



# The Role of Cardiovascular and Inflammatory Markers in Elucidating the Etiology of Respiratory Distress in the Newborn

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## Abstract

**Introduction:** Respiratory distress is at the top of the reasons for admission to the neonatal intensive care unit. This study aims to investigate the role of cardiovascular markers, such as N-terminal pro-brain natriuretic peptide (NT-proBNP), asymmetric dimethylarginine (ADMA) and an inflammatory marker interleukin-6 (IL-6) in elucidating the etiology of respiratory distress, severity of disease and early morbidity.

**Methods:** Infants born at  $\geq 32$  gestational weeks in our hospital with Downes scores  $>5$  between January 2014-June 2014 and admitted to the neonatal intensive care unit because respiratory distress were enrolled. Blood samples were obtained for this study, Pro-BNP, IL-6 and ADMA levels and Downes scores were calculated at the hospital admission and also 48-72 hours later. Gestational age-matched newborns without respiratory distress and congenital heart disease constituted the control group. Demographic data, medical treatments, type and duration of respiratory support and duration of admission were recorded.

**Results:** A total of 95 infants were enrolled in this study. Fifty-three (75.7%) of them had transient tachypnea, 9 (12.8%) of them had respiratory distress syndrome and 8 (11.4%) of the patients diagnosed as neonatal pneumonia. The control group consisted of 25 healthy infants. There was no statistically significant difference between ADMA, proBNP, IL-6 levels on days 1 and 3. ADMA levels were found to be positively correlated with Downes scores, duration of mechanical ventilation and inotrope requirement.

**Discussion and Conclusion:** There is no single reliable marker that can be used in the differential diagnosis of conditions that arise as the cause of respiratory distress in newborns. Especially on Day 3, ADMA levels may be a guide in determining the severity of the disease since it is associated with inotrope need and prolonged respiratory support requirements.

**Keywords:** Asymmetric dimethylarginine; respiratory distress; newborn.

Respiratory distress is one of the leading causes of hospitalization in the neonatal intensive care unit <sup>[1]</sup>. In 15% of term infants followed up in neonatal intensive care unit and in 29% of late preterm infants, morbidities related to respiratory causes have been observed <sup>[2]</sup>. Diagnosis should be made within the first hours of life and rapid and

effective treatment should be started. Delay in diagnosis and treatment of respiratory distress can cause respiratory failure and cardiac arrest. Clinical findings are very similar at first admission in term, near-term and moderately premature infants patients with transient tachypnea (TTN), pneumonia, respiratory distress syndrome (RDS) and often

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it is difficult to make a differential diagnosis.

Nitric oxide (NO) is a molecule synthesized by the endothelium and has important functions from vasodilation to the release of inflammatory cytokines. ADMA is an endogenous nitric oxide synthase (NOS) inhibitor [3,4]. Lungs have also been reported to be the main source of ADMA [5]. NO synthesized from the lungs in the newborn is important for normal lung development and maturation. As the blood level of ADMA increases, NO synthesis decreases. In the light of these findings, it can be said that there is a relationship between ADMA levels and pulmonary functions of newborns.

Enzymatic cleavage of the peptide named Pro-BNP results in the formation of brain natriuretic peptide (BNP) and amino terminal proBNP (NT-ProBNP). BNP is released from myocytes in response to the ventricular strain. The vasodilator BNP has a natriuretic effect and suppresses the renin-angiotensin and aldosterone system [6,7]. BNP level increases in cases of heart failure and hypervolemia. It is used to differentiate cardiac and lung related respiratory distress in the newborn. NT-proBNP has a longer half-life and is more practical to measure than BNP.

Interleukin-6 (IL-6) plays a role in the production of acute phase reactants, such as CRP and fibrinogen in the liver. Therefore, IL-6 rises before CRP and may be useful in the diagnosis of infections, such as pneumonia in the newborn [8].

This study aims to investigate the role of cardiovascular markers, such as NT-proBNP, ADMA and IL-6 levels in elucidating the etiology of respiratory distress, and the relationship between the severity of the disease and early-stage morbidities.

## Materials and Methods

This study was conducted prospectively between January 2014 and June 2014 in Neonatal Intensive Care Unit of our hospital. Infants who were hospitalized in the neonatal intensive care unit (NICU) due to respiratory distress, with Downes score >5 and born at  $\geq 32$  gestational weeks were consecutively included in the study group. Newborn babies without respiratory distress and known heart disease born during the same gestational week interval whose

parents gave their permissions to participate in this study were included in the control group. Necessary permission was obtained from the ethics committee of our hospital.

Newborns with congenital heart disease, meconium aspiration syndrome, diaphragmatic hernia, perinatal asphyxia, gastrointestinal malformation and syndromic findings were excluded from this study.

The diagnosis of respiratory distress was established if one or more of the following criteria exist:

1. Tachypnea: respiratory rate  $\geq 60$ /min
2. Participation of auxiliary respiratory muscles in the respiration: retractions of subcostal, intercostal, sternal, and suprasternal muscles
3. Whining, stridor or wheezing during respiration
4. Presence of cyanosis

Downes scoring [9] (Table 1) was used to determine the degree of respiratory distress.

After the patients were admitted to the neonatal intensive care unit, all newborns underwent standard care and treatment. The babies were monitored in the incubator with their body temperatures maintained between 36.5 and 37.5 °C with humidification performed with a skin probe suitable for their gestational week at birth. The diagnosis and treatment of the patients were made by the attending service physician. As respiratory support noninvasive, or conventional mechanical ventilation in SLE 5000 ventilator was delivered to infants. Downes scoring was performed by the physician conducting this study on the first admission of the patient and within 48-72 hours.

In addition to the examinations requested by the patient's attending physician, peripheral venous blood samples were taken from the patients twice within the first 12-24 and 48-72 hours for the measurement of pro-BNP, IL-6 and ADMA levels. IL-6 and proBNP levels were determined by immuno-chemiluminescent method. The blood samples drawn for the measurement of the ADMA level were centrifuged at 3000 rpm for five minutes, and their serum portions were stored at -80 °C. Serum ADMA level (ELISA) was studied by enzyme-linked immunosorbent assay (Sunred-bio, China).

**Table 1.** Downes Scoring

Scores	Respiratory rate	Cyanosis	Air entry	Whining	Retraction
0	< 60/min	None	Normal	None	None
1	60-80/min	At room air	Decreased	Heard during auscultation with stethoscope	Moderate
2	>80/min/apneic	>40% FiO <sub>2</sub>	Hardly heard	Heard during auscultation with stethoscope	Marked

RDS was diagnosed with typical radiological findings and requirement of oxygen supply of >40% to maintain saturation in the range of 90-95%.

The diagnosis of neonatal pneumonia was made in the presence of consolidation on the chest X-ray, increased acute phase markers and maternal facilitating causes.

All patients underwent echocardiography by an independent pediatric cardiologist to show that there was no hemodynamically significant PDA and congenital heart disease.

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS for Windows 11.0; Chicago, USA) program. Parametric variables were evaluated with Student's t-test and non-parametric variables with the Mann-Whitney test. One way ANOVA analysis was used to compare the independent means of more than two groups. P-value <0.05 was considered statistically significant.

## Results

The data of a total of 95 infants, including 70 infants in the study and 25 infants in the control group, were analyzed. In

the study group, 53 (75.7%) patients were diagnosed with transient tachypnea of the newborn (TTN), nine (12.8%) patients with respiratory distress syndrome (RDS), and eight (11.4%) patients with neonatal pneumonia. Twenty-five healthy infants constituted the control group. Gestational age and birth weights were significantly lower in the RDS group, and were similar between TTN, pneumonia and control groups. There was no statistically significant difference between sex distribution, mode of delivery and 5th minute APGAR scores (Table 2).

There was no statistically significant difference between TTN, RDS, pneumonia and control groups as for ADMA, proBNP, IL-6 levels measured on the 1<sup>st</sup> and 3<sup>rd</sup> days. IL-6 levels measured on the first and third days were significantly higher in the pneumonia group when compared with RDS and TTN groups (Table 3). There was a weak positive correlation between ADMA levels and Downes scores, mechanical ventilation time and inotropic drug requirement ( $r=0.39$ ,  $r=0.23$ ,  $r=0.28$ ;  $p<0.05$ ) (Table 3). Invasive and noninvasive respiratory support was needed for significantly longer periods in the pneumonia group (Table 3).

**Table 2.** Demographic data of the study and control groups

	TTN (n=53)	N. pneumonia (n=8)	RDS (n=9)	Control (n=25)	p
Gestational week (mean±SD)	36±2	36.7±1.9	32.2±0.8	36.2±2	<0.01
Birth weight (gr) (mean±SD)	2765±558	2861±414	1717±423	2767±707	<0.01
Gender (M) (n,%)	28 (52.8)	6 (75)	5 (55.6)	14 (56)	>0.05
Type of delivery (C/S) (n, %)	43 (81)	6 (75)	8 (89)	18 (72)	>0.05
Apgar 5. min score (median min-max)	9 (7-10)	9 (8-10)	8 (6-9)	9 (9-10)	>0.05

**Table 3.** Respiratory morbidities and laboratory findings

	TTN (n=53)	N. pneumonia (n=8)	RDS (n=9)	Control (n=25)	p
nCPAP hrs) (median)	36 (0-160)	36.5 (4-90)	54.3 (26-218)		<0.01
Mechanical ventilation (hrs) (median)	4.4 (0-88)	68 (0-210)	5.5 (0-58)		<0.01
ADMA (nmol/ml)	1.32 (0.2-3.38)	1.46 (0.7-3.25)	1.09 (0.53-2.8)	1.37 (0.69-3.5)	>0.05
The first value					
ADMA (nmol/ml)	1.2 (0.25-4.2)	1.4 (0.7-3.2)	1.06 (0.6-3.2)	1.3 (0.1-2.5)	>0.05
The second value					
ProBNP (pg/ml)	2195 (17-8245)	3037 (89-9098)	4814 (45-13641)	1827 (42-6315)	>0.05
The first value					
ProBNP (pg/ml)	2823 (9-13470)	4865 (44-15774)	1696 (97-5584)	1393 (21-3142)	>0.05
The second value					
IL-6 (pg/ml)	152 (7-1417)	802 (14.7-5000)	42.2 (1.5-125)	40.4 (5.6-172)	<0.01
The first value					
IL-6 (pg/ml)	106 (2-1759)	404 (6.4-2600)	18.6 (3.9-83)	24.7 (9-70)	<0.01
The second value					

## Discussion

It was found that the majority of newborn infants born between 32-40 gestational weeks and hospitalized consecutively in the tertiary NICU due to respiratory distress had TTN. In this study, it has been investigated whether the differential diagnosis among RDS, pneumonia and TTN morbidities, which frequently caused respiratory distress using biomarkers, could be made and found that ADMA and proBNP were not significant. However, levels of IL-6 were significantly higher in cases with pneumonia.

Nitric oxide (NO) is a free radical gas synthesized by nitric oxide synthetase (NOS) from L-arginine in the vascular endothelium<sup>[10]</sup>. Endogenous NO plays a role in the neuronal bronchodilator and vasodilator mechanisms in the lung<sup>[11]</sup>. L-arginine analogs secreted from endothelial cells inhibit NOS activity, leading to many cardiovascular diseases. The most potent endogenous NOS inhibitor is ADMA. Very few studies related to ADMA levels and respiratory distress in the neonatal period have been performed so far. In a study by Richir MC et al.,<sup>[12]</sup> ADMA levels were found to be higher in patients with respiratory distress needing mechanical ventilation than the patients not requiring mechanical ventilation.

Similarly, in our study, a significant relationship was found between the duration of mechanical ventilation and ADMA levels. In a study conducted by Işık DU et al.,<sup>[13]</sup> ADMA levels were found to be significantly higher in patients diagnosed with TTN compared to the control group. However, in our study, no significant difference was observed between the TTN and the control group concerning ADMA levels. In another recent study, ADMA levels in preterm infants have been shown to be a useful marker in determining the need for surfactant and predicting the development of bronchopulmonary dysplasia<sup>[14]</sup>. In our study, ADMA levels of infants diagnosed with RDS were found to be similar to the control group and patients with other morbidities.

Since moderate preterm and term babies were included in this study, moderate to long-term morbidities, such as BPD, were not evaluated. According to our findings, we think that ADMA levels, especially on the 3<sup>rd</sup> day, are related to the need for inotropic support and prolonged respiratory support.

Although ProBNP is a well-studied marker for the diagnosis of heart failure and hSPDA in pediatric and neonatal patients, there are few studies investigating its association with respiratory distress in the literature. In a study by Aydemir et al.,<sup>[7]</sup> a significant relationship was found between proBNP levels and the severity of respiratory distress and

the duration of mechanical ventilation in TTN. However, in the study of Kara et al.,<sup>[15]</sup> any significant relationship was not found between proBNP levels in the pathophysiology of TTN. In our study, proBNP levels were found to be higher in the study group patients with respiratory distress compared to the control group without any statistically significant intergroup difference. In a study of proBNP levels in preterm infants born at <34 gestational weeks with RDS, proBNP levels were shown to be associated with RDS severity<sup>[16]</sup>. Although the proBNP level was found to be higher in patients diagnosed with RDS compared to all other groups, this difference did not reach statistical significance in our study ( $p=0.06$ ).

In the diagnosis of neonatal sepsis, IL-6 has become increasingly accepted as an early stage marker. When used in combination with C-reactive protein, it is known to achieve high specificity and sensitivity in the diagnosis of sepsis<sup>[17]</sup>. Studies investigating IL-6 levels in the diagnosis of congenital pneumonia have not been found in the literature. According to our findings, the detection of high levels of IL-6 may be a guide for the diagnosis of pneumonia and initiation of antibiotic therapy.

The main limitations of this study were the relatively small number of patients, the unequal number of patients per group because this was a prospective observational cohort study and the investigation of a wide range of gestational weeks.

There is no reliable single biomarker that can be used in the differential diagnosis of conditions that cause respiratory distress in the newborn. There is still a need for better designed large-scale studies to support these findings.

**Ethics Committee Approval:** The Ethics Committee of Zekai Tahir Burak Women Health and Diseases Training and Research Hospital provided the ethics committee approval for this study (23.09.2013, 2013-11).

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**Conflict of Interest:** None declared.

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