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Short Communication

THE ACTIVITY OF OXIDATIVE STRESS MARKERS IN ACUTE RESPIRATORY DISTRESS SYNDROME

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Acute respiratory distress syndrome is a common complication characterised by severe hypoxemia, which leads to high mortality rates in ICU patients. Imbalance between oxidative stress markers like oxidants and antioxidants may play an important role in pathophysiology of the syndrome. We observed 17 ARDS patients during seven days after inclusion, with the main goal to describe dynamic changes in the level of oxidative stress markers in patients with acute respiratory distress syndrome. We found that there are dynamic differences in the level of malondialdechyde (MDA) and nitric oxide (NO) in patients with acute respiratory distress syndrome. There were also different levels of oxidative stress markers in non-survivor compared with survivor groups. Increased level of an oxidant like a thiobarbituric acid substance with malondialdechyde (TBS_MDA) and antioxidant glutathionperoxidase (GPx) at the first day after inclusion was related with poor outcome in patients with acute respiratory distress syndrome.

Key words: *ARDS, oxidative stress, reactive oxygen species.*

Acute respiratory distress syndrome (ARDS) is the complication of many acute conditions, which can be characterised with a non-cardiogenic lung edema and hypoxemia. The most common risk factors for developing ARDS can be grouped into direct (acute severe pneumonia, aspiration of gastric content) and indirect (sepsis, acute severe pancreatitis, massive blood component transfusion) effects (Galvin *et al.*, 2011). The incidence of ARDS since 1990 has doubled (Sigurdsson *et al.*, 2013), and the level of mortality can reach even 50% (Ferguson *et al.*, 2007) in intensive care unit (ICU) patients. The treatment of these patients can be very complicated, and can include serious fluid management, feeding, extra corporal membrane oxygenation, and mechanical lung ventilation advanced techniques.

Imbalance between oxidants and antioxidants may play an important role in pathogenesis of ARDS. The reactive oxygen molecules can be produced by all cells in the organism due to enzymatic and non-enzymatic processes. Lists of oxidative stress markers associated with development of many disorders are already known (Kumar *et al.*, 2000; Bowler *et al.*, 2003; Crimi *et al.*, 2006), but their predictive role in ARDS is still underestimated. Reactive oxygen species initiate cellular tissue death by lipid peroxidation, and as a result also modulation of proteins and DNA.

Oxidative stress related molecules, such as malondialdechyde and 4-hydroxinonenal, are important products of non-enzymatic reactions. On the other hand, protective function is provided by antioxidative molecules, for example, superoxiddismutase, glutathionperoxidase, and tocopherol.

The main aim of this study was to investigate the dynamic changes of the level of oxidative stress markers in patients with acute respiratory distress syndrome. The secondary goal was to determine the relationship of the level of oxidative stress markers to the outcome in patients with acute respiratory distress syndrome.

This prospective study was conducted in the ICU of Pauls Stradiņš Clinical University hospital during six months in 2013 according to the following inclusion criteria: mechanical lung ventilation 24 hours in patients over 18 years of age and acute severe pneumonia, pancreatitis, sepsis or massive blood component transfusion. The study was approved by the Ethics Committee of Rīga Stradiņš University.

Patients with ARDS were monitored for seven days. The ARDS was diagnosed according to the Berlin definition (Ranieri *et al.*, 2012) criteria, where: ARDS developed within seven days due to direct or indirect cause, bilateral

opacities were obeserved on chest X-ray or CT scan and origin of lung edema was not fully explained by cardiac failure or fluid overload. There are three stages of ARDS: 1) mild — PaO2/FiO2 200–300 with PEEP or CPAP ≥ 5 cmH2O, 2) moderate — PaO2/FiO2 101–199 with PEEP ≥ 5 cmH2O, and severe — PaO2/FiO2 100 with PEEP ≥ 5 cmH2O. Blood samples were taken on the 1st and 4th day after inclusion

Oxidative stress biomarkers — MDA (malondialdechyde), TBS (thiobarbituric acid reactive substance), 4-HNE (4-hydroxinonenal), and NO (nitric oxide) — were determined spectrophotometrically using a Microplate reader Infinity 2000 (Tecan Ltd.).

The assay of MDA, 4-HNE is based on the reaction of a chromogenic reagent, N-methyl-2-phenylindole with MDA and 4-hydroxyalkenals at 45 °C. One molecule of either MDA or 4-hydroxyalkenal reacts with two molecules of reagent R1 to yield a stable chromophore with maximal absorbance at 586 nm (Esterbauer *et al.*, 1990; Janero, *et al.*, 1990).

The Thiobarbituric Acid Reactive Substances (TBARS) Assay Kit is a tool for the direct quantitative measurement of MDA in biological samples. Unknown MDA containing samples or MDA standards were first reacted with TBA at 95 °C. After a brief incubation, the samples and standards were read spectrophotometrically. The MDA content in unknown samples was determined by comparison with a predetermined MDA standard curve (Armstrong *et al.*, 1994; 1998).

Antioxidative biomarkers — tocopherol, SOD (superoxid-dismutase), and GPx (glutathionperoxidase) — were determined spectrophotometrically using a clinical biochemistry analyser RX Daytona (Randox lab., Ltd).

The method of GPx determination was based on catalysation of oxidation of glutathione by cumene hydroperoxide. In the presence of glutathione reductase and NADPH the oxidised glutathione is converted to a reduced form.

To determine SOD we used heparinised whole blood samples, which were centrifuged at 3000 rpm 10 minutes followed by aspiration of the plasma. Erythrocytes were then washed three times with isotonic saline solution with centrifuging for 10 minutes at 3000 rpm after each wash. The solution of washed centrifuged erythrocytes (0.2 ml) was then made up to 2.0 ml with cold redistilled water, mixed and left to stand at +4 °C for 15 minutes. Xanthine and xanthine oxidase were used to generate superoxide radicals that react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride. The superoxide dismutase activity was then measured by the degree of inhibition of this reaction.

Clinical and laboratory parameters were collected according to the study protocol.

Medians and interquartile ranges (IQR) were calculated for biomarker serum levels. The Wilcoxon Signed Rank Test was used for testing significant differences of dependent events and the Mann–Whitney U Test for respective independent events. Statistically significance was assumed $p < 0.05$. Statistical calculations were performed using the SPSS 21.0 programme.

Forty patients were observed, including 17 ARDS patients with acute severe pneumonia, pancreatitis, sepsis and different stages of ARDS according to the Berlin definition and inclusion criteria.

Oxidants. Statistically significant dynamic changes were shown for MDA, from median value of 2[2-3] μM in the 1st day and 2[1-2] μM on the 4th day ($p = 0.05$, Wilcoxon Signed Rank Test).

Level of the biomarker nitric oxide decreased from 22.7[15.3-43.7] μM on the 1st day to 20.0[9.9-39.9] μM on the 4th day, but this change was not significantly different ($p = 0.062$, Wilcoxon Signed Rank Test).

Among antioxidants, GPx, the most important biomarker investigated, significantly decreased from 4749[4026-5568] U/L on the 1st day to 4034[3797-4784] U/L on the 4th day ($p = 0.03$, Wilcoxon Signed Rank Test) (Table 1).

Six patients of 17 included suffered mortality. Deceased patients had twice higher level of the oxidant TBS_MDA (9.5[8.0-10.0] μM) at the 1st day if compare with survivors, where the level of TBS_MDA was 4.0[3.0-6.0] mkM ($p = 0.035$, Mann–Whitney U Test). On the other hand the activity of antioxidant GPx at the 1st day was higher in non-survivors 5179[4638-6365] μM if compare with survivors 4585[3728-5023] μM ($p = 0.019$, Mann–Whitney U Test) (Table 2).

The results of our study shows a relationed between increased level of TBS_MDA and activity of GPx and poor outcome in ARDS patients, likely via cellular oxidative stress related tissue damage. In critically ill patients ARDS develops into multi-organ dysfunction and inflammation. Due to this, oxidative stress in ARDS patients can be a secondary reason for outcome. Our study shows changes in levels of biomarkers of oxidative stress, especially on the

Table 1

DYNAMIC CHANGES OF OXIDATIVE STRESS MARKERS

Biomarker	Median value [IQR] 1st day	Median value [IQR] 4th day	p value[1]
Oxidants			
MDA, mkM	2[2-3]	2[1-2]	0.05
4-HNE, mkM	3[2-4]	3[1-3]	0.69
NO, mkM	23[15-44]	20[10-40]	0.06
MDA+4-HNE	5[4-6]	4[3-5]	0.39
TBS_MDA	6[4-8]	5[4-6]	0.53
Antioxidants			
GPx, U/L	4749[4026-5568]	4034[3797-4784]	0.03
SOD, U/gHb	1627[1386-1697]	1527[1352-1692]	0.21

[1] Wilcoxon Signed Rank Test

Table 2

RELATION OF THE LEVEL OF OXIDATIVE STRESS MARKERS WITH OUTCOME IN ARDS PATIENTS

Biomarker	Non survivors (n = 6)		Survivors (n = 11)	
	Median value [IQR] 1st day	*p* value[1]	Median value [IQR] 1st day	*p* value[1]
Oxidants				
4-HNE, mkM	3[2-4]	0,94	4[1-4]	0.94
NO, mkM	33[15-72]	0,31	23[12-40]	0.31
TBS_MDA	10[8-10]	0,03	4[3-6]	0.03
Antioxidants				
GPx, U/L	5179[4638-6365]	0,02	4585[3728-5023]	0.02
SOD, U/gHb	1641[1384-1697]	0,89	1470[1386-1725]	0.89

[1] Mann–Whitney U Test

1st day after inclusion, which suggests that they play an important role in pathophysiology of ARDS.

According to recent studies, oxidative stress is an important process also in patients with hepatic malfunction, where lipid peroxidation is the main mechanism (Lichtenstern *et al.*, 2011). Antioxidants showed greater activity in septic patients, which is in contrast with the results of our study and may be explained by different changes in levels of antioxidants for various diseases (Alonso de Vega *et al.*, 2002).

Based on the results of our study we concluded that there are dynamic differences in the level of oxidants and antioxidants in patients with acute respiratory distress syndrome. Increased levels of the oxidant TBS_MDA and antioxidant GPx on the first day after inclusion was related with poor outcome in patients with acute respiratory distress syndrome.

Due to the fact that ARDS is still underdiagnosed and is associated with high mortality rate, it is important to study the pathogenesis of the syndrome in the Latvian population.

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REFERENCES

Alonso de Vega, J. M., Díaz, J., Serrano, E., Carbonell, L. F. (2002). Oxidative stress in critically ill patients with systemic inflammatory response syndrome. *Crit. Care Med*., **30**, 1782–1786.

Armstrong, D.. Browne, R. (1994). The analysis of free radicals, lipid peroxidases, antioxidant enzymes and compounds related to oxidative stress as applied to the clinical chemistry laboratory. *Free Radicals Diagn. Med*., **366**, 43–58.

Armstrong, D. , Hiramitsu, T., Ueda, T. (1998). *In vitro* screening for antioxidant activity. *Free Radical and Antioxidant Protocols*, **108**, 315–324.

Bowler, R. P., Velsor, L. W., Duda, B., Chan, E. D., Abraham, E., Ware, L. B., Matthay, M. A., Day, B. J. (2003). Pulmonary edema fluid antioxidants are depressed in acute lung injury. *Crit. Care Med*., **31** (9), 2309–2315.

Crimi, E., Sica, V., Williams-Ignarro, S., Zhang, H., Slutsky, A. S., Ignarro, L. J., Napoli, C. (2006). The role of oxidative stress in adult critical care. *Free Radical Biol. Med*., **40** (3), 398–406.

Esterbauer, H., Cheeseman, K. H. (1990). Determination of aldehydic lipid peroxidation products: Malonaldehyde and 4-hydroxynonenal. *Meth. Enzymol*., **186**, 407–421.

Ferguson, N. D., Frutos-Vivar, F., Esteban, A., Gordo, F., Honrubia, T., Penuelas, O., Algora, A., Garcia, G., Bustos, A., Rodriguez, I. (2007). Clinical risk conditions for acute lung injury in the intensive care unit and hospital ward: A prospective observational study. *Crit. Care* (London), **11** (5), R96.

Galvin, I., Ferguson, N. D. (2011). Acute lung injury in the ICU: Focus on prevention. *Annu. Update Int. Care Emerg. Med*., **1**, 117–128.

Janero, D. R. (1990). Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. *Free Radic. Biol. Med.,* **9** (6), 515–540.

Kumar, K. V., Rao, S. M., Gayani, R., Mohan, I. K., Naidu, M. U. (2000). Oxidant stress and essential fatty acids in patients with risk and established ARDS. *Clin. Chim. Acta: Int. J. Clin. Chem*., **298** (1–2), 111–120.

Lichtenstern, C., Hofer, S. Möllers, A., Snyder-Ramos, S., Spies-Martin, D., Martin, E., Schmidt, J., Motsch, J., Bardenheuer, H. J., Weigand, M. A. (2011). Lipid peroxidation in acute respiratory distress syndrome and liver failure. *J. Surg. Res*., **168**, 243–252.

Ranieri, V. M., Rubenfeld, G. D., Thompson, B. T., Ferguson, N. D., Caldwell, E., Fan, E., Camporota, L., Slutsky, A. S. (2012). Acute respiratory distress syndrome: The Berlin Definition. *JAMA* (*Journal of the American Medical Association*), **307** (23), 2526–2533.

Sigurdsson, M. I., Sigvaldason, K., Gunnarsson, T. S., Moller, A., Sigurdsson, G. H. (2013). Acute respiratory distress syndrome: Nationwide changes in incidence, treatment and mortality over 23 years. *Acta Anaesthesiol. Scand*., **57**, 37–45.

OKSIDATĪVĀ STRESA MARĶIERU AKTIVITĀTE AKŪTA RESPIRATORA DISTRESA SINDROMA GADĪJUMĀ

Akūts respirators distresa sindroms (ARDS) ir viena no biežākajām komplikācijām, kas saistīta ar smagu hipoksēmiju un augstu mirstību intensīvās terapijas un reanimācijas nodaļas pacientu vidū. Līdzsvara traucējumi starp oksidantiem un antioksidantiem var nopietni ietekmēt ARDS patofizioloģiju. Dinamikā tika novēroti 17 pacienti ar ARDS septiņu dienu laikā kopš iekļaušanas pētījumā ar mērķi sekot izmaiņām oksidantu un antioksidantu aktivitātē. Saskaņā ar iegūtajiem rezultātiem tika secināts, ka pacientiem ar ARDS dinamiski izmainās tādu oksidantu aktivitāte kā malondialdehīds un slāpekļa oksīds. Mirušo pacientu grupā oksidantu un antioksidantu līmenis bija atšķirīgs salīdzinājumā ar izdzīvojušiem pacientiem. Paaugstināta tiobarbitūrskābes atvasinājumu un glutationperoksidāzes aktivitāte pirmajā dienā kopš iekļaušanas pētījumā pozitīvi korelē ar nelabvēlīgu slimības iznākumu pacientiem ar akūtu respiratoru distresa sindromu.