-alpha or IL-6 and the other organs.

A detrimental consequence of ventilation strategy was validated by histological examination of the lung parenchyma

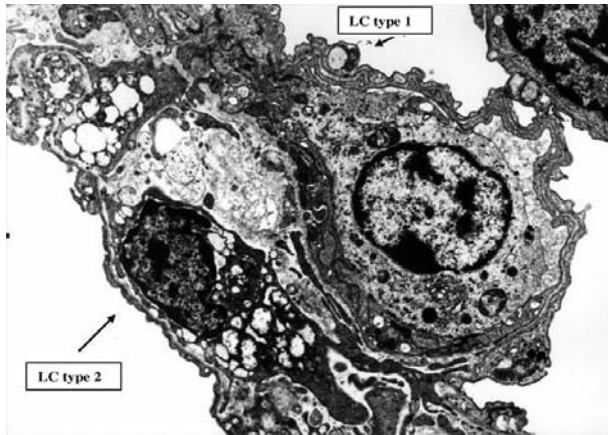


Figure 1. Electron photomicrograph of injured high volume (V_T 10 ml/kg) mechanically ventilated lung tissue in group C. There is severe alveolar deconfiguration with damage to type-1 lung cells (LC type 1; arrow), increase in type-2 cell numbers (LC type 2; arrow) and alveolar septal infiltration of macrophages and venule obliteration. Ultra structure, uranyl acetate, magnification 4000 \times .

of the animals from group C, as shown in Figure 1. Histological examination of tissue samples of liver (Figure 2), myocardium (Figure 3) and kidney (Figure 4) did not show pathological morphological changes in any of the groups.

Discussion

Recently several clinical and experimental studies have focused on organ interaction during mechanical ventilation of diseased. Airway pressure release ventilation had a protective influence on renal function in patients with acute lung injury (11); renal failure resulted from of cardio-renal syndrome (12); patients with renal failure required optimal interventional treatment strategy (13); experimental studies with lung impairment in a murine model identified the effect of a mechanical ventilation on the development of systemic organ inflammation (14) and mechanical ventilation induced inflammatory reactions leading to pulmonary, hepatic and renal dysfunctions in experimental pneumonia (1). Experimental evidence had also demonstrated renal functional impairment in dogs with acute lung injury after aspiration of gastric content (15) and acute lung injury caused by non-protective strategy of mechanical ventilation and exclusively renal functional changes without morphological changes (16).

In the present study, mechanical ventilation using inappropriate higher tidal volumes caused alveolar distension and led to progressive changes in inflammatory response, and supported previous report that induced such mechanical stimulus has been shown to induce expression of soluble adhesive molecules and cytokine activation (3, 4, 7). No data published so far has, however, been able to fully exclude the

Table V. Correlation, regression, and confidence intervals of targeted dependent and independent variables in the total dataset.

Dependent variables	Independent variables	R^2	95% CI interval	P-values
TNF-alpha	RIMP	0.601	129.35 to 257.49	<0.01
	OI	0.394	60.45 to 99.10	<0.05
	FeNa	0.605	41.54 to 63.04	<0.01
	GFI	0.564	41.13 to 63.62	<0.01
IL-6	AaDO ₂	0.394	59.23 to 186.86	<0.05
	V_D/V_T	0.367	0.38 to 8.26	<0.05
	VI	0.570	0.71 to 2.26	<0.01
	AST	0.395	10.76 to 121.67	<0.05
BNP	CrCl	0.372	0.11 to 2.08	<0.05
ICAM	Fibrinogen	0.395	2.73 to 30.88	<0.05
	RIMP	0.350	9.84 to 258.24	<0.05

TNF-alpha, Tumor necrosis factor alpha (pg/ml); IL-6, interleukin 6 (pg/ml); BNP, brain natriuretic peptide (ng/ml); ICAM, intercellular cell adhesion molecule-1 (ng/ml). RIMP, right ventricle index of myocardial performance; OI, oxygenation index; FeNa, fractional excretion of sodium; GFI, glomerular filtration index; AaDO₂, arterio-alveolar oxygen tension difference (kPa); V_D/V_T , dead space to tidal volume ratio (%); VI, ventilation index; AST, aspartate-aminotransferase (microkat/l); CrCl, creatinine clearance (ml/min). R^2 , Spearman's multiple correlation coefficient of the data; CI, confidence intervals; P-values calculated by paired *t*-test; NS, not significant.

Table V₁. Pro-inflammatory cytokines and correlations with the function of vital organs.

Dependent variable	Independent variable Parameters of function	n	F	R	P-value
TNF-alpha	Blood circulation time-2	15	11.181	0.904	<0.001
	Kidney time-2	15	5.471	0.867	<0.05
	Liver time-2	15	6.171	0.844	<0.05
	Respiratory tract time-2	15	15.682	0.977	<0.001
IL-6	Liver time-1	20	3.585	0.575	<0.05
	Liver time-2	15	11.030	0.903	<0.01
	Respiratory tract time-1	20	3.786	0.740	<0.01

TNF-alpha, Tumor necrosis factor alpha (pg/ml); IL-6, interleukin 6 (pg/ml); F, Fourier's factor; R-Spearman's correlation.

effect of underlying lung pathology and/or abnormal cardiopulmonary interactions on the function of extrapulmonary organ systems.

In the present study, remarkable changes were found after only one hour of mechanical ventilation with the development of a systemic inflammatory response and worsening performance of the right heart ventricle. The early inflammatory response was demonstrated by the significantly higher plasma levels of soluble adhesive molecules in both the high tidal volume target groups in comparison with the

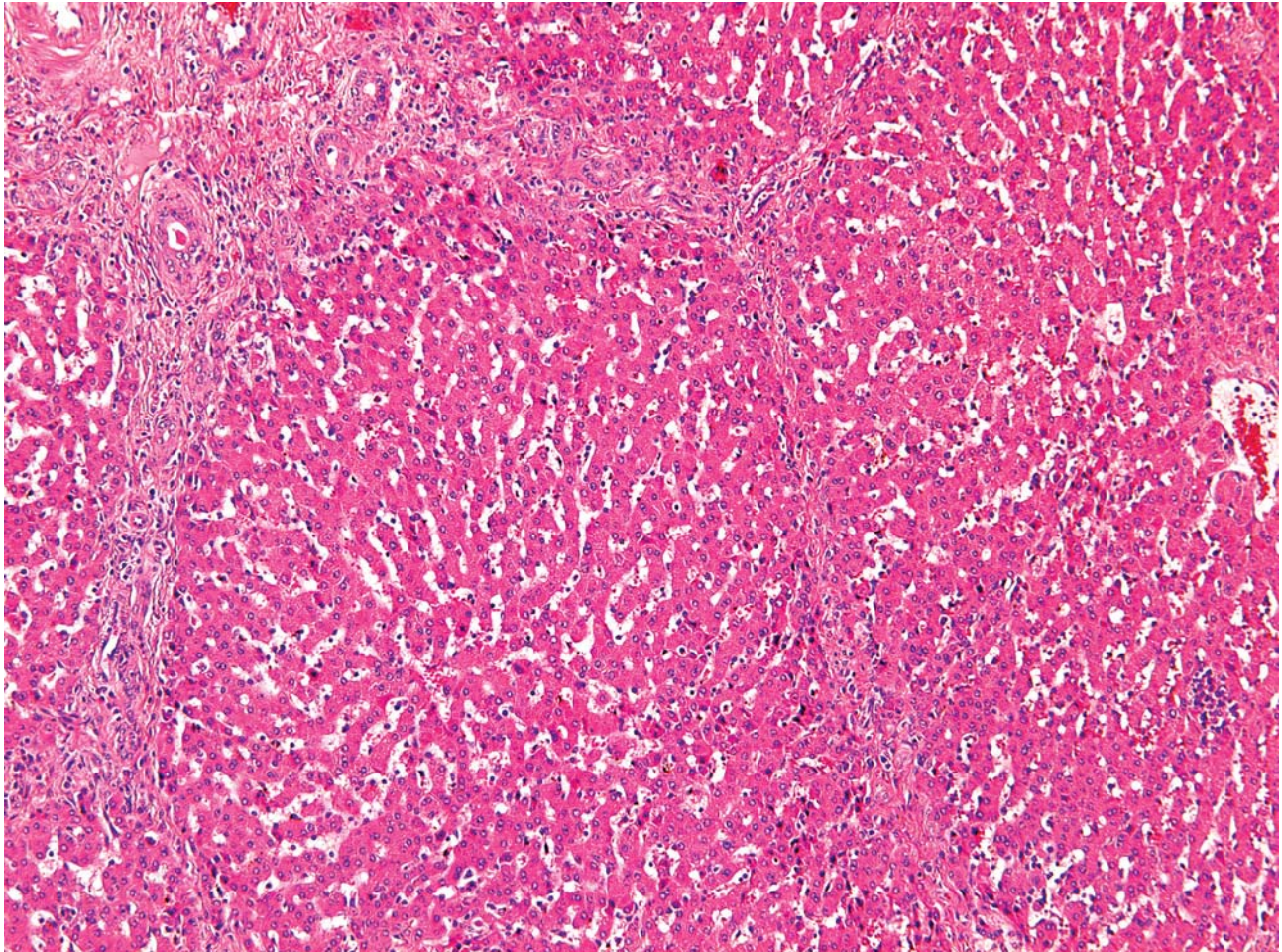


Figure 2. Optical photomicrograph of liver tissue from an animal from group C. Sampled from the right lobe of the liver of the same animal as in Figure 1, showing normal morphology of hepatocytes. Blood vessels and bile ducts were also normal. Fixed with 10% formalin solution, dyed with hematoxylin eosin. Magnification $\times 40$.

group of spontaneously breathing animals. After the first hour, the plasma levels of VCAM-1 and ICAM-1 molecules were higher in the group with the incorrect strategy in comparison with the protective strategy of mechanical ventilation in animals with lung injury (see Table III). Regression analysis of the total dataset demonstrated that changes in the signaling molecules were not organ-specific. In the first hour of the study, mechanical ventilation in the two target groups C and D had a minimal effect on blood oxygenation, systemic blood pressure, liver function and urine output. Urine output was, as expected, dependent on the mean systemic arterial pressure.

The deterioration of the right heart ventricular functional during the first hour of mechanical ventilation did not correlate with plasma levels of brain natriuretic peptide. This fact could only be explained by subtle changes to the right ventricular myocardial performance and/or activation of

other neuro-humoral autoregulation preventing cardiac atrial dilatation. The index of myocardial performance assesses both the systolic and diastolic function of the cardiac ventricles and appears to be sensitive to changes of preload and afterload in an acute experimental setting (17). It is possible to speculate that an inappropriate strategy of mechanical ventilation affects the systolic function and ventricle afterload more than the preload and diastolic function of the ventricles. Consequently atrial distention and increased levels of BNP could occur.

After twelve hours of mechanical ventilation, minimal differences in the parameters of vital organ function, such as a significant decrease in urine output were observed, although as expected, adverse interactions did occur. These minimal changes in function corresponded to changes in the expression of the signaling molecules. The incorrect, injurious mechanical ventilation contributed to a reduction

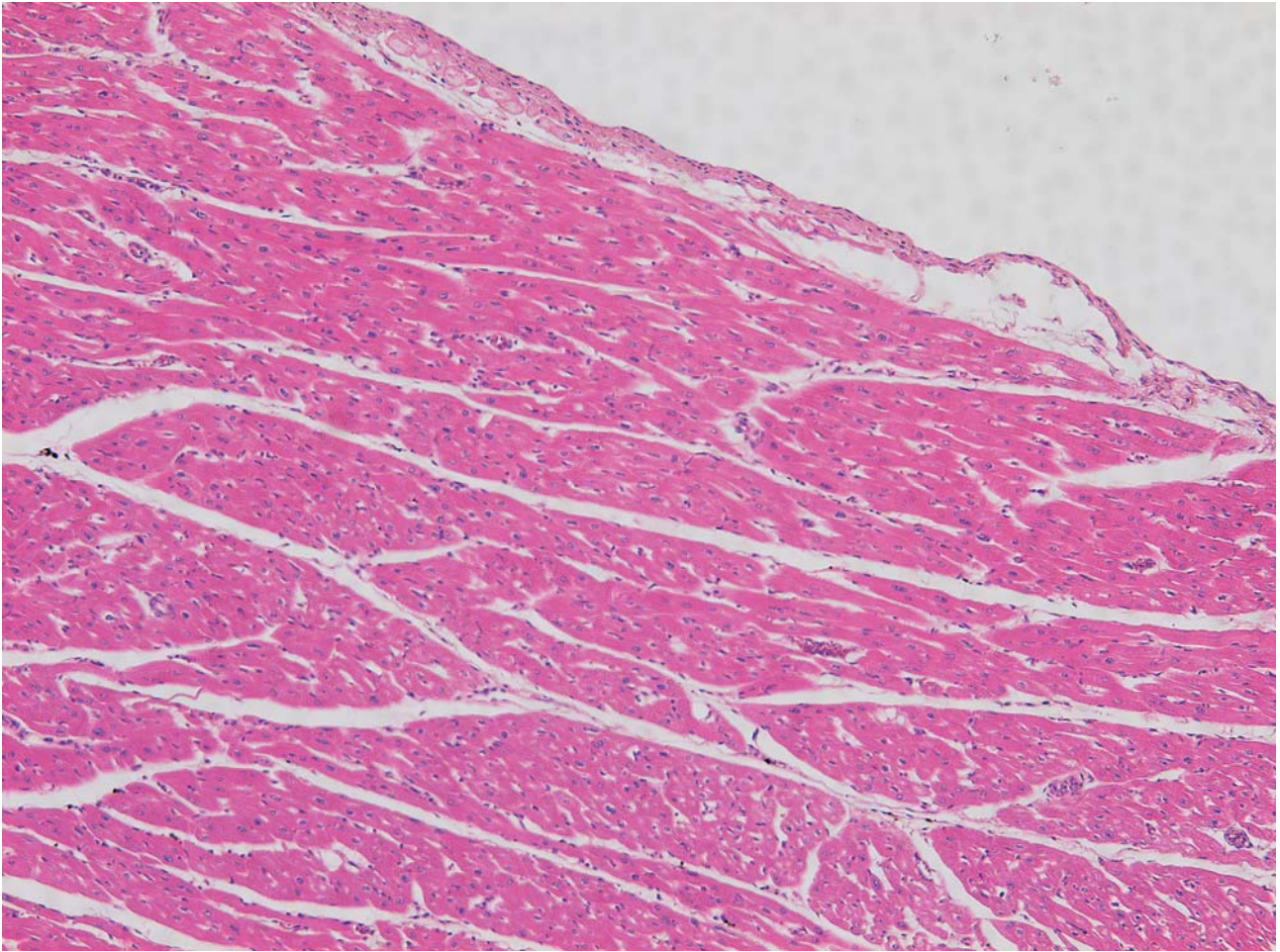


Figure 3. Optical photomicrograph of myocardium. Tissue sample of the left ventricle from the same animal from group C: Endocardium without pathological findings, myocytes were normal and free of degenerative or hypoxemic changes. Tissue fixed with 10% formalin solution, dyed with hematoxylin eosin. Magnification $\times 100$.

in performance of the heart ventricles, venous congestion, an increase in the oxygenation index, a decrease in urine output and a significant elevation of serum levels of both soluble cell adhesion molecules (see Table III). Lung injury and the protective setting of mechanical ventilation did not significantly affect the function of the monitored organ systems, but led to increased levels of IL-6 in the serum (see Table IV). Over time, the non-protective strategy of mechanical ventilation had a strong influence on organ function.

TNF- α , IL-6, ICAM-1, VCAM-1 and BNP are all good potential markers for the early detection of adverse interactions during mechanical lung ventilation. Liver damage correlated with TNF- α and IL-6, while kidney damage correlated with IL-6. In the present study, the possibility of primary disability was excluded by using only clinically healthy animals. Mechanical lung ventilation

prevented hypoxia and hypercapnia during the course of the experiment. The results could have been partially affected by dispersion of the data for renal indices (Anderson-Darling; $p=0.05$) caused by post-analytical mathematical calculations of the laboratory values and also by pre-analytical stress from the invasive interventions in individual animals. The presented data were sufficiently accurate (Bland-Altman; $p<0.01$) as the comparisons were not based on the absolute values assessment but on their differences.

Conclusion

An early inflammatory response is activated in mechanically ventilated animals with acute lung injury or non-protective ventilation strategy. Rapid and reliable heralding of adverse interactions during mechanical ventilation is provided by all five of the studied molecules.

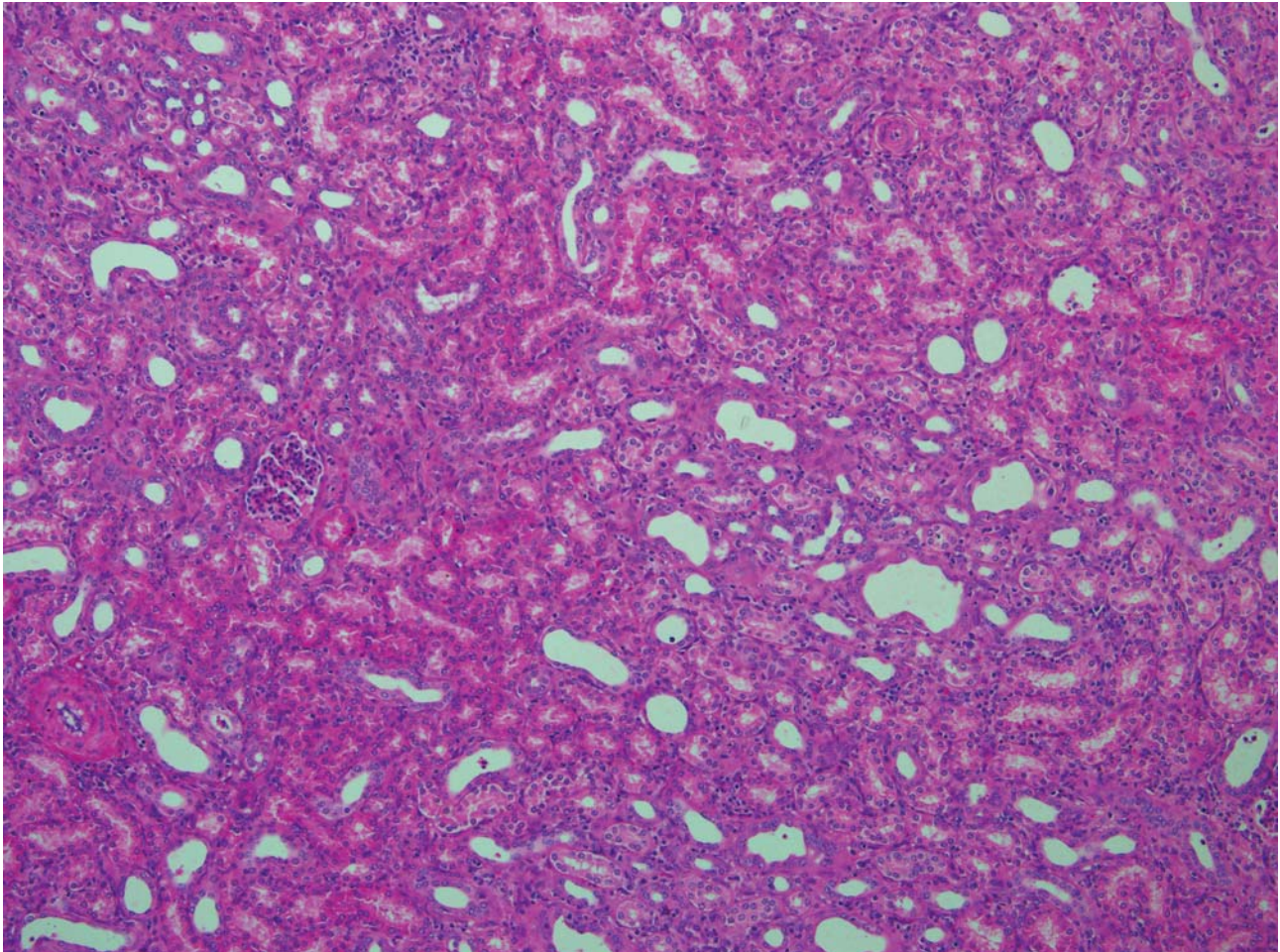


Figure 4. Optical microscope image of kidney tissue. Cortex and renal medulla from the same animal from group C without pathological findings. Tissue fixed with 10% formalin solution, dyed with hematoxylin eosin. Magnification $\times 40$.

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