DÜSTAD
Dünya Sağlık ve Tabiat Bilimleri Dergisi
JOGHENS
Journal of Global Health & Natural Science
ISSN: 2687-637X Yıl: 2022 Cilt: 5 Sayı: 1

Diagnostic Values of Nitric Oxide and Hydrogen Peroxide Content in Inspired Air in Patients with Pneumonia Under Respiratory Device

Amine AKTAR KARAKAYA[1]
Yusuf TÜRKÖZ[2]
Ünsal ÖZGEN[3]

Abstract: Free radicals, especially nitric oxide (NO), are of increasing importance in the management of lung diseases. In this study, we aimed to measure the inflammatory parameters with a method that is easy to sample and does not carry any risk to the patient**.** 37 cases connected to ventilators were included (27 patients, 10 controls). On the first day and the third day, expiratory air was removed with an exhaled breath condenser, and NO, hydrogen peroxide (H_2O_2), and serum C-reactive protein (CRP) were examined. First day; The NO level of the pneumonia group was statistically higher than the control group ($p<0.0001$). No significant difference was found between the patients in terms of hydrogen peroxide levels on the first day ($p>0.05$). The decrease in NO level between the first and third days of the pneumonia group was found to be statistically significant ($p<0.05$). Serum CRP and exhaled air NO levels were significantly higher in the pneumonia group on the first day compared to the control group, but no difference was found between the H_2O_2 values. In our study, it is thought that NO is very significant in showing inflammation and may be valuable in early diagnosis, especially in respiratory device-associated pneumonia.

Keywords: exhaled air, H_2O_2, mechanical ventilation, nitric oxide, pneumonia

Solunum Cihazına Bağlı Pnömonili Hastalarda Soluk Havasındaki Nitrik Oksit ve Hidrojen Peroksitin Tanısal Değerleri

Özet: Serbest radikallerin özellikle nitrik oksitin (NO), akciğer hastalıklarının takibinde önemi giderek artmaktadır. Çalışmada, hasta için herhangi bir risk taşımayan, örneklemesi kolay bir yöntemle, enflamatuvar parametrelerin ölçülmesi planlandı. Solunum cihazına bağlı 37 olgu alındı (27 hasta, 10 kontrol). Olguların birinci ve üçüncü günlerde soluk hava yoğunlaştırıcısı ile ekspiryum hava numunesi alınarak, NO, hidrojen peroksit (H_2O_2) ayrıca serumdan C-reaktif protein (CRP) düzeyleri ölçüldü. Birinci gün; pnömoni grubunun NO düzeyi kontrol grubundan istatistiksel olarak daha yüksekti ($p<0,0001$). Birinci gün H_2O_2 düzeyi açısından gruplar arasında anlamlı bir farklılık yoktu ($p>0,05$). Pnömoni grubunun birinci ve üçüncü gün arasındaki NO düzeyindeki düşüş istatistiksel olarak anlamlı bulundu ($p<0,05$). Birinci gün bakılan serum CRP ve ekspiryum hava NO düzeyleri pnömoni grubunda anlamlı derecede yüksekti, ancak H_2O_2 düzeyleri arasında fark bulunmadı. Yapılan çalışmada, enflamasyonu göstermede NO ölçümünün oldukça anlamlı olduğu, özellikle solunum cihazına bağlı pnömonide erken tanıda değerli olabileceği düşünülmektedir.

Anahtar kelimeler: soluk havası, H_2O_2, solunum cihazı, nitrik oksit, pnömoni

[1] Corresponding author, Department of Pediatric Endocrinology, Mardin Education and Research Hospital, Mardin, Turkey, aktarkarakaya@gmail.com, 0000-0001-7004-6803
[2] Department of Medical Biochemistry, Faculty of Medicine, Inonu University, Malatya, Turkey, yusuf.turkoz@inonu.edu.tr, 0000-0001-5401-0720
[3] Department of Pediatric Hematology, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Turkey, unsal.ozgen@omu.edu.tr, 0000-0002-4896-7435

INTRODUCTION

Ventilator-associated pneumonia (VAP) is an important cause of mortality and morbidity in the pediatric intensive care units and is reported to affect approximately one-tenth of children in mechanical ventilation (Chang and Schibler, 2016). In the literature, the mortality rate from VAP was found to be 13% (Kalil et al., 2016). Ventilator-associated pneumonia is defined as pneumonia that occurs 48 hours after intubation (Türk toraks derneği, 2018). New infiltrations on chest X-ray, fever and change in secretions, and increase in respiratory support requirement should warn the clinicians about VAP. Pneumonia occurring during the first four days of mechanical ventilation was called early-onset VAP, and pneumonia developed after day 4 was called late-onset VAP (Xu et al., 2019). The importance of nitric oxide (NO) is increasing in terms of early identification of patients with pneumonia under the respiratory device. Free oxygen radicals (FOR) and NO, in particular, play an important role in the development of lung diseases as well as other organs. However, the detection of these markers in the blood is not very reliable. Therefore, in patients with inflammation, to monitor the degree of the inflammatory reaction, the exhaled breath is condensed and examined. This method is preferred because of its easy applicability in evaluating the response to treatment, in the differential diagnosis, and in assessing the severity of lung diseases (Lee and Thomas 2009). It is hoped that identifying metabolites in the exhaled breath will be useful in understanding the pathophysiology of lung diseases and in the diagnosis (Bjermer et al., 2014). Increased lung stress and increased reactive oxygen radicals and NO in lung diseases have been shown in many studies (Balint et al., 2001; Kelekçi et al., 2013; Karsten et al., 2014).

To date, many studies have been conducted by collecting samples by direct inhalation of patients with asthma (Ricciardolo et al., 2015), cystic fibrosis (Balint et al., 2001), chronic obliterative bronchitis (Brugière et al., 2005) to exhaled breath condenser. In the routine follow-up of patients, the use of NO (Balint et al., 2001; Dweik et al., 2001), and hydrogen peroxide (H_2O_2) (Teng et al., 2011) parameters is recommended. Although the availability of the breath air condensation method has been shown in patients with the respiratory device (Cheah et al., 2003), as far as we can review the literature, there are only few studies on the measurement of inflammatory parameters in children with mechanical ventilator needs.

In this study, we aimed to measure the inflammatory parameters by using the EBC method which is easy to sample and does not carry any risk to the patient. In this way, we aim to demonstrate the usefulness of NO and H_2O_2 detected in exhaled breath of patients, especially in the early diagnosis of ventilator-associated pneumonia.

MATERIALS and METHODS

This study was conducted in Inönü University Faculty of Medicine, on patients under respiratory device support in pediatric and neonatal intensive care units. The patient group was composed of a total of 27 patients (19 males and 8 females) hospitalized in intensive care units, aged between one day and 10 years. As a control group, a total of 10 patients (8 boys, 2 girls) whose ages ranged from one day to 10 years without infection were evaluated. In the pneumonia group; the day on which pneumonia was diagnosed and in the control group; the day of adherence to the respiratory device was accepted as the first day. Samples were taken on the first day and the third day NO and H_2O_2 in the exhaled breath taken by the EBC method and CRP in serum were studied in the patients who were included in the study. The informed approval was taken from the parents. In this study, the approval of the ethics committee was obtained.

Clinical Pulmonary Infection Score

This scoring system is performed based on the body temperature, white blood cell count, characteristics and amount of tracheal secretions, oxygenation, chest X-ray, and tracheal aspirate culture of the patients (Table 1). This scoring system was also used in later studies. The total score of the patients in the control group was calculated as 1 point.

Table 1. Clinical pulmonary infection score (Türk Toraks Derneği, 2018).

Parameters	0 point	1 point	3 points
Body temperature (^{0}C)	≥36.1 - ≤38.4	≥38.5- ≤38.9	≥39, ≤36
White blood cell count	≥4000-≤11000	<4000, >11000	
Tracheal secretion	Absent	Non-purulent	Purulent
Oxygenization (PaO_2/FiO_2)	>240 or ARDS		<240 and no ARDS
Chest radiograph	No infiltration	Diffuse /patch infiltration	Localised infiltration
Microbiology	Insignificant microbial growth	Significant microbial growth	

ARDS: Acute respiratory distress syndrome, PaO_2: Partial arterial oxygen pressure, FiO_2: Fraction of inspired oxygen

This scoring was especially performed with the aim of early diagnosis and effective treatment of VAP, and it was stated that antibiotic use would be appropriate and effective in this way (Zilberberg and Shorr, 2010). According to the VAP report published by the American Chest Association, the diagnostic criteria for VAP are;

1-Fever

2-Infiltration in chest x-ray

3-Increased number of fragmented cells in the blood

4-Inflammatory character in tracheal secretion

The above criteria were used to diagnose pneumonia in our patients (van Oort et al., 2019). Clinical pulmonary infection scoring was used as a guide for these patients.

Exhaled Breath Condenser

The exhaled breath condenser consists of a nested cold tube assembly made of polyvinyl chloride. Patients connected to the respiratory device were assessed for their vital functions before being connected to the exhaled breath condenser. The patients were connected to EBC after humidification of the lungs at 37 °C with a humidifier in the respiratory device and after 30 minutes of opening the condenser to cool. The patient's breathing hose was connected to this system and a hose coming out of it was connected to the expiration outlet of the respiratory device. The air was condensed in about 30 minutes. While the condenser is connected, to evaluate the vital functions; pulse, oxygen saturation, blood pressure, and blood gas were monitored. For optimal evaluation, no procedures such as aspiration were performed. Since there was no gas exchange during breathing, there was no risk of infection.

Taking the Samples

After connecting the EBC, samples of 2-4 ml of exhaled air concentrate taken over 30 minutes were placed in siliconized Eppendorf tubes and stored at approximately –80°C. All chemical materials were of analytical purity. The chemical materials were obtained from Sigma Aldrich (Sigma Aldrich Chemie Gmbh, Steinheim, Germany).

Nitric Oxide Analysis

Nitric oxide is an unstable molecule. It has a short half-life, rapidly converting to nitrite (NO_2) and nitrate (NO_3). For this reason, the total nitrite (NO_2) level in biological fluids is usually suggested as an indicator of NO production (Jungersten et al., 1996). So, NO levels of the breath condensate samples were measured as total nitrite after the conversion of nitrate to nitrite. The total nitrite assay procedure was partly adapted from the method described by Ozbek et al. (2000). The total nitrite assay procedure is based on spectrophotometric measurement at a wavelength of 548 nm after the conversion of nitrite to a purple-colored azo-dye with Greiss reagent.

Hydrogen Peroxide Analysis

The hydrogen peroxide assay procedure was partly adapted from the method described by Loukides et al. (1998). The hydrogen peroxide assay procedure is based on spectrophotometric measurement of the oxidized-end product at a wavelength of 450 nm after the oxidation of tetramethylbenzidine by Horseradish Peroxidase (HRP) using hydrogen peroxide in the sample. The minimal detection limit of the method was approximately 0.1 μmol/L H_2O_2.

Ethical Statement

In this study, 2004/71 numbered ethics of İnönü University School of Medicine board approval was obtained. This study is İnönü University Scientific Research Projects Management Unit Supported by the 2005-12 project number. The study was conducted in design with the Declaration of Helsinki Ethical Principles. The informed consent form was signed by the families participating in the study.

Statistical Analyses

Statistical analyses were performed with the SPSS version 18.0 package program compatible with Windows. Results were given as mean ± standard deviation. Kolmogorov-Smirnov Test was used to determine the normal distribution of the variables in the groups ($p<0.05$). Mann-Whitney U test was used in the comparison of the patient group and the control group. Wilcoxon test was used to evaluate the change in the patient group over time. $p<0.05$ was considered to be statistically significant.

RESULTS

The age of the 27 pediatric patients under respiratory device support who were included in the study ranged from one day to 10 years, 70% were male and 30% were female. 80% of the control group was male and 20% was female. The mean age of the children in the patient group was 13.7±5.1 months and the control group was 17.8±4.2 months (Table 2). 44% of the patient group and 60% of the control group were in the newborn period (0-28 days). There was no significant difference in terms of age between the control and patient groups ($p>0.05$).

Table 2. The demographic data of the patient and control groups.

Parameters	Patient group	Control group
Age (month)	13.7±5.1	17.8±4.2
Gender (F/M)	8/19	2/8
Feeding (NG%)	52	60
Body temperature (^{0}C)		
36.5-38.4	19	10
38.5-38.9	4	-
36.4<veya 39>	4	-
White blood cell(10^3/mm^3)		
4-11	9	3
<4 or >11	18	7
Tracheal secretion		
No secretions	-	6
Serous secretion	14	4
Purulent secretion	13	-

F: Female; M: Male; NG: Nasogastric

Pneumonia was detected in 18 (66.6%) of the cases within the first 4 days after being connected to the respiratory device and 9 (33.3%) after the 4th day. The agent was detected in one of the patients with early-onset pneumonia and 6 of the patients with late-onset pneumonia. The most common microorganisms were *Pseudomonas auroginosa, Candida albicans,* and Staphylococcus. Staphylococcus was isolated in the patient with early-onset pneumonia. The most common microorganism was found to be *P. auroginosa* in late-onset pneumonia (57%). *Pseudomonas auroginosa* was detected especially in neurological patients with long-term respiratory support. *Candida albicans* was also detected in a patient with neurological problems due to long-term respiratory device support and in a patient with congenital heart disease (28%). In the control group, four patients (40%) were diagnosed with hypoxic-ischemic encephalopathy, two (20%) with congenital heart disease, two (20%) with intracranial hemorrhage, one (10%) with subacute sclerosing panencephalitis, and one (10%) with epilepsy. Two patients had CRP positivity. Patients who had a score of 1 point according to the clinical pulmonary infection scoring were considered as the control group. Body temperature was measured as 36.5-38.4 °C in 70.4% of the pneumonia group and 100% of the control group. 40 °C was the highest measured body temperature and was measured in 1 patient. Respiratory secretion was detected in patients with pneumonia. The purulen secretion ratio was evaluated as 48.2%. Increased airway secretion was observed as the primary symptom. There was a significant difference between serum CRP values in the statistical analysis of the pneumonia group and control group on the first day; p= 0.021, (Fig. 1).

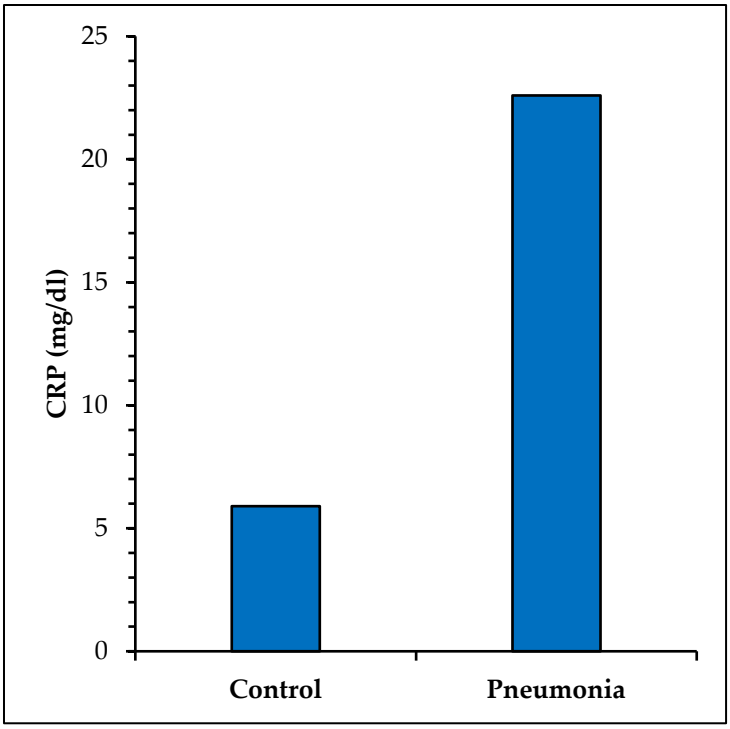

Figure1. Comparison of the first day CRP values of the pneumonia and control group.

In the statistical analysis of the pneumonia group and control group on the first day; there was a significant difference between exhaled breath NO values ($p<0.0001$) (Fig. 2).

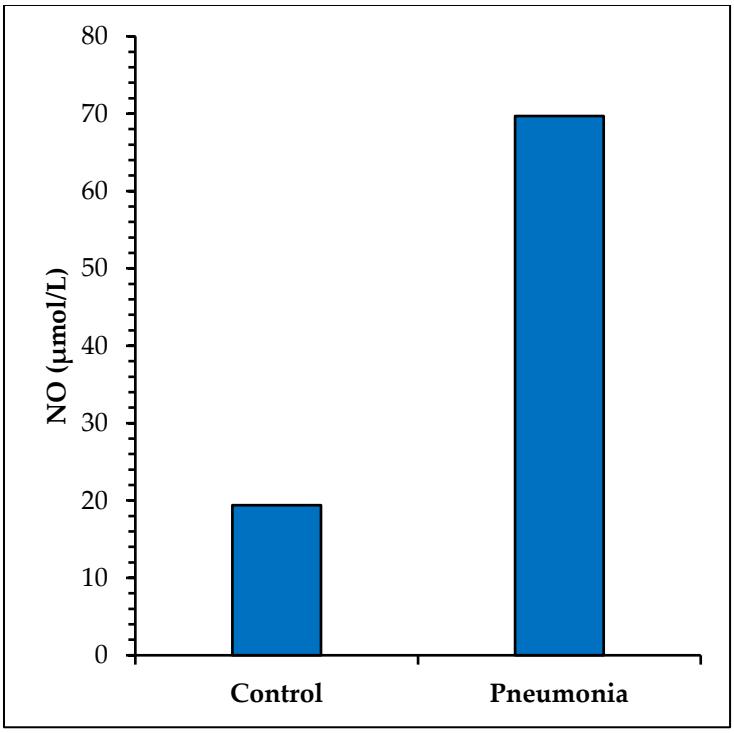

Figure 2. Comparison of the first day NO values of pneumonia and control group.

In the statistical analysis of the pneumonia group and control group on the first day; no significant difference was found in H_2O_2 analysis in exhaled breath ($p>0.05$) (Fig. 3).

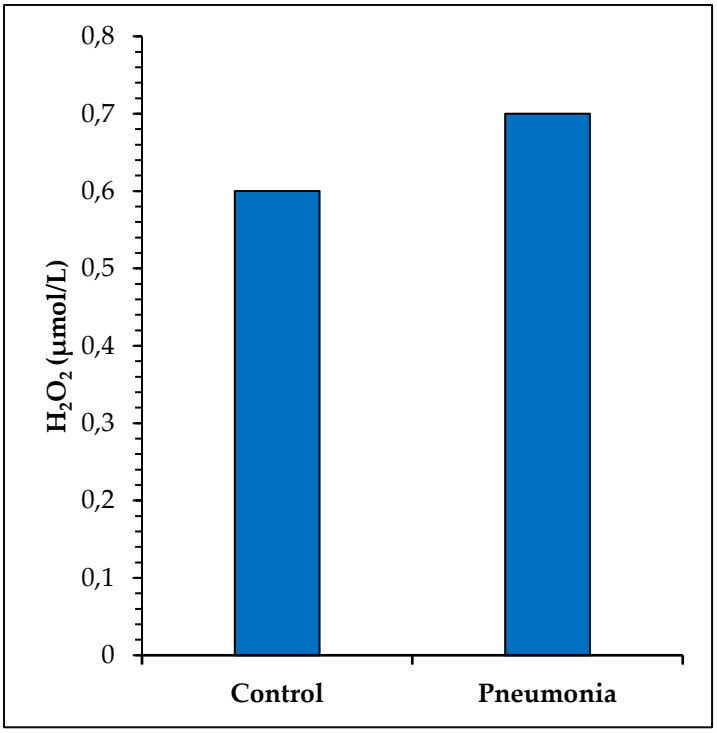

Figure 3. Comparison of H_2O_2 values of pneumonia and control group on the first day.

There was no statistically significant difference between the exhaled breath H_2O_2 values of the patients with pneumonia on the first day and the serum white blood cell count on the first day ($p>0.05$). On the first day, NO analysis was performed between the pneumonia and control groups and NO was statistically higher in the pneumonia group than the control group (69.7 ± 9.6 versus 19.4 ± 4.6) ($p<0.0001$) (Table 3). In the comparison of NO measurements on the first day and 3rd day of patients with pneumonia (69.7 ± 9.6 versus 29.5 ± 4.7), a decrease in NO during the days was statistically significant ($p<0.05$) (Table 4).

In the comparison of pneumonia and control group; there was no significant difference between them in terms of H_2O_2 values on the first day (0.7 ± 0.13 versus 0.6 ± 0.18) ($p>0.05$) (Table 3). In the comparison of H_2O_2 on the first day and 3rd day of the patients with pneumonia (0.7 ± 0.13 versus 0.2 ± 0.06), the decrease in H_2O_2 value was found to be statistically significant ($p<0.05$) (Table 4). According to the comparison of CRP levels on the first day between the pneumonia and the control group; the higher CRP value in the pneumonia group was found to be statistically significant compared to the control group (22.6 ± 6.66 versus 5.9 ± 1.77) ($p=0.021$) (Table 3). CRP values of the patients with pneumonia on the first and 3 rd day (22.6 ± 6.66 vs. 24.5 ± 7.86) were not statistically significant ($p>0.05$) (Table 4).

Table 3. The statistical comparison of the inflammatory parameters in the pneumonia and control group on the first day.

Parameters	Control	Pneumonia	*P* value
NO (µmol/L) $\bar{x}$±SD	19.4 ± 4.6	69.7 ± 9.6	0.0001
H_2O_2 (µmol/L) $\bar{x}$±SD	0.6 ± 0.18	0.7 ± 0.13	0.677
CRP (mg/dl) $\bar{x}$±SD	5.9 ± 1.77	22.6 ± 6.66	0.021

$\bar{x}$: Arithmetic mean; SD: Standard deviation

Table 4. The statistical comparison of the inflammatory parameters in the pneumonia group on the first day and 3rd.

Group	Parameters	First day	Third day	*P*
Pneumonia	NO (µmol/L) x̄±SD	69.7 ± 9.6	29.5 ± 4.7	<0.05
	H_2O_2 (µmol/L) x̄±SD	0.7 ± 0.13	0.2 ± 0.06	<0.05
	CRP (mg/dl) x̄±SD	22.6 ± 6.66	24.5±7.86	>0.05

x̄: Arithmetic mean; SD: Standard deviation

DISCUSSION and CONCLUTION

Ventilator-associated pneumonia is one of the important hospital infections that cause morbidity and mortality in patients with respiratory devices in intensive care units. There are no reliable markers to predict the onset of VAP (4). Free oxygen radicals play an important role in the development of lung diseases. The oxidant molecules cause harmful effects by disrupting the organism's structural elements, proteins, lipids, carbohydrates, nucleic acids, and necessary enzymes (Eşsizoğlu and Yıldırım, 2009; Lee and Thomas 2009). In some studies conducted so far, plasma nitrate / total nitrite ratio has been used to have an idea about NO status. Plasma nitrite and nitrate are end products of endogenous NO metabolism. In a study performed on 133 newborns, NO measurements in breath air and plasma nitrite/nitrate measurements were compared and it was concluded that NO level in breath air did not correlate with plasma nitrite/nitrate value and thus this result could not represent NO in breath air (Biban et al., 2001). Several methods have been developed over time to measure inflammation and oxidative stress of the airway. One method is induced sputum. This method is not appropriate for children and patients with severe dyspnea as it can cause bronchoconstriction and inflammatory reactions. Bronchoalveolar lavage is another method that can be used in patients, but is not preferred since it is an invasive method. Therefore, exhaled breath condensation, which is a noninvasive method, has been used to show airway inflammation and oxidative stress (Lee and Thomas 2009). EBC is used in many pulmonary diseases in both adults and children. With this method, many markers such as NO, H_2O_2, isoprostane, prostaglandins, and leukotrienes can be detected (Lee and Thomas 2009).

In our study, the NO level in the exhaled breath of children with VAP on the first day was found to be higher than the control group. Based on this data, NO was interpreted as a reliable marker in the early diagnosis of pneumonia. The fact that the NO value in the exhaled breath of the patients with pneumonia was highest on the first day and decreased on the third day was thought to be the result of suppression of inflammation in the lung due to antibiotic treatment. In a study, of 24 patients with community-acquired pneumonia who were not on a respiratory device, exhaled breath NO levels were measured before and after treatment, and it was found that NO showed a significant decrease after antibiotic treatment. In this study, it was stated that exhaled NO can be used to evaluate inflammation during pneumonia treatment and to define lung infection (Karsten et al., 2014). In another study performed on adult patients in the intensive care unit; NO, nasal NO, and plasma nitrate concentrations were measured in the exhaled breath of 49 intubated patients. In the 21 patients (43%) with pneumonia, the exhaled breath NO and nasal NO levels were found to be high when compared to the non-pneumonia group, but no difference was found between plasma nitrate levels (Adrie et al., 2001). In a study conducted on groups such as asthma, bronchiolitis obliterans, bronchiectasis, acute bacterial pneumonia, and pulmonary tuberculosis, exhaled breath NO levels of the patients were found to be significantly higher than the control group (Kelekçi et al., 2013). In all studies, no increase in NO was

shown in the case of inflammation (Carraro et al., 2008; Dikener et al., 2018). In the group of 18 community-acquired pneumonia and 17 healthy children, NO measurements were performed by examining the exhaled breath air and no significant difference could be obtained between the two groups[24]. Hydrogen peroxide can be found in expiratory air in many pulmonary diseases (ARDS, COPD, bronchiectasis, smoking) because it is an indicator of oxidative stress (Loukides et al., 1998; Ueno et al., 2008).

In our study, the statistical analysis of the first day H_2O_2 levels of pneumonia and control group was not significant ($p>0.05$), but the first day H_2O_2 values of patients with pneumonia were higher than those of 3th day ($p<0.05$). These results suggest that H_2O_2 is not a valuable enough marker in the early diagnosis of pneumonia. The fact that hydrogen peroxide was not a stable molecule was seen as a factor in the formation of this result. Similarly, in a study conducted with newborns with ventilator-dependent nasal CPAP, H_2O_2 in the exhaled breath was studied from the sample and no significant difference was found between the patient groups (Cheah et al., 2003). However, in a study on adult patients; 36 patients with a respiratory device associated with ARDS and 10 patients having respiratory device support due to extrapulmonary reasons were taken as the control group and daily hydrogen peroxide levels were compared. It was found that H_2O_2 levels increased 5 times more in the ARDS group than the control group. In the determination of hydrogen peroxide in the exhaled breath of these patients, increased levels of these oxygen radicals were associated with pulmonary infiltrative pathologies. But; it was concluded that the correlation between lung injury level and H_2O_2 level was incomplete due to the instability of this molecule and its diffusion into intact tissues (Kietzman et al., 1993).

In our study, no statistically significant correlation was found between the exhaled breath H_2O_2 values on the first day and serum white blood cell counts on the first day of the patients with pneumonia. It is assumed that the hydrogen peroxide released from the phagocytes can reach the lungs by circulation and can be detected in the exhaled breath. However, it is stated that some antioxidants in the pulmonary endothelium of pneumonia patients may prevent the passage of hydrogen peroxide from the blood to the lungs. We think that this statement may explain the correlation between hydrogen peroxide and white blood cells (Majewska et al., 2004). Our study showed that CRP values were higher in the pneumonia group compared to the control group ($p=0.021$) on the first day and CRP could be used in the early diagnosis of pneumonia. As a result; the limitation of our study was the small number of patients with pneumonia and the measurement of NO in exhaled breath and serum CRP were found to be more significant than H_2O_2 in exhaled breath. In addition, there was no statistically significant difference between exhaled breath NO and serum CRP measurements in patients with pneumonia on first day, suggesting that exhaled breath NO measurement in patients with pneumonia had no superiority to serum CRP. However, CRP is less specific than NO in pneumonia because it is not specific for lung infections and increases in the presence of infection in any part of the body. Therefore, although NO and CRP increase in pneumonia in the early period, the specificity of NO is higher and CRP is easier to perform.

In this study, it is suggested that NO measurement in exhaled breath by the EBC method can be used in the early diagnosis of pneumonia in pediatric patients with the respiratory device. Thus, it was thought that the mortality and morbidity related to VAP could be reduced and unnecessary antibiotic usage and cost could be prevented. Studies of NO in the exhaled breath in pediatric patients with respiratory devices have been reported in a small number in the literature. More comprehensive studies with a greater number of subjects are needed.

Conflict of interest: There is no conflict of interest among the authors.

REFERENCES

Adrie, C., Monchi, M., Dinh-Xuan, A. T., Dall'Ava-Santucci, J., Dhainaut, J. F., & Pinsky, M. R. (2001). Exhaled and nasal nitric oxide as a marker of pneumonia in ventilated patients. *Am J Respir Crit Care Med, 163*(5), 1143-1149.

Balint, B., Kharitonov, S. A., Hanazawa, T., Donnelly, L. E., Shah, P. L., Hodson, M. E. … Barnes, P. J. (2001). Increased nitrotyrosine in exhaled breath condensate in cystic fibrosis. *Eur Respir J, 17*(6), 1201-1207.

Biban, P., Zangardi, T., Baraldi, E., Dussini, N., Chiandetti, L., & Zachello, F. (2001). Mixed exhaled nitric oxide and plasma nitrites and nitrates in newborns infants. *Life Sciences, 68*(25), 2789-2797.

Bjermer, L., Alving, K., Diamant, Z., Magnussen, H., Pavord, I., Piacentini, G. … Usmani, O. (2014). Current evidence and future research needs for FeNO measurement in respiratory diseases. *Respir Med, 108*(6), 830-841.

Brugière, O., Thabut, G., Mal, H., Marceau, A., Dauriat, G., Marrash-Chahla, R. … Fournier, M. (2005). Exhaled NO may predict the decline in lung function in bronchiolitis obliterans syndrome. *Euro Res J, 25*(5), 813-819.

Carraro, S., Andreola, B., Alinovi, R., Corradi, M., Freo, L., Da Dalt, L. … Baraldi, E. (2008). Exhaled leukotriene B4 in children with community acquired pneumonia. *Pediatr Pulmonol, 43*(10), 982-986.

Chang, I., & Schibler, A. (2016). Ventilator associated pneumonia in children. *Paediatr Respir Rev, 20*, 10-16.

Cheah, F. C., Darlow, B. A., & Winterbourn, C. C. (2003). Problems associated with collecting breath condensate for the measurement of exhaled hydrogen peroxide from neonates on respiratory support. *Biol Neonate, 84*(4), 338-341.

Dikener, A. H., Özdemir, H., Ceylan, A., Ünlü, A., & Artaç, H. (2018). Serum periostin level and exhaled nitric oxide in children with asthma. *Asthma Allergy Immunology,16*(2), 97-103.

Dweik, R.A., Boggs, P. B., Erzurum, S. C., Irvin, C. G., Leigh, M. W., Lundberg, J. O. … Taylor, D. R. (2011). An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FENO) for clinical applications. *Am J Respir Crit Care Med,184*(5), 602-615.

Eşsizoğlu, A., & Yıldırım, E. A. (2009). Nitric oxide in the psychobiology of mental disorders. *Dicle Med J, 36*(1), 67-74.

Jungersten, L., Edlund, A., Petersson, A. S., Wennmalm, A. (1996). Plasma nitrate as an index of nitric oxide formation in man: analyses of kinetics and confounding factors. *Clin Physiol,16*(4), 369-379.

Kalil, A. C., Metersky, M. L., Klompas, M., Muscedere, J., Sweeney, D. A., Palmer, L. B., … Brozek, J. L. (2016). Management of adults with hospital-acquired and ventilator-associated pneumonia: 2016 Clinical Practice Guidelines by the Infectious Diseases Society of America and the American Thoracic Society. *Clin Infect Dis, 63*(5), 575-582.

Karsten, J., Krabbe, K., Heinze, H., Dalhoff, K., Meier, T., & Drömann, D. (2014). Bedside monitoring of ventilation distribution and alveolar inflammation in community-acquired pneumonia. *J Clin Monit Comput, 28*(4), 403-408.

Kelekçi, S., Sen, V., Yolbas, I., Uluca, Ü., Tan, I., & Gürkan, M. F. (2013). FeNO levels in children with asthma and other diseases of the lung. *Eur Rev Med Pharmacol Sci, 17*(22), 3078-3082.

Kietzman, D., Kahl, R., Müller, M., Burchardi, H., & Kettler, D. (1993). Hydrogen peroxide in breath condensate of patients with acute respiratory failure and with ARDS. *Intensive Care Med, 19*(2), 78-81.

Lee, W., & Thomas, P. S. (2009). Oxidative stress in COPD and its measurement through exhaled breath condensate. *Clin Transl Sci, 2*(2), 150-155.

Loukides, S., Horvath, I., Wodehouse, T., Cole, P. J., & Barnes, P. J. (1998). Elevated levels of expired breath hydrogen peroxide in bronchiectasis. *Am J Respir Crit CareMed, 158*(3), 991-994.

Majewska, E., Kasielski, M., Luczynski, R., Bartosz, G., Bialasiewicz, P., & Nowak, D. (2004). Elevated exhalation of hydrogen peroxide and thiobarbituric acid reactive substances in patients with community acquired pneumonia. *Respir Med, 98*(7), 669-676.

Ozbek, E., Turkoz, Y., Gokdeniz, R., Davarci, M., & Ozugurlu, F. (2000). Increased nitric oxide production in the spermatic vein of patients with varicocele. *Eur Urol, 37*(2), 172-175.

Ricciardolo, F. L., Sorbello, V., & Ciprandi, G. (2015). FeNO as biomarker for asthma phenotyping and management. *Allergy Asthma Proc, 36*(1), 1-8.

Teng, Y., Sun, P., Zhang, J., Yu, R., Bai, J., Yao, X., … Barnes, P. J. (2011). Hydrogen peroxide in exhaled breath condensate in patients with asthma: a promising biomarker? *Chest, 140*(1), 108-116.

Türk Toraks Derneği (2018). Erişkinlerde hastanede gelişen pnömoni. Tanı ve tedavi uzlaşı raporu, 1-4.

Ueno, T., Kataoka, M., Hirano, A., Iio, K., Tanimoto, Y., Kanehiro, A., … Tanimoto, M. (2008). Inflammatory markers in exhaled breath condensate from patients with asthma. *Respirology, 13*(5), 654-663.

van Oort, P. M., Bos, L. D., Póvoa, P., Ramirez, P., Torres, A., Artigas, A., … Martin-Loeches, I. (2019). Soluble urokinase plasminogen activator receptor for the prediction of ventilator-associated pneumonia. *ERJ Open Res, 5*(1), 00212-2018.

Xu, Y., Lai, C., Xu, G., Meng, W., Zhang, J., Hou, H., … Pi, H. (2019). Risk factors of ventilator-associated pneumonia in elderly patients receiving mechanical ventilation. *Clin Interv Aging, 7*(14), 1027-1038.

Zilberberg, M. D., & Shorr, A. F. (2010). Ventilator-associated pneumonia: the clinical pulmonary infection score as a surrogate for diagnostics and outcome. *Clin Infect Dis, 51*(S1), 131-135.