Early Cardiac Injury in Acute Respiratory Distress Syndrome: Comparison of Two Experimental Models

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Summary

Acute respiratory distress syndrome (ARDS) is characterized by diffuse lung damage, inflammation, oedema formation, and surfactant dysfunction leading to hypoxemia. Severe ARDS can accelerate the injury of other organs, worsening the patient´s status. There is an evidence that the lung tissue injury affects the right heart function causing cor pulmonale. However, heart tissue changes associated with ARDS are still poorly known. Therefore, this study evaluated oxidative and inflammatory modifications of the heart tissue in two experimental models of ARDS induced in New Zealand rabbits by intratracheal instillation of neonatal meconium (100 mg/kg) or by repetitive lung lavages with saline (30 ml/kg). Since induction of the respiratory insufficiency, all animals were oxygen-ventilated for next 5 h. Total and differential counts of leukocytes were measured in the arterial blood, markers of myocardial injury [(troponin, creatine kinase - myocardial band (CK-MB), lactate dehydrogenase (LD)] in the plasma, and markers of inflammation [tumour necrosis factor (TNF)α, interleukin (IL)-6], cardiovascular risk [galectin-3 (Gal-3)], oxidative changes [thiobarbituric acid reactive substances (TBARS), 3-nitrotyrosine (3NT)], and vascular damage [receptor for advanced glycation end products (RAGE)] in the heart tissue. Apoptosis of heart cells was investigated immunohistochemically. In both ARDS models, counts of total leukocytes and neutrophils in the blood, markers of myocardial injury, inflammation, oxidative and vascular damage in the plasma and heart tissue, and heart cell apoptosis increased compared to controls. This study indicates that changes associated with ARDS may contribute to early heart damage what can potentially deteriorate the cardiac function and contribute to its failure.

Key words

Acute respiratory distress syndrome • Cardiac injury • Oxidative stress • Inflammation • Apoptosis

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Introduction

Acute respiratory distress syndrome (ARDS) represents a serious life-threatening situation with a mortality rate about 40 % in adults, however, different forms of ARDS may manifest in all age groups including neonates (De Luca 2019). In spite of growing knowledge on ARDS, the treatment is only supportive, with protective ventilation by low tidal volumes and prone positioning as the only interventions minimizing iatrogenic effects and decreasing mortality (Silva *et al.* 2020). Nevertheless, even in patients who had survived

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ARDS, the long-term quality of life is adversely affected (Johnson and Matthay 2010). High mortality of ARDS and worsened quality of life may be also attributed to dysfunction of other organs (heart, liver, kidneys, brain etc.), which are hit secondarily in the primary lung injury.

ARDS could be initiated by direct lung injury (e.g. pneumonia, gastric contents aspiration, near-drowning, toxic inhalation) or indirectly due to nonpulmonary reasons (e.g. sepsis, multiple trauma, acute pancreatitis, blood transfusion) (Rocco and Pelosi 2008). Regardless of the site of injury, ARDS is a heterogeneous syndrome with deterioration and disruption of the lung endothelial and epithelial barriers leading to diffuse alveolar damage, increased vascular permeability, and pulmonary oedema formation (Pierrakos *et al.* 2012). Dysfunction of alveolar-capillary lining enables excessive transepithelial migration and activation of neutrophils with release of toxic mediators, including proteases, reactive oxygen and nitrogen species (RONS), pro-inflammatory cytokines, and pro-coagulant molecules (Zemans *et al.* 2009). Deterioration of pulmonary surfactant together with oedema formation, ventilation-perfusion mismatch, and inflammation lead to reduced lung compliance and hypoxemia (Pierrakos *et al.* 2012, Zebialowicz Ahlstrom *et al.* 2019).

However, ARDS affects not only the alveolar compartment but also the pulmonary circulation and, thereby, may negatively influence the hemodynamics and cardiac function, especially of the right ventricle. Mean pulmonary artery pressure may increase in ARDS as a consequence of structural alteration of the pulmonary circulation, with inflammation, vasoconstriction, oedema, thrombi, and vascular remodelling leading to excessive muscularization of pulmonary arteries (Moloney and Evans 2003). However, elevation in pulmonary artery pressure may be also caused by unadapted positive pressure ventilation, e.g. in the use of positive end-expiratory pressure (PEEP) >10 cm H_2O (equal to 1.0 kPa), when alveolar distending pressure reduces the flow in the pulmonary capillaries (Repesse *et al.* 2015). Pulmonary vascular dysfunction defined by an elevated transpulmonary pressure gradient (pulmonary artery diastolic pressure minus pulmonary capillary wedge pressure) may induce acute cor pulmonale, which occurs in >70 % of cases of ARDS and contributes to increased mortality (Bull *et al.* 2010, Repesse *et al.* 2015).

Additional deterioration of extra-pulmonary organs including heart in ARDS can be associated with lung tissue injury, excessive and/or prolonged activation of neutrophils, increased apoptosis of lung cells, and systemic inflammatory response. Numerous potentially injurious products, such as pro-inflammatory cytokines, RONS, components of complement, as well as extracellular histones, heat-shock proteins and other damage-associated molecular patterns (DAMPs), released from the affected lung through injured alveolar-capillary membrane into the circulation could contribute to multiorgan failure (MOF) (Slutsky and Tremblay 1998, Ranieri *et al.* 1999, Plotz *et al.* 2004). Loss of integrity of alveolar-capillary membrane could be further worsened by excessive mechanical ventilation, contributing to decompartmentalization of the inflammatory response and spreading injury from the lung to the distal organs (Del Sorbo and Slutsky 2011).

Thus, there is a strong evidence that ARDS can ultimately affect the heart function. However, extent of deterioration of the heart tissue and relationship between the ARDS and direct cardiac injury have not been fully explained. Therefore, this study evaluated effects of direct lung injury induced in rabbits by intratracheal neonatal meconium instillation (meconium-induced model of ARDS) or repetitive saline lung lavage (surfactant-depleted model of ARDS) on the heart presented as changes in inflammatory, oxidative and vascular damage markers, and apoptotic markers in the heart tissue, and as changes in the plasma markers of myocardial injury.

Methods

Animal instrumentation

This study was authorized by the National Veterinary Board of Slovak Republic and the local Ethics Committee of Jessenius Faculty of Medicine in Martin. Adult New Zealand white rabbits of both genders with a body weight (b.w.) of 2.3±0.3 kg were instrumented in accordance with previous studies (Kosutova *et al.* 2016, Mikolka *et al.* 2018). The animals were sedated by infusion of anesthetics (tiletamine and zolazepam, 10 mg/kg/h i.v.) and paralyzed with pipecuronium bromide (0.3 mg/kg/0.5 h). Subsequently, animals were ventilated (Aura V, Chirana, Slovakia) with tidal volume (V_T) of 6 ml/kg, time of inspiration (Ti) 50 %, respiratory rate (RR) of 40 breaths/min, fraction of inspired oxygen (FiO_2) of 1.0, and PEEP of 0.5 kPa. The animals were euthanized by an overdose of anaesthetics.

Gas exchange and acid-base balance were measured in arterial blood samples using analyser

RapidLab TM348 (Siemens AG, Germany). The following parameters were calculated: P/F = calculated as a ratio between arterial oxygen partial pressure (PaO_2) and fraction of inspired oxygen (FiO_2); oxygenation index (OI) = (mean airway pressure × FiO_2)/PaO_2; and alveolar–arterial gradient (AaG) = [FiO_2 (P_{atm} - P_{H2O}) - $PaCO_2$/0.8] - PaO_2.

Experimental models of ARDS

After 15 min of stabilisation, animals were randomly divided into 3 groups (n=7 for each): (i) Control group, healthy ventilated controls without any other intervention; (ii) ARDS-MAS, meconium-induced ARDS; (iii) ARDS-LAV, surfactant-depleted ARDS.

Meconium-induced model of ARDS (ARDS-MAS), mimicking the neonatal meconium aspiration syndrome, was induced by intratracheal instillation of meconium suspension (4 ml/kg) in semi-upright lateral positions of animal to supply a homogenous distribution of meconium throughout the lungs (Mikolka *et al.* 2018). First-pass meconium was obtained from 25 healthy term neonates born in University Hospital (Martin, Slovakia), then lyophilized and suspended in saline (25 mg/ml).

Surfactant-depleted model of ARDS (ARDS-LAV) was induced by repeated lung lavage with saline (30 ml/kg, 37 °C) with 2 min intervals of ventilation between the lavages (Kosutova *et al.* 2016).

For both models, respiratory failure was defined as a decrease of P/F<26.7 kPa, what equals moderate ARDS. After the criteria of lung injury were fulfilled, all animals were oxygen-ventilated (V_T 6 ml/kg, Ti 50 %, RR 40/min, FiO_2 1.0, PEEP 0.5 kPa) for additional 5 h.

Total and differential leukocyte counts in the arterial blood

Total leukocyte count was determined microscopically in a counting chamber after staining by Türck and expressed in absolute values. Differential leukocyte counts were estimated microscopically after staining by May-Grünwald/Giemsa-Romanowski and expressed as percentage (%).

Post-mortem tissue sampling and assays

At the end of experiment, arterial blood was taken and centrifuged (3000 rpm, 15 min, 4 °C) for plasma collection. The thorax was opened and the heart was excised. Tissue samples from right ventricle were either immediately shock frozen and stored at -70 °C until biochemical analyses were performed, or fixed in 10 % buffered formalin.

In the heart homogenate prepared as 10 % (weight/volume) tissue homogenate, oxidative modification was determined using OxiSelect™ Nitrotyrosine ELISA Kit for proteins and OxiSelect™ TBARS Assay Kit for lipids (both Cell Biolabs Inc., USA). ELISA assays were also used for determination of damage to the heart endothelial cells (receptor for advanced glycation end products (RAGE), MyBioSource, USA), tumor necrosis factor alpha (TNFα), interleukine-6 (IL-6; both Cloud-Clone Corp., USA) and galectin-3 (GAL-3; BlueGene, China). The plasma markers of myocardial injury: cardiac troponin T (cTnT), MB fraction of creatine kinase (CK-MB), and lactate dehydrogenase (LD) were determined using standard biochemical analysers (Olympus AU640/AU680, Mitsubishi Pathfast). Levels of lactate in plasma was determinated using lactate meter (Statsensor Novavet, NOVA Biomedical, USA).

In situ labeling of DNA strand breaks by TUNEL method was performed on paraffin embedding microtome slides from the formalin fixed heart samples using DeadEnd™ Colorimetric TUNEL System (Promega, USA). For immunohistochemical investigation of activated caspase-3 in the heart, primary antibody rabbit anti-caspase 3 (1:500; Bioss, USA), subsequently biotinylated anti-rabbit secondary antibody and peroxidase-labelled streptavidin conjugated to HRP (DAKO LSAB®2 System-HRP; Dako, Denmark) were used. For both analyses, the count of affected cells was calculated from three sites within each section as described previously (Kosutova *et al.* 2018).

Statistical analysis

Statistical analysis was performed using statistic software Graph Pad Prism 8 (USA). The differences among the groups were analysed by Kruskal-Wallis nonparametric test with Dunn's multiple all pairs comparison test. A $p<0.05$ was considered for statistically significant. The results are presented as means ± SD.

Results

Lung function parameters

Induction of lung injury using both insults, meconium instillation (ARDS-MAS) or lung lavage (ARDS-LAV), led to a significant worsening in the lung function parameters (Table 1). In both models, all respiratory parameters; P/F ratio, oxygenation index (OI),

and alveolar-arterial gradient (AaG) were deteriorated and reached the pre-determined limit for ARDS (P/F<26.7 kPa) compared to controls, while this deterioration persisted till the end of experiment (ARDS-MAS & ARDS-LAV vs. Control $p<0.001$). Significant differences between the models were observed in P/F and OI, with worse oxygenation in ARDS-MAS model (Table 1).

Table 1. Respiratory parameters: the ratio of arterial oxygen partial pressure to fraction of inspired oxygen (P/F), oxygenation index (OI), alveolar-arterial gradient (AaG), and pH before (basal value, BV) and after induced ARDS (Model) and within 5 h in Control group, meconium-induced (ARDS-MAS) and lavage-induced (ARDS-LAV) acute respiratory distress syndrome (ARDS) models.

	BV	Model	30'	1 h	2 h	3 h	4 h	5 h
***P/F** (kPa)*								
Control	78.8±2.7	81.2±5.7	82.1±6.0	82.1±5.8	87.4±4.6	86.4±3.1	81.0±8.3	81.0±4.5
ARDS-MAS	76.4±4.3	6.9±1.4$^{\varphi\dagger}$	8.6±1.7$^{\varphi\dagger}$	7.6±1.5$^{\varphi}$	7.9±1.0$^{\varphi}$	7.4±1.3$^{\varphi\dagger}$	6.4±0.9$^{\varphi\dagger}$	7.0±1.0$^{\varphi\dagger}$
ARDS-LAV	76.4±9.8	14.9±3.8$^{\varphi}$	14.9±6.1$^{\varphi}$	12.0±4.2$^{\varphi}$	11.5±5.4$^{\varphi}$	15.8±6.7$^{\varphi}$	15.9±7.2$^{\varphi}$	14.3±7.0$^{\varphi}$
OI								
Control	1.0±0.1	0.9±0.1	0.9±0.1	0.9±0.1	0.8±0.1	0.8±0.1	0.9±0.1	0.8±0.1
ARDS-MAS	0.8±0.2	13.0±2.0$^{\varphi\dagger\dagger}$	11.6±2.6$^{\varphi\dagger\dagger}$	11.5±3.9$^{\varphi}$	12.5±2.1$^{\varphi\dagger}$	13.1±2.3$^{\varphi\dagger\dagger}$	15.2±2.2$^{\varphi\dagger\dagger\dagger}$	13.7±1.6$^{\varphi\dagger\dagger\dagger}$
ARDS-LAV	1.1±0.2	7.8±1.9$^{\varphi}$	8.0±3.2$^{\varphi}$	10.0±5.3$^{\varphi}$	9.6±4.3$^{\varphi}$	7.9±2.5$^{\varphi}$	8.0±3.2$^{\varphi}$	8.7±1.2$^{\varphi}$
***AaG** (kPa)*								
Control	11.0±2.4	8.3±5.7	7.8±5.7	7.7±5.7	3.4±4.1	3.7±2.8	9.4±7.8	5.2±2.8
ARDS-MAS	10.6±0.7	75.7±3.0$^{\varphi}$	74.8±2.5$^{\varphi}$	74.7±4.8$^{\varphi}$	75.8±2.2$^{\varphi}$	76.0±2.8$^{\varphi}$	76.9±3.4$^{\varphi}$	75.4±3.3$^{\varphi}$
ARDS-LAV	12.9±1.1	70.8±4.8$^{\varphi}$	66.6±15.9$^{\varphi}$	68.7±17.8$^{\varphi}$	66.8±21.8$^{\varphi}$	63.7±24.0$^{\varphi}$	64.5±21.6$^{\varphi}$	63.6±22.1$^{\varphi}$
pH								
Control	7.47±0.01	7.42±0.02	7.41±0.01	7.37±0.01	7.31±0.03	7.28±0.02	7.26±0.02	7.25±0.02
ARDS-MAS	7.48±0.02	7.17±0.03$^{\varphi}$	7.17±0.02$^{\varphi}$	7.19±0.03$^{\varphi}$	7.14±0.04$^{\varphi}$	7.11±0.04$^{\varphi}$	7.12±0.06$^{\varphi}$	7.07±0.05$^{\varphi}$
ARDS-LAV	7.55±0.01	7.23±0.02$^{\varphi}$	7.22±0.02$^{\varphi}$	7.2±0.03$^{\varphi}$	7.15±0.02$^{\varphi}$	7.11±0.03$^{\varphi}$	7.07±0.02$^{\varphi}$	7.06±0.04$^{\varphi}$

Statistical comparisons: ARDS-MAS & ARDS-LAV vs. Control: $^{\varphi}$ $p<0.001$; ARDS-MAS vs. ARDS-LAV † $p<0.05$, †† $p<0.01$, ††† $p<0.001$.

Markers of vascular, inflammatory and oxidative modifications in the heart tissue

For determination of the heart vascular damage, receptor for advanced glycation end products (RAGE) was analysed. Significantly increased levels of RAGE were observed in ARDS-MAS group ($p<0.01$ vs. Control), while just negligible increase was found in ARDS-LAV group ($p>0.05$ vs. Control). Similarly, the concentration of galectin-3 (Gal-3), a marker of cardiovascular risk, elevated only in ARDS-MAS group ($p<0.05$ vs. Control). Pro-inflammatory cytokines TNFα and IL-6 increased in the heart tissue homogenates in both ARDS models compared to controls; for TNFα and IL-6, $p<0.05$ for both ARDS-MAS & ARDS-LAV vs. Control. Oxidative damage to lipids expressed by TBARS was higher only in ARDS-MAS group compared to controls ($p<0.05$). Oxidative damage to proteins expressed by 3NT increased in both models (for both $p<0.05$ vs. Control). There were no significant differences between the ARDS models (Fig. 1).

Plasma markers of myocardial injury

For determination of myocardial injury in the plasma, cardiac troponin T (cTnT); MB fraction of creatine kinase (CK-MB); and lactate dehydrogenase (LD) were analysed. Levels of these markers significantly increased in both ARDS models compared to controls except for CK-MB in ARDS-MAS group vs. controls (Fig. 2).

Lactate, markers of inflammatory and oxidative modifications in the plasma

The levels of plasma lactate elevated over time in both ARDS groups (BV vs. 300' after model induction $p<0.001$), for ARDS-MAS also in 180' vs. BV ($p<0.01$). At the end of experiment, lactate levels were significantly higher in both ARDS models compared to controls (for both $p<0.001$). Similarly to the heart tissue, inflammatory

and oxidative markers elevated in the plasma, too. TNFα significantly increased only in ARDS-LAV group compared to controls (p<0.05). TBARS as a marker of lipid oxidation elevated in ARDS-LAV group (p<0.01) and 3NT as a marker of protein modification increased in ARDS-MAS group (p<0.01) compare to controls. There were no significant differences between the ARDS groups (Fig. 3).

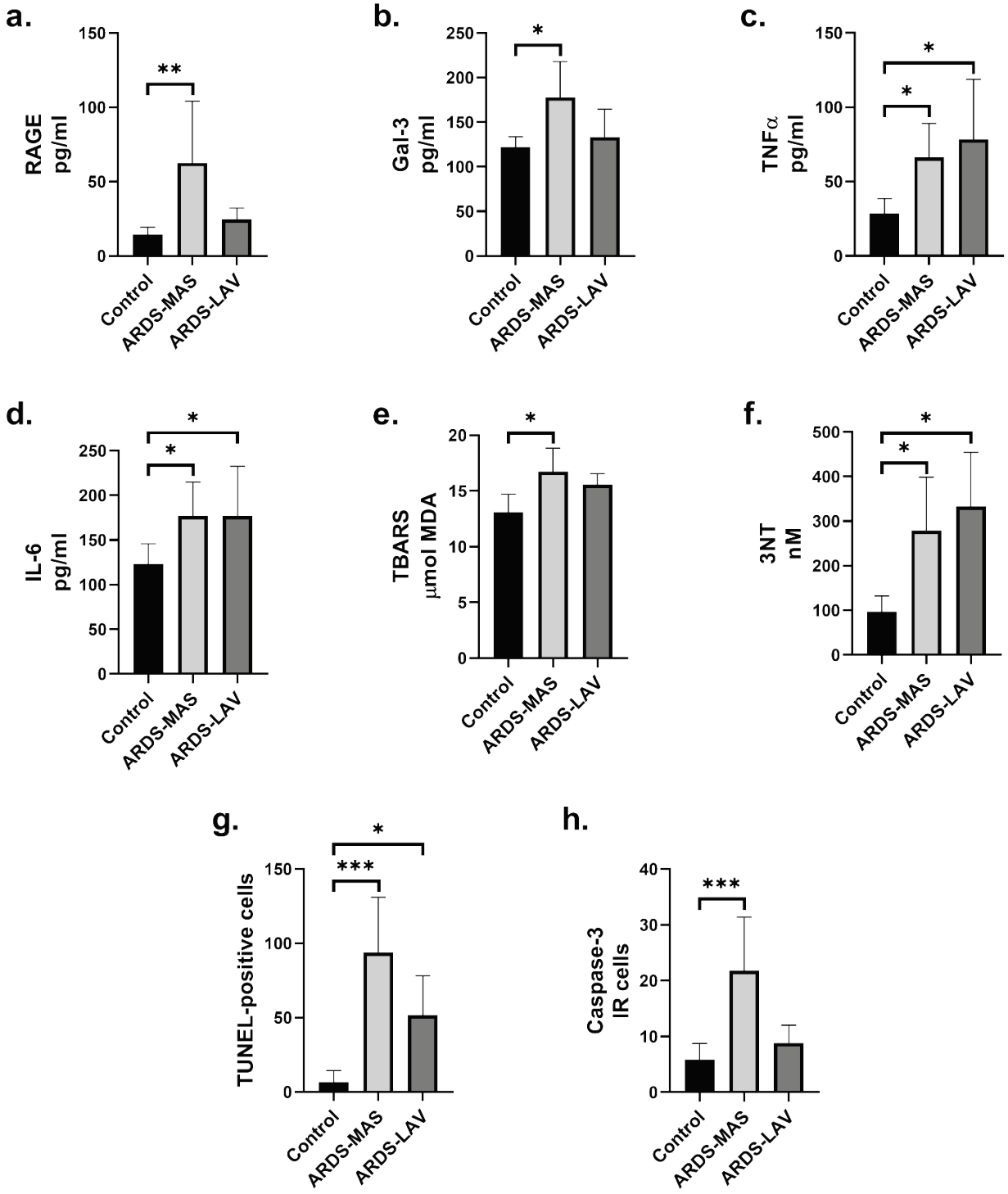

Fig. 1. Vascular damage, inflammatory, oxidative and apoptotic markers in the heart tissue in the control group, meconium-induced (ARDS-MAS) and lavage-induced (ARDS-LAV) acute respiratory distress syndrome (ARDS) models. (**a**) Receptor for advanced glycation end products (RAGE); (**b**) Galectin-3 (Gal-3); (**c**) Tumor necrosis factor alpha (TNFα); (**d**) Interleukin 6 (IL-6); (**e**) Thiobarbituric acid-reactive substances (TBARS); (**f**) 3-nitrotyrosine (3NT); Number of (**g**) Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL)-positive cells and (h) Caspase-3 positive immunoreactive (IR) cells. Statistical comparisons: ARDS-MAS & ARDS-LAV vs. Control: * p<0.05, ** p<0.01.

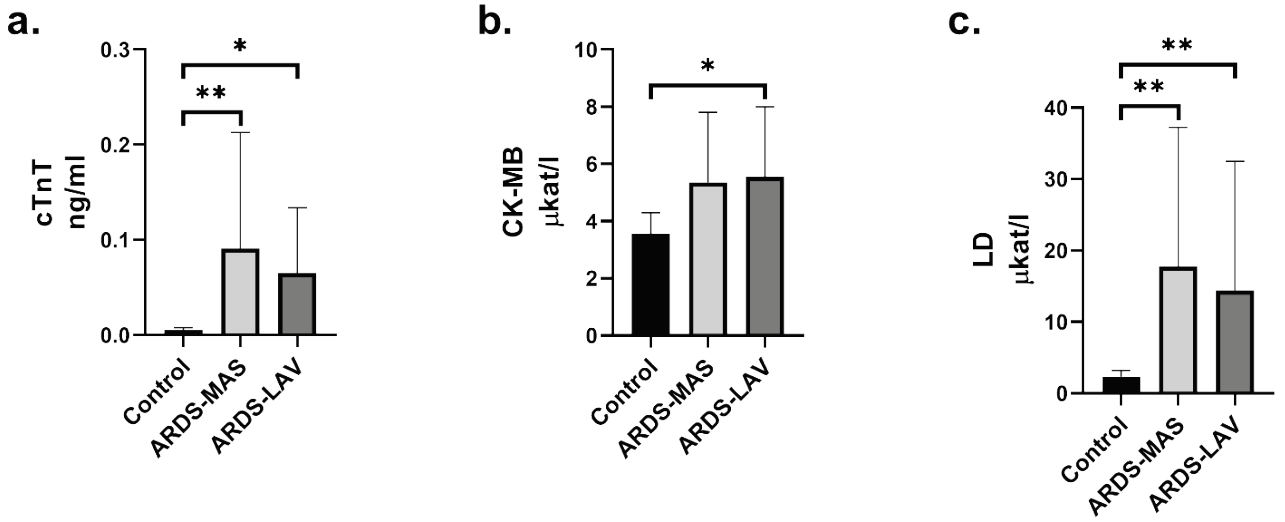

Fig. 2. The plasma markers of myocardial injury in the control group, meconium-induced (ARDS-MAS) and lavage-induced (ARDS-LAV) acute respiratory distress syndrome (ARDS) models. (**a**) Cardiac troponin T (cTnT); (**b**) Creatine kinase isoenzyme (CK-MB); (**c**) Lactate dehydrogenase (LD). Statistical comparisons: ARDS-MAS & ARDS-LAV vs. Control: * $p<0.05$, ** $p<0.01$.

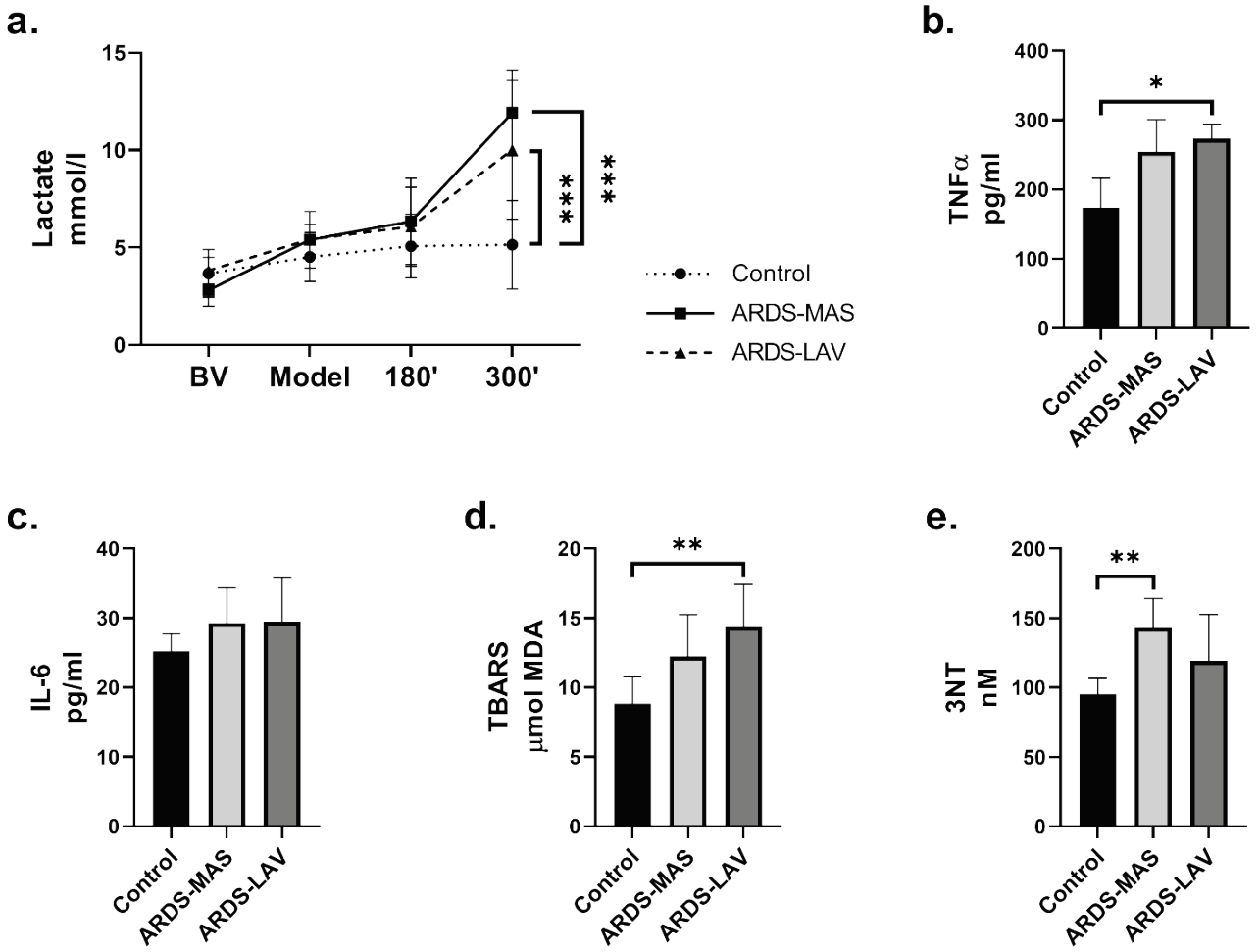

Fig. 3. Levels of lactate (**a**), inflammatory and oxidative markers in the plasma in the control group, meconium-induced (ARDS-MAS) and lavage-induced (ARDS-LAV) acute respiratory distress syndrome (ARDS) models. (**b**) Tumor necrosis factor alpha (TNFα); (**c**) Interleukin 6 (IL-6); (**d**) Thiobarbituric acid-reactive substances (TBARS); (**e**) 3-nitrotyrosine (3NT). Statistical comparisons: ARDS-MAS & ARDS-LAV vs. Control: * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Apoptosis of the heart cells

The number of TUNEL-positive cells in the heart tissue significantly increased in both ARDS models with more obvious increase in ARDS-MAS group vs. controls (p<0.001). Similarly, number of caspase-3 immunoreactive cells massively elevated in ARDS-MAS group compared to Control group (p<0.001) (Fig. 1g, h).

Total and differential leukocyte counts in the arterial blood

As shown in Table 2, total leukocytes decreased with time in both ARDS models compared to controls. More potent effect was observed in ARDS-MAS group with a significant decrease of leukocytes already at 30 min after model induction compared to controls (p<0.05) and this change had gradually pronounced (at 5 h for ARDS-MAS vs. Control p<0.001). Significant differences in the neutrophil percentages for baseline values vs. values at the end of the experiment in Control (p<0.05) and in both ARDS-MAS and ARDS-LAV groups (for both p<0.001) were observed (Table 1).

Table 2. Total and differential leukocyte counts in the arterial blood before (basal value, BV) and within 5 h after induced ARDS ('hit') in Control group, meconium-induced (ARDS-MAS) and lavage-induced (ARDS-LAV) acute respiratory distress syndrome (ARDS) models.

		Control	ARDS-MAS	ARDS-LAV
Total count of leukocytes ($\times 10^{-9}$)				
	BV	2.69±0.23	2.50±0.19	2.30±0.19
	30' hit	2.26±0.29	1.67±0.18*	2.47±0.27
	1 h hit	2.50±0.26	1.28±0.08***	2.04±0.24*
	3 h hit	2.14±0.23	1.31±0.10**	1.61±0.15*
	5 h hit	2.37±0.36	1.10±0.10***	1.73±0.20**
Differential count (%)				
Neu (%)	BV	2.35±1.09	2.06±0.60	2.65±0.78
	5 h hit	21.46±7.45†	28.19±3.04†††	30.66±3.28†††
Lym (%)	BV	91.14±4.70	95.58±0.99	95.46±0.91
	5 h hit	76.12±7.67	70.99±2.84	71.35±3.34
Mono (%)	BV	1.47±0.17	1.64±0.23	1.19±0.11
	5 h hit	1.31±0.19	1.41±0.15	0.90±0.09
Eos (%)	BV	0.51±0.16	0.69±0.35	0.69±0.21
	5 h hit	0.49±0.01	0.46±0.11	0.37±0.09

Neutrophils (Neu), Lymphocytes (Lym), Monocytes (Mono), Eosinophils (Eos). Significant comparisons: ARDS-MAS & ARDS-LAV vs. Control: * p<0.05, ** p<0.01; *** p<0.001; BV vs. 5 h hit: † p<0.05, ††† p<0.001.

Discussion

ARDS is associated with a diffuse alveolar epithelial and endothelial damage leading to surfactant alteration, oedema formation, ventilation-perfusion mismatch, and inflammation. This leads to reduced lung compliance and profound hypoxemia (Pierrakos *et al.* 2012, (Hernandez-Beeftink *et al.* 2019, Mikolka *et al.* 2019). Current treatment of ARDS is mainly supportive with emphasis on management of hypoxemia using lung-protective ventilation (Confalonieri *et al.* 2017). Mechanical ventilation could contribute to spreading of acute-phase reactants from the lung to peripheral circulation, and together with action of activated neutrophils, could target the extra-pulmonary organs and contribute to multiple organ failure (MOF) (Plotz *et al.* 2004, Zemans *et al.* 2009, Del Sorbo and Slutsky 2011, Grommes and Soehnlein 2011). Moreover, pulmonary vascular injury in ARDS has direct impact on the heart, affecting hemodynamics and function of the right ventricle (Repesse *et al.* 2015). Because the mechanisms and time relations of the heart injury secondary to ARDS need to be elucidated, the present study has focused on direct changes in the heart tissue associated with two

experimental models of ARDS, surfactant-depleted model of ARDS (ARDS-LAV) and meconium-induced model of ARDS (ARDS-MAS). However, meconium aspiration syndrome in the text should be treated with caution, because adult rabbits with the associated limitation were used. We expect, that effect of hypoxia induced by meconium-induced lung injury on heart tissue was observed. And the same effect may vary in the neonatal heart. Used adult animals avoided postnatal changes of the lung, heart and hemodynamics. Due to ethical and technical problems with instrumentation of the neonatal animals, use of adult animals is preferred, especially in the studies where artificial ventilation is used (Calkovska *et al.* 2008, Kopincova *et al.* 2018). In addition, some inter-species differences in the immune system between humans and animals may result in some differences in response to induction of the model and effect on heart tissue. Considering all limitations, using the rabbit provides an animal model that resembles humans in anatomical structure (Kamaruzaman *et al.* 2013) and we feel that the results of our study bring new information for research on ARDS.

The main hallmark of ARDS is diffuse alveolar damage resulting in respiratory failure. In this study, situation resembling ARDS was induced by two approaches: intratracheal instillation of meconium or repeated lung lavage with saline. Both interventions led to deterioration of the lung function parameters (P/F, OI and AaG) which occurred within several minutes and remained stable until the end of experiment, in agreement with previous studies (Kamiyama *et al.* 2015, Ricci *et al.* 2017). Instillation of meconium showed more negative impact on the respiratory parameters compared to lung lavages, while at some timepoints the differences for P/F and OI between the ARDS models were significant. This finding is consistent with previous experience that aspiration of neonatal meconium cause severe ARDS (Szymankiewicz *et al.* 2004, Mikolka *et al.* 2018, Thomas *et al.* 2018). Reduced amount of oxygen in the blood (hypoxemia), clearly visible at the P/F ratio in both ARDS models, leads to tissue hypoxia which can rapidly progress to MOF and death (Bakker *et al.* 1996, Kamo *et al.* 2019). Measurement of blood lactate concentration has been used for monitoring tissue oxygenation, because cells deprived of adequate oxygen produce excessive quantities of lactate. Finding of significantly increased lactate in both ARDS models vs. Control in our study indicates serious tissue hypoxia in these lung-injured animals.

In response to the lung injury, neutrophils are rapidly recruited into the lung (Grommes and Soehnlein 2011). In our study, increased percentage of neutrophils at 5 h vs. BV was observed also in Controls where it could attribute to neutrophil demarginalization due to anaesthesia, surgical interventions, and mechanical ventilation. However, in ARDS groups the increase in neutrophils was more remarkable and was associated with gradual decrease in a total count of leukocytes, confirming transmigration of neutrophils into the lung.

After activation, neutrophils release various bioactive agents into the lung tissue as well as into the systemic circulation, such as chemokines, pro-inflammatory cytokines, acute-phase reactants, matrix remodelling enzymes, and reactive oxygen species (ROS) through their oxidant-generating systems comprising the phagocyte NADPH oxidase and nitric oxide synthase (Grommes and Soehnlein 2011, Parekh *et al.* 2011). In our study, significantly increased protein nitrosylation (3NT) in ARDS-MAS group and lipid peroxidation (TBARS) in ARDS-LAV group were observed in the plasma and heart tissue compared to Control. These findings are in agreement with other authors (Li *et al.* 2013, Antonucci *et al.* 2014, Lv and Wang 2016), who confirmed relation between ARDS- or sepsis-induced oxidation stress with myocardial mitochondrial damage.

Inflammatory processes in ARDS can multiply the oxidative stress with ROS overproduction (Tasaka *et al.* 2008, Confalonieri *et al.* 2017) and vice versa. In this study, pro-inflammatory cytokines TNFα and IL-6 were evaluated as biomarkers of early phase of ARDS (Mokra and Kosutova 2015). TNFα mediates various acute and chronic inflammatory reactions such as systemic inflammation or initiation of acute phase reaction, and along with IL-6 represent the key regulators of gene expression of acute phase proteins (Bavunoglu *et al.* 2016). However, increased concentrations of proinflammatory cytokines of the early phase are associated with greater mortality (Bhatia and Moochhala 2004, Del Sorbo and Slutsky 2011, Aisiku *et al.* 2016). In this study, plasma TNFα and IL-6 increased in both ARDS groups compared to Control with statistical significance only for TNFα in ARDS-LAV, what is consistent with our previous studies (Kosutova *et al.* 2018, Mikolka *et al.* 2019). In the heart tissue, levels of TNFα and IL-6 significantly increased in both ARDS models compared to controls. In accordance with cytokines, other inflammatory marker galectin-3 (Gal-3) significantly elevated in the heart tissue homogenate in

ARDS-MAS model vs. controls. In the heart, Gal-3 regulated by paracrine signalization stimulates proliferation of myofibroblasts and accumulation of pro-collagen in the extracellular matrix, causing heart fibrosis what suggests galectin for a predictive marker of the heart failure (Suarez and Meyerrose 2014).

Spreading of inflammation markers by circulation can affect the endothelium of extra-pulmonary organs. Inflammatory stimuli can indirectly upregulate production of receptor for advanced glycation end products (RAGE) *via* NF-κB activation. Increased RAGE, as a result of vascular damage, maintains and amplifies inflammatory responses in the vasculature if ligand for the receptor is present. Interestingly, RAGE binding by circulating advanced glycation end products or S100 protein released by activated leukocytes results in the generation of ROS and further activation of NF-κB. This leads to upregulation of adhesion molecules for circulating monocytes and further upregulation of RAGE itself (Farmer and Kennedy 2009). In our study, ARDS-induced inflammation led to overproduction of RAGE in the heart tissue with more serious changes in ARDS-MAS vs. Control. Activation of endothelium in distant organs was previously confirmed by increased expression of adhesion molecules (Hegeman *et al.* 2009).

Additional evidence of direct myocardial injury has been provided by increased plasma concentrations of cardiac specific troponin N (cTnT), creatine kinase isoenzyme MB (CK-MB) and lactate dehydrogenase (LD) in both ARDS-MAS and ARDS-LAV groups compared to controls. The cTnT levels are frequently elevated in patients with ARDS what is associated with adverse outcomes, including death, organ failure, and need for mechanical ventilation (Rivara *et al.* 2012). CK-MB and LD have been also widely used as clinical markers of cardiac injury or failure (Nigam 2007), Danese and Montagnana 2016). There are several mechanisms potentially contributing to myocardial injury: tissue hypoxia due to reduced coronary flow, cardiac depressant effects of ARDS-induced circulating pro-inflammatory mediators, nitric oxide, matrix metalloproteinases, mitogen-activated protein kinase activity, metabolic alterations including increased lactate, mitochondrial dysfunction or oxidative stress, induction of cell apoptosis leading to myocardial cell death etc. (Flierl *et al.* 2008). Increased apoptosis of myocardial cells as one of the reasons for direct heart injury was also found in our study, as indicated by increased number of apoptotic cells detected by TUNEL method and increased activation of caspase-3 in the heart tissue of ARDS affected animals. These findings are in agreement with other authors (Li *et al.* 2013, Lv and Wang 2016).

Concluding, this study demonstrated significant inflammatory, oxidative and vascular modifications in the heart tissue in two distinct models of ARDS. Meconium-induced lung injury (ARDS-MAS) had more serious impact and significantly affected more markers compared to surfactant-depleted lung injury (ARDS-LAV). We can speculate, that delivered meconium induced stronger respiratory failure and stronger inflammation process than lung lavage. Aspirated meconium triggers the inflammation *via* TLR4/MD-2 CD14 signaling complex (Salvesen *et al.* 2010) and is a rich source of cytokines (de Beaufort *et al.* 2003). Whereas these changes occurred very early, at 5 h after induction of experimental ARDS, potential functional and morphological changes of the heart should be considered, because they can influence the status and future prognosis of patients. However, further investigation is warranted to define the relationship between ARDS and early myocardial injury more in detail.

Conflict of Interest

There is no conflict of interest.

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