International Journal of Health Services Research and Policy
www.dergipark.org.tr/ijhsrp

IJHSRP

e-ISSN: 2602-3482

Research Article

PROGNOSTIC VALUE OF DYNAMIC THIOL-DISULFIDE HOMEOSTASIS IN PREDICTING HOSPITAL MORTALITY IN HYPOXEMIC RESPIRATORY FAILURE

Bilal İLANBEY*[*1] Duygu ZORLU KARAYİĞİT[2] Haci Mehmet ÇALIŞKAN[3]
Zamir Kemal ERTÜRK[4] Burak ÇELİK[5] Suleyman ERSOY[3] Kenan GÜÇLÜ[1]

[1]Kirsehir Ahi Evran University, Faculty of Medicine, Department of Medical Biochemistry, Kirsehir, Turkey
[2]Kirsehir Ahi Evran University, Faculty of Medicine, Department of Pulmonology, Kirsehir, Turkey
[3]Kirsehir Ahi Evran University, Faculty of Medicine, Department of Emergency Medicine, Kirsehir, Turkey
[4]Etimesgut Sehit Sait Erturk State Hospital, Department of Emergency Medicine Ankara, Turkey
[5]Kirsehir Training and Research Hospital, Department of Emergency Medicine, Kirsehir, Turkey
[*] Corresponding author; bilalilanbey@hotmail.com

Abstract: *Hypoxemic respiratory failure (HRF) is a PaO_2 < 60 mmHg with normal or subnormal $PaCO_2$. The gas exchange is impaired at the level of the alveolo-capillary membrane. HRF is associated with a high mortality rate in hospitals, and there is no diagnostic laboratory test to predict this mortality. This study evaluates the possibility of predicting mortality in HRF patients with dynamic thiol-disulfide homeostasis parameters, which are indicators of oxidation state. Sixty-two patients with HRF and 40 healthy individuals in the control group were included in the study. Dynamic thiol-disulfide parameters were studied from the serum of all participants. Total and native thiol levels were significantly lower in the patients than in the control group (p <0.05). Disulfide levels were higher in patients who died than in survivors (p <0.01). The logistic regression analysis determined that the rise in disulfide values increased the mortality risk by 1.57 times. Progressive hypoxemia increases oxidation. Serum disulfide level is a valuable parameter in predicting mortality in hypoxemia.*

Keywords*: Hypoxemia; thiol-disulfide; oxidative stress; mortality*

Received: June 10, 2021 *Accepted: May 11, 2022*

1. Introduction

Hypoxemic respiratory failure (HRF), also referred to as type I respiratory failure, is a clinical picture of the respiratory system's oxygenation function failing [1]. HRF can be seen in almost all lung diseases, usually involving fluid accumulation or collapse in the alveoli, and many extrapulmonary disorders such as obesity and heart failure [2]. Recently, the importance of HRF has increased due to the effects of the disease associated with SARS-CoV-2 infection (coronavirus disease 2019; COVID-19) in the lungs [3]. Acute HRF is among the most common causes of hospital mortality, with an approximately 30% rate [4]. Nowadays, the diagnosis of HRF is made by clinical findings, arterial blood gas tests, and chest radiography. Various scores such as APACHE II are currently used for the severity of such diseases, and there is no single laboratory parameter routinely used to predict mortality [5].

Insufficient oxygenation can result in hypoxia in the lung and other tissues [6]. There are conflicting studies in the literature between hypoxia and oxidative stress. Cell culture and experimental animal studies show both the increase and decrease of reactive oxygen species (ROS) in hypoxia [7, 8].

Thiols are organic compounds containing the sulfhydryl group. These compounds have general antioxidant properties [9]. During oxidation, intramolecular and intermolecular disulfide bonds are

formed between two sulfhydryl groups, and since this is a reversible reaction, it is called the dynamic thiol-disulfide homeostasis [9]. In addition, it can also be an index for oxidant-antioxidant states in many pathological conditions, such as heart disease, diabetes, cancer, and neurological diseases [10].

This study aimed to investigate the relationship between hypoxemia and oxidative stress based on the dynamic thiol-disulfide balance. Also, we aimed to determine whether dynamic thiol-disulfide homeostasis parameters effectively predict mortality in patients diagnosed with HRF.

2. Material and Methods

2.1. Study population

This cross-sectional study included 62 HRF with chronic obstructive pulmonary disease (COPD) patients admitted to the Kırşehir Training and Research Hospital emergency department and 40 healthy individuals who were admitted to the emergency department and had no health problems. Those using antioxidant drugs, chronic liver disease, malignancies, or active smokers were excluded from the study. The diagnosis of HRF was made based on clinical findings, chest radiography, and arterial blood gas test measurements. Fourteen of the 62 HRF patients died soon after being admitted to the hospital; ~~3~~ three from sepsis, six from cardiac arrest, and five from pulmonary disease; ~~48~~ forty-eight patients survived. All participants provided written informed consent. The investigation conforms to the principles outlined in the Declaration of Helsinki.

2.2. Ethical Consideration

The study was approved by the Ethics Committee of Ahi Evran University Faculty of Medicine (Ethical committee approval number and date: 2018-03/32, 13/02/2018).

2.3. Laboratory Parameters

Venous blood samples were collected from all participants into anticoagulant-free serum tubes and tubes containing K2EDTA at the first admission. Serum tubes were centrifuged at 2000 x g for 10 minutes. Routine biochemistry tests were performed immediately from a portion of the serum collected, and the remaining serum was transferred to microcentrifuge tubes and stored at −80 °C.

Routine biochemistry tests, including glucose, urea, creatinine, aspartate transaminase (AST), alanine transaminase (ALT), and C-reactive protein (CRP), were measured in a Cobas 501 (Roche Diagnostics®, Germany) autoanalyzer. Complete blood count parameters from the blood samples collected in tubes with K_2EDTA have been studied on the Sysmex XN-1000 (Sysmex Corporation, Kobe, Japan) device. Blood samples collected from the radial or femoral artery to dry lithium heparin injectors were immediately analyzed for pH, O_2 saturation, PaO_2 (partial pressure of oxygen), $PaCO_2$ (partial pressure of carbon dioxide), and HCO_3 (bicarbonate) parameters using the RAPIDLab® 348EX (Siemens Healthcare GmbH, Germany) instrument at room temperature.

The parameters of thiol-disulfide homeostasis were measured as previously described by Erel and Neseliolu [11]. Total thiol and native thiol tests were run with commercial kits (Rel Assay Diagnostics, Gaziantep, Turkey) using the Cobas 501 (Roche Diagnostics®, Germany) biochemistry autoanalyzer. Disulfide levels were calculated as follows:

$$[total\ thiol\ values - native\ thiol\ values]/2 \quad (1)$$

2.4. Statistical Analysis

All statistical analyses were performed using SPSS software (version 21.0, SPSS Inc., Chicago, IL, USA) and Excel (Microsoft Turkey). The normality of variables was determined by Kolmogorov-Smirnov and Shapiro-Wilks tests. Results were presented as mean ± standard deviation or median (at

the 25th and 75th percentiles) for continuous variables and frequency for categorical variables. To compare two groups, an unpaired t-test or Mann-Whitney U-test was used for continuous variables, a χ^2 test was used for categorical data, and Pearson's / Spearman's test was used for correlation analysis. One-way analysis of variance (ANOVA) was used to compare more than two normally distributed groups. Bonferroni comparison was made as a post hoc test after the ANOVA test. The Kruskal-Wallis test was performed to compare more than two groups that were not normally distributed. Dunn's nonparametric comparison was performed as a post hoc test after the Kruskal-Wallis test. A linear regression model was used to examine the independent effects of different predictors. Logistic regression analysis was used to find other predictors of mortality in the study. In this analysis, variables were chosen using the backward elimination method. The receiver operating characteristic (ROC) curve analysis was used to evaluate the diagnostic value for mortality of variables found to contribute to the logistic regression model significantly. Values of $p < 0.05$ were considered statistically significant.

3. Results

The demographic characteristics and laboratory findings of the study group are summarized in Table 1. There was no statistically significant difference in demographic characteristics between the groups.

Table 1. Demographic characteristics and laboratory results of the groups.

Parameters	Deceased (n = 14)	Survivor (n = 48)	Control (n = 40)	p-value
Age, years	71.5 ± 13.1	68.5 ± 10.8	66.7 ± 7.3	0.292
Sex, male %	57.1	70.8	67.5	0.628
BMI, kg/m^2	27.5 ± 6.4	27.5 ± 6.7	26.7 ± 3.8	0.783
Hypertension, %	42.9	50.0	47.5	0.892
Diabetes mellitus, %	42.9	27.1	27.5	0.493
Glucose, mg/dL	127 (96-193)	124 (104-145)	109 (98-183)	0.662
Urea, mg/dL	66 (46-111)	49 (35-69)	49 (37-58)	0.247
Creatinine, mg/dL	1.43 (0.90-1.88)	0.89 (0.77-1.11)	0.90 (0.74-1.23)	0.113
ALT, IU/L	11 (6-21)	15 (9-23)	15 (8-21)	0.322
AST, IU/L	17 (11-32)	22 (14-28)	19 (12-28)	0.811
CRP, mg/L	10.3 (5.52-13.4) *†	1.49 (0.53-4.31)	1.04 (0.41-2.55)	**<0.001**
Leukocyte, x10^{3}µL	9.80 ± 4.7	9.24 ± 4.3	9.68 ± 4.0	0.860
Total thiol, µmol/L	296 ± 61*†	309 ± 56†	424 ± 57	**<0.001**
Native thiol, µmol/L	254 ± 56*†	274 ± 54†	381 ± 58	**<0.001**
Disulfide, µmol/L	21.8 ± 3.8*†	17.4 ± 4.1	17.9 ± 3.0	**0.001**
O_2 Sat, %	66.6 ± 9.9	71.6 ± 8.2	-	0.061[a]
pH	7.37 ± 0.05	7.35 ± 0.05	-	0.213[a]
$PaCO_2$, mmHg	40.0 (37.2-42.0)	40.5 (34.0-44.8)	-	0.760[b]
PaO_2, mmHg	43.2 ± 8.3	43.6 ± 8.8	-	0.863[a]
HCO_3, mEq/L	24.2 ± 2.8	24.6 ± 3.4	-	0.736[a]

Note: AST, aspartate transaminase; ALT, alanine transaminase; BMI, body mass index; CRP, C-reactive protein; HCO_3, bicarbonate; O_2 Sat, oxygen saturation; PaO_2, partial pressure of oxygen; $PaCO_2$, partial pressure of carbon dioxide.
[a,b]: p values were obtained via unpaired t-test and Mann-Whitney U-test, respectively. *p<0.05 versus Survivor,†p<0.05 Control

There was a statistically significant difference in CRP levels between the patients who were deceased and survived and the control group; CRP levels were approximately ten times higher in patients deceased than others (p<0.001, Table 1). Other biochemical tests in the three groups were statistically

similar. There were statistically significant differences in thiol-disulfide homeostasis parameters between the groups. Both total and natural thiol levels were significantly lower in surviving patients and deceased, both from each other and in the control group. In contrast, disulfide levels were significantly higher only in those who were deceased (p=0.001, Table 1, Figure 1). Mean leukocyte count and blood gas parameter values in the three groups were statistically similar.

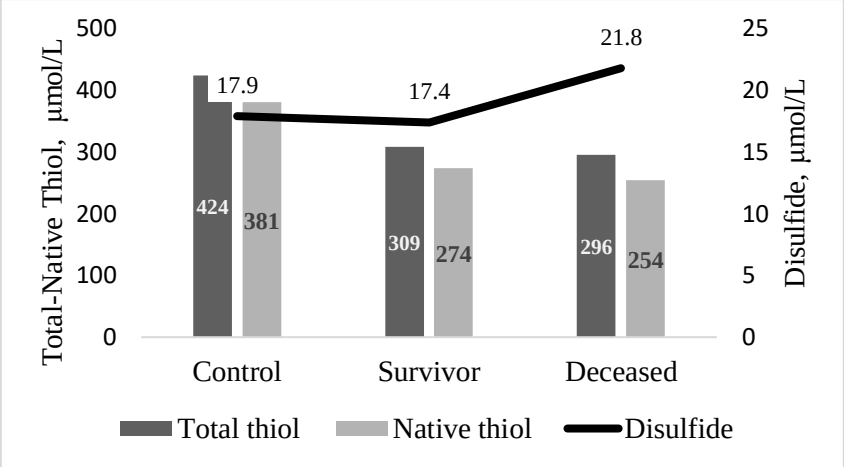

Fig. 1 Average disulfide values in control, survivor, and deceased groups

We also evaluated the correlations of laboratory findings of deceased patients. The correlation between CRP, an important marker for inflammation, and disulfide levels were not significant (r = -0.180, p = 0.538). There was an inverse correlation between disulfide and O_2 saturation levels, an indicator of hypoxemia (r = -0.632, p = 0.015; Figure 2). In the linear regression analysis for disulfide and oxygen saturation in the deceased group, it was found that oxygen saturation was responsible for approximately 40% (r^2 = 0.339) of the increase in serum disulfide levels. There was no statistically significant relationship between other parameters.

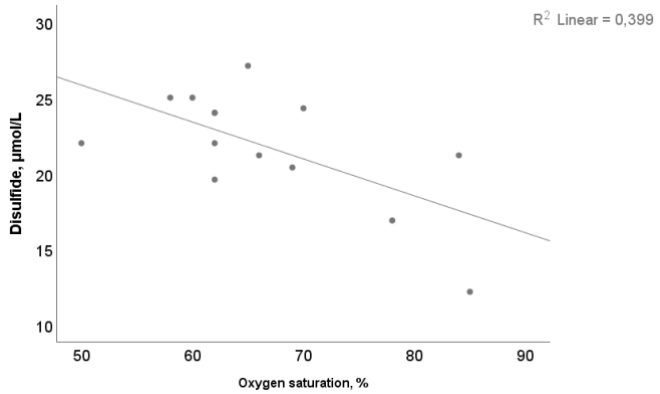

Fig. 2 Scatter plot showing disulfide levels and oxygen saturation in the deceased group.

Table 2 shows the logistic regression analysis to determine the variables that predict mortality. According to the logistic regression analysis results, an increase in total thiol level decreased the mortality probability by 0.98 times, and an increase in disulfide and CRP levels increased the mortality probability by 1.57 and 1.19 times, respectively. There was no statistically significant prediction of the effect of gender on mortality.

Table 2. Logistic regression analysis to estimate the probability of death.

Risk Factor	OR (95% C.I.)	p-value
Total thiol (μmol/L)	0.98 (0.96–0.99)	0.044*
Disulfide (μmol/L)	1.57 (1.18–2.08)	0.002**
CRP, (mg/L)	1.19 (1.04–1.35)	0.011*
Gender (1)	0.19 (0.03–1.24)	0.083

CRP, C-reactive protein; * :p<0.05; **: p<0.01

ROC analysis was performed to determine whether CRP, total thiol, and disulfide variables had a cut-off value in predicting mortality in patients with HRF. As there was no statistically significant cut-off value of total thiol values on death, the area between the reference line and the line of total thiol values was not statistically significant (AUC: 0.537, p = 0.674). In the ROC analysis, cut-off values of 4.18 mg/dL for CRP and 19.5 μmol/L for disulfide were found in predicting mortality, respectively (Figure 3).

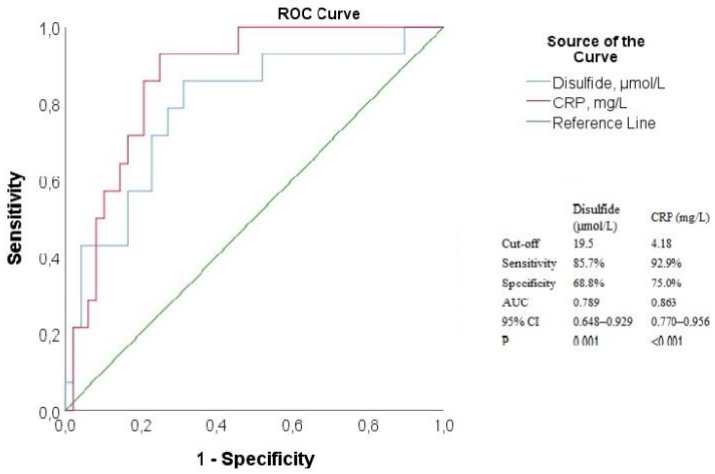

Fig. 3 ROC curve for disulfide and CRP values

4. Discussion

In this study, we found that due to the oxidative stress caused by hypoxemia, the dynamic thiol-disulfide balance was preserved in survivors in patients with HRF, but the balance shifted towards disulfide in patients who died. We found that the disulfide parameter is more valuable than other routine laboratory tests in predicting mortality.

During normal cellular metabolism in aerobic respiration organisms, ROS and reactive nitrogen species (RNS) are released, which can be harmful to the organism [12, 13]. At the same time, these species deliberately produce these compounds at low levels for use in many physiological processes, such as the killing of pathogens, autophagy, apoptosis, and some signaling pathways [14]. Organisms have developed various antioxidant defense systems to keep ROS under control and prevent damage to themselves, including non-enzymatic peptides containing thiol, such as glutathione, and enzymatic antioxidants, such as catalase and superoxide dismutase (SOD). Thus, an oxidant-antioxidant balance is achieved in the body [9, 15]. In any case, if this balance shifts in favor of ROS increase, oxidative damage occurs. Oxidative damage is associated with many diseases, such as cardiovascular, neurodegenerative, metabolic, and inflammatory diseases [16]. An increase in ROS production under hypoxemic conditions has been found in various experimental studies. Among these studies, there are

studies in the literature that show that both the direct ROS itself and the number of oxidation products it causes and the antioxidants decrease against it [17-21]. The increase in ROS in hypoxia is thought to be the signaling function required by the cells to respond to hypoxia [22].

The levels of various compounds and enzyme activities are measured to assess the oxidant-antioxidant levels in the body. One of these measurements is thiol-disulfide homeostasis [10]. In case of ROS increase, thiols are oxidized to disulfides and the equilibrium shifts towards disulfide. If the ROS increase is relatively small and short-lived, the antioxidant responses can be activated to reduce excess ROS and restore equilibrium. However, when the ROS increase is at high levels and persistent (in cases of chronic oxidative stress), antioxidant responses are insufficient, and restoration cannot be achieved [23]. In our study, there was a slight increase in serum disulfide levels in HRF patients than in the control group, while there was a decrease in total and native thiols, suggesting that oxidation is at controllable levels. We found that the increase in hypoxia is responsible for the increase in disulfide levels. It was observed that oxygen saturation decreased, and disulfide levels increased in patients who died (Figure 2). The high disulfide values of the deceased patients indicate that the oxidation was severe in those individuals and that the antioxidant thiols were insufficient to prevent oxidant damage. In addition, since CRP is a good marker for inflammation, we examined whether there is a relationship between CRP and disulfide levels. However, we could not find a relationship. It suggests that inflammation did not contribute to disulfide levels in patients who deceased.

The hospital mortality rate from acute HRF is about 30% [4], and the leading causes of death are sepsis, pulmonary dysfunction, and neurological dysfunction [24]. In the current study, 14 of the patients (23%) died after admission to the emergency department. In our study, when biochemical tests, blood gas, and dynamic thiol-disulfide parameters were compared between those who are deceased and those who survived, there were only significant increases in disulfide and CRP levels but no significant differences in all the remaining parameters. These results show that routine parameters and blood gases may not be so beneficial in predicting the mortality of these patients. On the other hand, serum disulfide levels are significant in predicting mortality in these patients.

Our study has some limitations, in that it was conducted in a single center, and the study group was relatively small in numbers. In addition, the underlying primer disease and comorbidities of the patients that caused HRF may also have affected disulfide levels and mortality.

5. Conclusion

In HRF, ROS increases due to hypoxia and progressive hypoxemia, oxidation level, and mortality risk. Our study demonstrated that the routine biochemical and blood gas parameters used in predicting the mortality of HRF patients were insufficient. However, we found that the serum disulfide parameter predicted mortality better in these patients.

We believe that the findings we obtained from our study will shed light on further studies. Antioxidant supplements that cause an increase in blood thiol levels, such as glutathione, can be given to patients with hypoxemic respiratory failure who are admitted to the emergency department with high disulfide levels. In this way, it can be investigated whether it has a positive effect on the mortality of patients.

Ethics approval

The study was approved by the Ethics Committee of Ahi Evran University Faculty of Medicine (Ethical committee approval number and date: 2018-03/32, 13/02/2018). The procedures used in this study adhere to the tenets of the Declaration of Helsinki.

Acknowledgments

The authors would like to thank the Scientific Research Projects Unit of Ahi Evran University.

Conflict of Interest

The authors declare that there is no conflict of interest.

Funding

This research was supported by a grant from Ahi Evran University Research Foundation (TIP.A4.19.004).

Author contributions

B. I: Conceptualization, writing - Original Draft preparation, Methodology, Formal analysis
D. Z. K: Conceptualization, Methodology, Investigation
H. M. .C: Conceptualization, Methodology, Writing - Original Draft preparation
Z. M. E: Writing- Reviewing and Editing, Visualization
B. C: Investigation
S. E: Investigation
K.G: Data curation, Formal analysis

References

[1] Gurka, D. P., and Balk, R. A., Acute respiratory failure, in *Critical Care Medicine*, (Ed. Parrillo J., Dellinger R.P.) Elsevier, Philadelphia, pp. 773-794, 2008.

[2] Roussos, C., and Koutsoukou, A., "Respiratory failure", *European Respiratory Journal*, 22 (47 suppl), 3s-14s, 2003.

[3] Radovanovic, D., et al., "Helmet CPAP to treat acute hypoxemic respiratory failure in patients with COVID-19: a management strategy proposal", *Journal of clinical medicine*, 9 (4), 1191, 2020.

[4] Vincent, J.-L., et al., "The epidemiology of acute respiratory failure in critically ill patients", *Chest*, 121 (5), 1602-1609, 2002.

[5] Kelley, M. A., et al., "Predictive scoring systems in the intensive care unit", *UpToDate. Available at: URL: http://www. uptodate. com/online/content/author. do*, 2012.

[6] Askew, E., "Work at high altitude and oxidative stress: antioxidant nutrients", *Toxicology*, 180 (2), 107-119, 2002.

[7] Prabhakar, N. R., and Kumar, G. K., "Oxidative stress in the systemic and cellular responses to intermittent hypoxia", *Biological Chemistry*, 385 (3-4), 217-221, 2004.

[8] McGarry, T., et al., "Hypoxia, oxidative stress and inflammation", *Free Radical Biology and Medicine*, 125, 15-24, 2018.

[9] Deneke, S. M., "Thiol-based antioxidants", *Curr Top Cell Regul*, 36 (151-180), 447, 2000.

[10] Erel, Ö., and Erdoğan, S., "Thiol Disulfide Homeostasis: An Integrated Approach with Biochemical and Clinical Aspects", *Turkish Journal of Medical Sciences*, 2020.

[11] Erel, O., and Neselioglu, S., "A novel and automated assay for thiol/disulfide homeostasis", *Clinical biochemistry*, 47 (18), 326-332, 2014.

[12] Circu, M. L., and Aw, T. Y., "Reactive oxygen species, cellular redox systems, and apoptosis", *Free Radical Biology and Medicine*, 48 (6), 749-762, 2010.

[13] Clanton, T. L., "Hypoxia-induced reactive oxygen species formation in skeletal muscle", *Journal of Applied Physiology*, 102 (6), 2379-2388, 2007.

[14] Paardekooper, L. M., et al., "Radical Stress Is More Cytotoxic in the Nucleus than in Other Organelles", *International journal of molecular sciences,* 20 (17), 4147, 2019.

[15] Bowler, E., and Ladomery, M. R., Working with Hypoxia, in: *Redox-Mediated Signal Transduction,* (Ed. Hancock J., Conway M). Springer, New York, pp. 109-133, 2019.

[16] Frijhoff, J., et al., "Clinical relevance of biomarkers of oxidative stress", *Antioxidants & redox signaling,* 23 (14), 1144-1170, 2015.

[17] Bernard, O., et al., "Mesenchymal stem cells reduce hypoxia-induced apoptosis in alveolar epithelial cells by modulating HIF and ROS hypoxic signaling", *American Journal of Physiology-Lung Cellular and Molecular Physiology,* 314 (3), L360-L371, 2018.

[18] Yadav, S., et al., "Activator protein-1 (AP-1): a bridge between life and death in lung epithelial (A549) cells under hypoxia", *Molecular and cellular biochemistry,* 436 (1-2), 99-110, 2017.

[19] Møller, P., et al., "Acute hypoxia and hypoxic exercise induce DNA strand breaks and oxidative DNA damage in humans", *The FASEB Journal,* 15 (7), 1181-1186, 2001.

[20] Ilavazhagan, G., et al., "Effect of vitamin E supplementation on hypoxia-induced oxidative damage in male albino rats", *Aviation, space, and environmental medicine,* 72 (10), 899-903, 2001.

[21] Li, N., et al., "The multiple organs insult and compensation mechanism in mice exposed to hypobaric hypoxia", *CELL STRESS & CHAPERONES,* 2020.

[22] Hernansanz-Agustín, P., et al., "Acute hypoxia produces a superoxide burst in cells", *Free radical biology and medicine,* 71, 146-156, 2014.

[23] Dröge, W., "Free Radicals in the Physiological Control of Cell Function", *Physiological Reviews,* 82 (1), 47-95, 2002.

[24] Ketcham, S. W., et al., "Causes and characteristics of death in patients with acute hypoxemic respiratory failure and acute respiratory distress syndrome: a retrospective cohort study", *Critical Care,* 24 (1), 1-9, 2020.