TOTAL BODY RESPONSE TO MECHANICAL VENTILATION OF HEALTHY LUNGS: AN EXPERIMENTAL STUDY IN PIGLETS.

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Summary:

Objectives: To assess the influence of mechanical ventilation on healthy body organs.

Type of the study: Experimental, comparative.

Material and Methods: 15 piglets, age 6 weeks, average weight 23kg (range 18.8-27kg). These animals were identically anesthetized, instrumented, and divided into 3 groups. Group A – spontaneously breathing, group B – mechanically ventilated with tidal volume 6ml/kg, and group C – ventilated with tidal volume 10ml/kg for 12 hours. The parameters of lung, heart, liver and kidney functions, neuro-humoral regulation and systemic inflammatory reaction were recorded initially (time-1) and after twelve hours (time-12) of mechanical ventilation.

The results: At time-1 levels of soluble adhesive molecules were higher (CAM; $P<0.01$), glomerular filtration index and free water clearance were lower ($P<0.05$) in both the ventilated groups when compared with group A. Right ventricle myocardial performance index was higher (RIMP; $P<0.05$) in group C when compared with group A. Levels of CAM ($P<0.05$) and creatinine clearance ($P<0.01$) were higher, free water clearance was lower ($P<0.05$) in group C when compared with group B.

At time-12 the RIMP ($P<0.05$) and levels of CAM increased ($P<0.01$), creatinine clearance decreased ($P<0.05$) in both the ventilated groups when compared with same parameter at time-1. Ventilation index was higher ($P<0.05$), and hypoxemic index was lower ($P<0.01$) in group C when compared against with group B.

Conclusions: This study showed that mechanical ventilation induced changes compatible with early inflammatory response in healthy animals. Higher tidal volumes had detrimental effect on ventilatory parameters, reduced myocardial performance and over time potentiated adverse reaction of other organs.

Key words: mechanical ventilation, inflammatory reaction, neuro-humoral regulation, organ function

Introduction:

In our clinical practice, we observe extravasal fluid retention in critically ill newborns and infants with pulmonary disease. The question is whether this fluid retention is a mediated response of extra-pulmonary organs associated with pulmonary pathology or whether it is a consequence of the ventilatory treatment.

Expression of soluble adhesive molecules and release of cytokines from alveolar macrophages, the endothelium of pulmonary vascular bed and lung tissue follow intrathoracic positive pressure changes during mechanical ventilation. Circulating inflammatory mediators induce molecular interactions, vascular permeability as well as organ microcirculation changes (Flori *et al.* 2003, Hiro *et al.* 2007, Thomas 1997). Intrathoracic volume and pressure changes limit diastolic cardiac ventricular filling and activate neuro-humoral regulation to stabilize the blood circulation (Zellers *et al.* 1997). Aldosteron decreases urinary excretion of sodium, whereas brain natriuretic peptide increases urinary sodium excretion. Activated vegetative nervous system receptors, angiotensin, and catecholamines influence renal perfusion whereas connexines influence microcirculation in the renal medulla. Renal microcirculation, membrane receptors, transport channels, and cell organels functions are regulated by signal molecules and influenced by organ, tissue, cell, and molecular interactions (Haeflinger *et al* 2004, Wölfle *et al.* 2007, and Schmit *et al.* 2008). The resulting renal functions are influenced mostly at the regional level (Hoste *et al.* 2006, Morgan 2007).

Our healthy animal model allowed for regular examination of global cardiac ventricular performance, function of lung, liver, and kidneys as well as neuro-humoral regulation and inflammatory response. To select representative parameters for assessment we followed our own previous experimental experience (Kobr *et al.* 2008).

Material and Method:

This experimental, comparative study was approved by the multidisciplinary ethical committee of Faculty of Medicine in Pilsen, Czech Republic according to the valid regulations of the Czech Republic and European Union (Helsinki Declaration 2004), and conducted at an EU accredited experimental laboratory of the Faculty of Medicine in Pilsen.

The study included 15 white piglets, age 6 weeks, average weight of 23kg (range 18.8-27kg), and sex ratio 6:9 in favor of females.

Animal Model

All animals were premedicated with atropine 0.07mg/kg (Atropin; Hoechst-Biotika, Slovakia) and azaperon 5.0mg/kg (Stresnil; Janssen Pharmaceutica N.V., Belgium) intramuscularly. Thiopental 10.0mg/kg (Thiopental, VUAB Pharma, Czech Republic) by was used intravenously for induction of anesthesia and tracheal intubation (endotracheal tube with internal diameter 5.5mm, Kendall; GPS Prague, Ltd.) (Jacson *et al.* 2007). Thiopental at 2.0mg/kg/hour by continuous intravenous administration and bolus doses of fentanyl at 5.0microg/kg (Fentanyl; Hexal AG, Germany) were used for continuous analgesia and sedation. Central venous catheter was inserted into the internal jugular vein (Certofix Paed 5F; B.Braun, Germany), arterial cannula (Arrow 22G; International CR, Germany) was surgically inserted into femoral artery. Urinary drainage was achieved by surgical cystostomy using permanent urinary catheter (Arrow Cystofix 10F; International CR, Germany). All animals were given intravenous infusion of Ringer`s solution at 2.0 ml/kg/hour (Ringer, Infusia, Czech Republic) for the duration of the study.

Animals were divided into three groups based on different strategies of ventilation. Group A used as the control group included tracheally intubated and spontaneously breathing piglets, connected to the ventilator for 1 hour to assess lung mechanics. Animals in Group B and C were tracheally intubated and mechanically ventilated in supine position for 12 hours using

different tidal volumes, in Group B 6ml/kg and in Group C 10ml/kg. Animals were mechanically ventilated using pressure controlled setting (Elema 900C, Siemens, Germany) with constant respiratory rate 26breaths/min, positive end-expiratory pressure 6cmH_2O, and fraction of inspired oxygen 0.21.

Measurements and Calculations

The following parameters were continuously monitored in all animals (Life Scope 9, Nihon Kohden, Japan): ECG, heart rate (HR), pulse oxymetry (SpO_2), end-tidal carbon dioxide ($etCO_2$; kPa), central venous pressure (CVP; mmHg), systemic systolic arterial blood pressure (SBP; mmHg), mean systemic arterial blood pressure (MABP; mmHg) and diastolic systemic arterial blood pressure (DBP; mmHg). Urine output (UO; ml/kg/h) and core temperature (°C) was recorded using permanent urinary catheter. The values of peak inspiratory pressure (PIP; cmH_2O), mean airway pressure (Paw; cmH_2O), respiratory rate (RR), end-expiratory pressure (PEEP; cmH_2O), tidal volume (V_T; ml/kg), minute ventilation (V_E; l/min), and fraction of inspired oxygen (FiO_2) were recorded. Calculation of the following indices was performed: alveolar-arterial oxygen tension difference ($AaDO_2$; kPa), arterio-alveolar oxygen tension difference (a/ADO_2; kPa), oxygenation index (OI), hypoxemic index (PaO_2/FiO_2), and dead space to tidal volume ratio (V_D/V_T ;%), ventilation index (VI), dynamic compliance of lung (C_{dyn}; ml/cmH_2O/kg), airway resistance (R_{aw}; cmH_2O/l/s). Echocardiographic examination was performed repeatedly (probe 3.5-5.0 Hz, Sono Line; Siemens, Germany) and the following indices were recorded: left ventricular shortening fraction (SF), and Tei-index of myocardial performance of the right (RIMP) and left (LIMP) ventricles, [IMP= (isovolumic relaxation time + isovolumic contraction time) / ejection time. Tei-index evaluates global systolic and diastolic function of each ventricle. Increase of the index value represents myocardial functional impairment (Tei *et al.* 1995).

The following parameters were measured in urine and serum samples: urea (mmol/l), creatinine (µmol/l), sodium (mmol/l), potassium (mmol/l), chloride (mmol/l), osmolality (mmol/kg). Alanin-aminotransferase (ALT; µkat/l), asparat-aminotransferase (AST; µkat/l), total bilirubin (µkat/l), glucose (mmol/l), and fibrinogen (g/l) were measured in serum and plasma samples. Blood gas and acid-base analysis were obtained from systemic arterial blood. Creatinine clearance [CrCl= (u-creatinine x urine output) / s-creatinine; ml/min], fractional excretion of sodium [FeNa= (u-Na / s-Na) / (u-creatinine / s-creatinine)], glomerular filtration index [GFI= u-Na / (u- creatinine / s-creatinine)] and free water clearance [Cfw= urine output – (urine output x u-osmolality) / s-osmolality] were calculated (Quigley *et al.* 1997).

The following immunoanalysis was performed in serum and plasma samples: interleukin 6 (IL-6; pg/ml; RD-ELISA), tumor necrotizing factor alpha (TNF-α; pg/ml; RD-ELISA), intercellular adhesion molecule-1 (ICAM; ng/ml; Bender-ELISA), vascular cell adhesion molecule-1 (VCAM; ng/ml; Bender-ELISA) and brain natriuretic peptide (BNP; ng/ml; Bachem-EIA).

Study Protocol

The recovery interval after endotracheal intubation and insertion of intravascular and urinary catheters took 60 minutes.

Clinical assessment and respiratory and circulatory parameters were recorded, blood samples were obtained after recovery interval (time-1) in all animal groups (A, B_1, C_1); and after twelve hours (time-12) in mechanically ventilated groups (B_{12}, C_{12}). The blood samples were collected from the arterial line, urine from the urinary catheter at the same time points.

All the animals were put down at the end of the study by intravenous administration of a bolus dose of cardioplegic solution at 15ml/kg (Infuse Thomas cum procain; Ardapharma, Czech Republic). The dead animal bodies were disposed of according to the regulations of the Czech Republic and European Union.

Statistical Analysis

Parametric data were expressed as mean, 95%CI, SEM, standard deviation. Non-parametric data were expressed as median, 95%CI, range, interquartile range. For qualitative analysis of accuracy of the variables reference interval dispersion (Wilkoxon-Shapiro), the linearity (linear fit), average (Anderson-Darling), reproducibility (Bland-Altman) agreement were used.

The data of groups B and C were compared to a control group A; within each group before and after the 12 hour interval and between groups B and C. *P* values <0.05 were considered statistically significant. All the data were analyzed using statistical software (Analyze-it 211 Software Ltd.).

Results:

All measured values, laboratory analysis results, and calculated parameters from spontaneously breathing animals in group A were used as control data.

Time-1 Experimental data acquired at time-1 of the study were analyzed by ANOVA analysis. Significant differences were found between Groups C and A (F= 6.12; $P<0.001$; Fcrit= 2.699), whereas no significant differences between Groups B and A were noted (F= 1.02; $P= 0.449$; Fcrit= 2.250).

In the parameters of ventilation a/ADO_2 was lower in Group C_1 when compared to Group A (0.55 ± 0.22 versus 0.89 ± 0.19; $P= 0.019$), and Rawe was higher in Group C_1 when compared to Group B_1 (1.21 ± 0.28 versus 1.01 ± 0.36; $P= 0.049$).

The observed extrapulmonary parameters and their differences between mechanically ventilated groups (B, C) and spontaneously breathing group (A) at time-1 are summarized in table 1.

Table 1

When data between groups B and C were compared the following differences were detected: higher levels ICAM in group C_1 compared to group B_1 (41.86 ± 1.34 versus 25.2 ± 0.33; $P= 0.032$), higher CrCl in group C_1 compared to group B_1 (0.609 ± 0.04 versus 0.234 ± 0.02; $P= 0.0002$), and lower Cfw in group C_1 compared to group B_1 (-0.12 ± 0.76 versus -0.78 ± 0.31; $P= 0.044$).

Data variations of other monitored parameters did not reach the level of statistical significance.

Time-12 Data acquired at time-12 of the study were analyzed by ANOVA analysis. Significant differences were found between Groups B and C (F= 4.595, $p<0.01$, Fcrit= 2.725).

In the parameters of ventilation a/ADO_2 was lower in group C_{12} compared to group B_{12} (0.67 ± 0.39 versus 0.87 ± 0.24; $P= 0.021$), PaO_2/FiO_2 was lower in group C_{12} compared to group

B_{12} (322.97 ± 173.50 versus 414.12 ± 107.62; *P*= 0.040), and higher OI in group C_{12} compared to group C_1 (4.33 ± 2.73 versus 2.10 ± 1.38; *P*= 0.041).

The observed extra pulmonary parameters and their differences between groups B and C at time-12 are summarized in table 2.

Table 2

Differences in renal indices were observed only within group C with Cfw lower in group C_{12} compared to group C_1 (*P*= 0.040), UO lower in group C_{12} compared to group C_1 (*P*= 0.008). Significant changes in myocardial function were observed in the left ventricular shortening fraction being lower in group B_{12} compared to group B_1 (*P*= 0.037) and group C_{12} compared to group C_1 (*P*= 0.041). RIMP was higher in group B_{12} compared to group B_1 (*P*= 0.0085), and in group C_{12} compared to group C_1 (*P*= 0.019). LIMP was higher in group C_{12} compared to group C_1 (*P*= 0.032).

The summarized data (n= 25) were analyzed and levels of relevance, casual relation, odds and the reliability were determined. Interesting correlation, regression, level of reliability and confidence interval of targeted dependent and independent variables are summarized in table 3.

Table 3

The multivariate analysis of renal parameters as target of dependent variables demonstrated the following results: free water clearance did not correlate with FeNa, GFI, CrCl or with UO; glomerular filtration index did not correlate with LIMP, UO or with MABP, and one-hour diuresis did not correlate with GFI, CrCl, LIMP or with SF.

Regression analysis clarified following findings. Glomerular filtration was correlated with creatinine clearance (*r*= 0.723; p<0.01) but not with one-hour diuresis (*r*= 0.029; *p*= 0.842), mean arterial pressure (*r*= 0.321; *p*= 0.588) or left ventricle performance (*r*= 0.097; *p*=

0.923). One-hour diuresis ware was influenced by mean of arterial pressure (r= 0.487; p<0.05) but not by glomerular filtration (r= 0.029; p= 0.772), shortening fraction or the left ventricle performance. An excretion of sodium correlated with glomerular filtration (r= 0.994; p<0.01). The influence of the aldosteron on decrease of sodium and increase of potassium urinary excretion was present after twelve hours demonstrated by their urine losses (ratio of values sodium / potassium in urine permanently bellow 1.0).

Figure 1

Discussion:

Respiratory failure is the most common indication of mechanical ventilation in the pediatric intensive care units. In contrast, indications for mechanical ventilation of otherwise healthy children are rare. Scientific literature does not provide enough information about the direct impact of mechanical ventilation on changes in the functions of extrapulmonary organs or systems.

The aim of our study was to assess the influence of mechanical ventilation of healthy lungs on the function of extrapulmonary organs. It used healthy experimental animals to exclude the influence of other primary organ pathology and to eliminate the confounding influence of hypoxia and/or hypercapnia.

Several clinical and experimental studies focusing on renopulmonary interaction during mechanical ventilation of diseased lungs were published in the last few years. Clinical series demonstrate a protective influence of the airway pressure release ventilation on renal function in patients with acute lung injury (Hering *et al.* 2002), renal failure as a result of cardio-renal syndrome in acute on chronic heart failure (Liang *et al.* 2007), and the need for optimal interventional treatment strategy including mechanical ventilation in patients with renal failure (Kuiper *et al.* 2005). Experimental studies with lung impairment in murine model referred to an effect of a particular strategy of mechanical ventilation on development of systemic organ inflammation (Gurkan *et al.* 2003) and mechanical ventilation induced inflammatory reaction leading to pulmonary, hepatic and renal dysfunctions in experimental pneumonia (Dhanireddy *et al.* 2006). There is also experimental evidence for renal functional impairment in dogs with acute lung injury after aspiration of gastric content (Hoang *et al.* 2008) or non-protective strategy of mechanical ventilation causing acute lung injury and exclusively renal functional changes without morphological changes (Wolthuis *et al.* 2009). All the so far published data from clinical and experimental studies could not fully exclude

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

The results of our study support the so far published data and bring some new findings too. Remarkable changes were found after only one hour of mechanical ventilation with the development of systemic inflammatory response, reduction of the right ventricular myocardial performance, and subsequent activation of neuro-humoral reaction. Early development of systemic inflammatory response was confirmed by higher serum levels of both soluble adhesive molecules compared with the group of spontaneously breathing piglets ($P<0.01$). Deterioration of the right ventricular myocardial performance was detected by higher values of Tei-index in comparison with spontaneously breathing piglets ($P<0.05$). Interestingly, this right ventricular functional myocardial deterioration during the first hour of mechanical ventilation did not correlate with brain natriuretic peptide levels. This could be explained by only subtle changes of right ventricular myocardial performance and/or activation of other neuro-humoral autoregulation preventing cardiac atrial dilatation.

After 12 hours of mechanical ventilation this situation changed completely. Over time, mechanical ventilation resulted in further reduction of biventricular myocardial performance ($P<0.01$), development of systemic and pulmonary venous congestion, and expected elevation of serum levels of brain natriuretic peptide. Tei index of myocardial performance assesses both systolic and diastolic function of cardiac ventricles and appears to be sensitive to changes of preload and afterload in the acute clinical and experimental setting (Tei *et al.* 1995, Cheung *et al.* 2004). One can also speculate about mechanical ventilation affecting systolic function or ventricular afterload to larger extent than preload and diastolic function, therefore not leading readily to atrial distension and increased brain natriuretic peptide levels. This explanation is supported by the fact that no correlation was found between the values of Tei index and levels of brain natriuretic peptide in our study.

Activation and time related changes of the neuro-humoral regulation can explain higher left ventricular contractility ($P<0.05$) and higher creatinine clearance; urinary sodium excretion as well as glomerular filtration ($P<0.05$) in the first hour compared with a group of spontaneously breathing animals.

Mechanical ventilation using higher tidal volumes led to progressive changes in inflammatory response in our study. Increased tidal volumes in the course of artificial ventilation cause alveolar distension. This mechanical stimulus induces expression of soluble adhesive molecules and cytokine activation (Barton *et al.* 1997, Thomas 1997). The effect of mechanical ventilation on oxygenation, systemic blood pressure, one-hour diuresis, and on liver function was minimal. One-hour diuresis was as expected dependent on the mean systemic arterial pressure.

We have to admit that our selected set of biochemical parameters provides only indirect information about activation of hormonal rennin – aldosteron – angiotensin system. The sodium excretion changes and presence of neuro-humoral regulation could be supported by direct measurement of peptides and hormones. Indices like free water clearance and fractional excretion of sodium became remarkable indicators of aldosteron effect.

We are aware that the results of our study could been partially limited by a dispersion of the renal indices data (Anderson-Darling; $p\geq0.05$) caused by post-analytical mathematical calculations of the laboratory values, and in individual animals influenced pre-analytically by stress from invasive interventions. Indexed data were sufficiently accurate (Bland-Altman; $p<0.01$) as the concept was not based on absolute values assessment but their differences.

Results of our study show that mechanical ventilation of healthy lungs activates early inflammatory response and triggers neuro-humoral regulation. After 12 hours of mechanical ventilation, effect of aldosteron persisted with reduction of free water clearance. Non-

protective, higher tidal volume mechanical ventilatory strategy contributed to both reduction of right ventricular myocardial performance and to reduction of endogenous creatinine clearance and one-hour diuresis. Based on our data, the use of protective strategy in mechanical ventilation and an early extubation have priority in daily pediatric clinical practice.

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Table 1: Summarized extrapulmonary parameters and their differences compared with group A in the time-1 (n= 15)

Variables	**A**	**B_1**	*P Values*	**C_1**	*P Values*
	Mean ± SD (95%CI)	*Mean ± SD (95%CI)*		*Mean ± SD (95%CI)*	
TNF-α *pg/ml*	72.96 ±7.08 (6.207)	68.65 ± 13.72 (12.026)	NS	96.94 ± 51.60 (45.200)	0.050
IL-6 *pg/ml*	32.82 ± 7.75 (6.795)	32.79 ± 6.75 (5.916)	NS	29.50 ± 1.10 (1.000)	NS
BNP *ng/ml*	1.04 ± 0.56 (0.492)	1.64 ± 0.22 (0.189)	NS	1.78 ± 0.42 (0.365)	<0.01
VCAM *ng/ml*	25.88 ± 1.09 (0.951)	37.70 ± 1.02 (0.896)	<0.05	38.86 ± 0.92 (0.808)	<0.01
ICAM *ng/ml*	6.9 ± 2.27 (1.993)	25.2 ± 0.33 (0.288)	<0.01	41.86 ± 1.34 (1.176)	<0.01
ALT *µkat/l*	0.63 ± 0.13 (0.115)	0.82 ± 0.21 (0.182)	NS	0.76 ± 0.23 (0.202)	NS
AST *µkat/l*	0.62 ± 0.30 (0.264)	1.16 ± 1.35 (1.183)	NS	0.50 ± 0.12 (0.103)	NS
Bilirubin *µmol/l*	4.00 ± 1.55 (1.358)	3.40 ± 0.49 (0.429)	NS	3.60 ± 0.80 (0.701)	NS
Fibrinogen *g/l*	1.17 ± 0.22 (0.194)	1.33 ± 0.49 (0.429)	NS	1.51 ± 0.23 (0.206)	NS
Cfw	-0.68 ± 0.51 (0.448)	-0.78 ± 0.31 (0.274)	NS	-0.12 ± 0.76 (0.664)	<0.05
GFI	1101 ± 1181.60 (1035.703)	3063 ± 4537.90 (3977.543)	<0.05	2885 ± 3057 (3572.70)	<0.05
FeNa	7.56 ± 7.78 (6.822)	22.67 ± 33.89 (29.707)	<0.05	25.36 ± 36.78 (32.240)	<0.05
UO *ml/kg/h*	2.12 ± 0.53 (0.465)	1.92 ± 1.01 (0.886)	NS	1.70 ± 0.60 (0.526)	NS
CrCl *ml/min*	0.183 ± 0.03 (0.195)	0.234 ± 0.02 (0.226)	NS	0.609 ± 0.04 (0.624)	<0.01
MABP *mmHg*	74 ± 10.46 (9.167)	77 ± 15.12 (13.251)	NS	73 ± 5.12 (4.490)	NS
SF	0.43 ± 0.12 (0.092)	0.64 ± 0.07 (0.063)	<0.05	0.57 ± 0.09 (0.082)	<0.05
RIMP	0.22 ± 0.02 (0.015)	0.24 ± 0.01 (0.012)	NS	0.25 ± 0.02 (0.020)	<0.05
LIMP	0.30 ± 0.03 (0.024)	0.30 ± 0.04 (0.031)	NS	0.30 ± 0.04 (0.034)	NS

Legend:

A - Spontaneous breathing, B_1 - Mechanical ventilation V_T 6 ml/kg in the time-1, C_1 - Mechanical ventilation V_T 10 ml/kg in the time-1

TNF-α - tumor necrotizing factor alpha, IL-6 - interleukin 6, BNP - brain natriuretic peptide, VCAM - vascular cell adhesion molecule, ICAM - intercellular adhesion molecule, ALT - alanin-aminotransferase, AST - asparat-aminotransferase, Bilirubin - total bilirubin value, Fibrinogen – fribrinogen value, Cfw - free water clearance, GFI - glomerular filtration index, FeNa - fractional excretion of sodium, UO - one-hour diuresis, CrCl – creatinine clearance, MABP - mean arterial blood pressure, SF - shortening fraction of left ventricle, RIMP - myocardial performance index of right ventricle, LIMP - myocardial performance index of left ventricle.

Values are stated as mean and standard deviation (mean ± SD), 95% confidence interval (95%CI).

NS - Not significant

Table 2: Summarized extrapulmonary parameters and their differences in the time-12

(n= 10)

Variables	**B_{12}**	**C_{12}**	*P Values*
	Mean ± SD (95%CI)	*Mean ± SD (95%CI)*	
TNF-α *pg/ml*	49.42 ± 24.24 (21.244)	36.58 ± 14.93 (13.083)	NS
IL-6 *pg/ml*	92.18 ± 15.14 (13.269)	37.50 ± 15.37 (13.468)	NS
BNP *ng/ml*	1.38 ± 0.39 (0.344)	1.46 ± 0.26 (0.226)	NS
VCAM *ng/ml*	37.52 ± 0.77 (0.673)	40.04 ± 0.66 (0.575)	<0.05
ICAM *ng/ml*	30.22 ± 1.77 (1.556)	66.66 ± 1.59 (1.393)	<0.01
ALT *µkat/l*	0.74 ± 0.26 (0.224)	0.72 ± 0.22 (0.190)	NS
AST *µkat/l*	1.18 ± 1.32 (1.156)	0.99 ± 0.14 (0.125)	NS
Bilirubin *µmol/l*	11.60 ± 13.88 (12.165)	4.60 ± 2.24 (1.968)	NS
Fibrinogen *g/l*	1.22 ± 0.39 (0.343)	1.39 ± 0.38 (0.330)	NS
Cfw	0.14 ± 1.53 (1.345)	-1.04 ± 0.23 (0.203)	NS
GFI	390.18 ± 588.33 (515.680)	251.10 ± 128.84 (112.930)	NS
FeNa	2.54 ± 4.35 (3.810)	1.89 ± 1.01 (0.890)	NS
UO *ml/kg/h*	1.30 ± 0.48 (0.419)	0.96 ± 0.70 (0.614)	NS
CrCl *ml/min*	0.123 ± 0.02 (0.661)	0.254 ± 0.05 (0.821)	<0.05
MABP *mmHg*	89 ± 6.97 (6.108)	81 ± 7.20 (6.311)	NS
SF	0.52 ± 0.05 (0.047)	0.43 ± 0.13 (0.118)	NS
RIMP	0.31 ± 0.03 (0.029)	0.30 ± 0.03 (0.028)	NS
LIMP	0.35 ± 0.05 (0.043)	0.38 ± 0.05 (0.047)	NS

Legend:

B_{12} - Mechanical ventilation V_T 6 ml/kg in the time-12, C_{12} - Mechanical ventilation V_T 10 ml/kg in the time-12

TNF-α - tumor necrotizing factor alpha, IL-6 - interleukin 6, VCAM - vascular cell adhesion molecule, ICAM - intercellular adhesion molecule, ALT - alanin-aminotransferase, AST - asparat-aminotransferase, Bilirubin - total bilirubin values, Fibrinogen – fribrinogen values, BNP - brain natriuretic peptide, Cfw - free water clearance, GFI - glomerular filtration index, FeNa - fractional excretion of sodium, UO - one-hour diuresis, CrCl - creatinine clearance, MABP - mean arterial blood pressure, SF - shortening fraction of left ventricle, RIMP – myocardial performance index of right ventricle, LIMP - myocardial performance index of left ventricle.

Values are stated as mean and standard deviation (mean ± SD), 95% confidence interval (95%CI).

NS - Not significant

Table 3: Correlation, regression and data reliability (n= 25)

Dependent variables	*Independent variables*	R^2	*p*	*t*	*95% CI intervals*	*F*	*P Values*
Cfw	FeNa	0.08	NS	0.007	-13.79 to 9.25	0.16	NS
	GFI	0.06	NS	0.004	-1412 to 1214	0.08	NS
	UO	0.11	NS	0.013	-0.27 to 0.47	0.30	NS
FeNa	GFI	0.99	<0.01	0.998	115.93 to 127.51	19.0	<0.05
	TNF-α	0.66	<0.01	0.444	0.48 to 1.36	18.3	<0.01
	$AaDO_2$	0.16	NS	0.027	-0.19 to 0.43	0.63	<0.05
GFI	UO	0.02	NS	0.001	-0.0001 to 0.0001	0.01	NS
	CrCl	0.72	<0.01	0.637	0.288 to 0.455	3.01	<0.05
	LIMP	0.09	NS	0.009	-0.001 to 0.002	0.21	NS
	MABP	0.32	NS	0.107	-0.003 to 0.0003	2.74	NS
UO	ICAM	0.42	<0.01	0.181	-0.03 to -0.001	5.09	<0.05
	MABP	0.48	<0.05	0.238	-0.01 to -0.06	7.16	<0.05
	CrCl	0.50	<0.05	0.331	0.388 to 0.405	4.02	<0.05
	GFI	0.02	NS	0.001	-1514 to 1732	0.01	NS
	LIMP	0.28	NS	0.083	-0.046 to 0.057	2.08	NS
	SF	0.06	NS	0.004	-0.077 to 0.288	0.09	NS

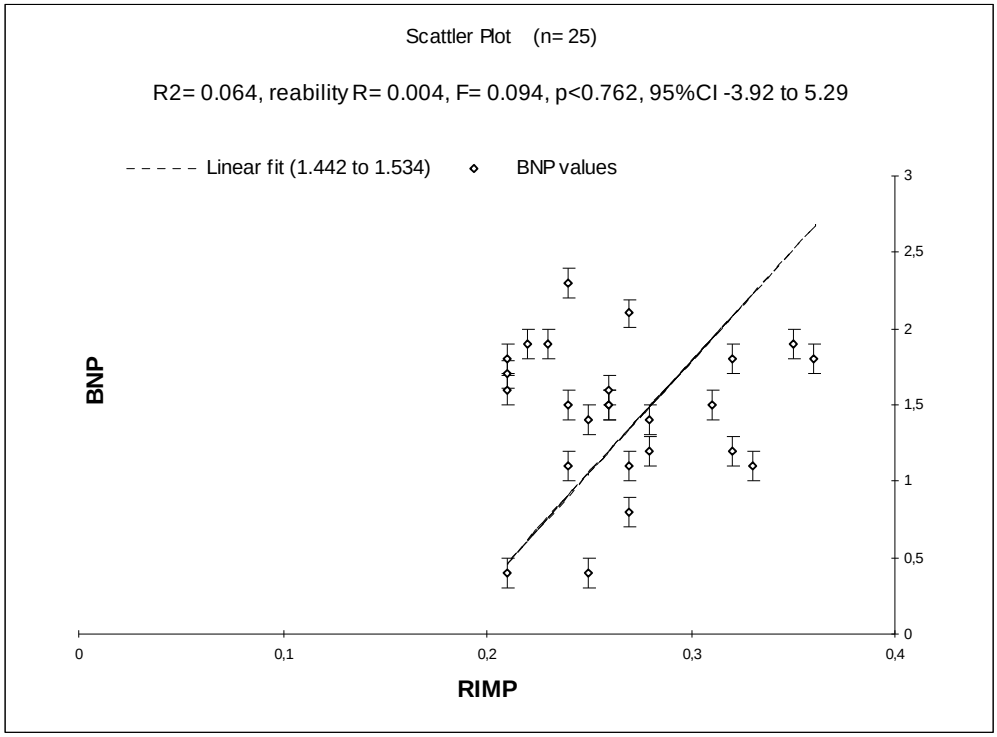
Legend:

Cfw - free water clearance, FeNa - fractional excretion of sodium, GFI - glomerular filtration index, UO - one-hour diuresis, CrCl – creatinine clearance.

TNF-α - tumor necrotizing factor alpha, $AaDO_2$ - alveolar-arterial oxygen tension difference, LIMP - myocardial performance index of left ventricle, MABP - mean arterial blood pressure, ICAM - intercellular adhesion molecule, SF - shortening fraction of left ventricle.

NS - Not significant

Figure 1: Regressions analysis of data RIMP versus BNP (n= 25)



Legend:

BNP – brain natriuretic peptide, RIMP – myocardial performance index of right ventricle