

Serum levels of nitric oxide as a predictor of survival in acute respiratory distress syndrome caused by H₁N₁ pneumonia?

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Abstract

A large number of studies show elevated levels of nitric oxide (NO) in infective syndromes, but there is an insufficient number of studies which have investigated serum levels of NO in patients with acute respiratory distress syndrome (ARDS), especially in relation to survival. Hence, we created a study with the aim of determining the NO levels in relation to ARDS survival.

Serum levels of NO were measured by Griess reaction in 29 patients [16 men (55%), mean age years 52.72±18]. All data were statistically analyzed using one way ANOVA.

Our results show significantly higher serum NO levels in ARDS survivors compared to ARDS non-survivors, ($p < 0.05$). We conclude that higher serum levels of NO are strongly associated with better clinical outcomes, including increased survival.

Key words: acute respiratory distress syndrome, nitrogen oxide species, outcome

Introduction

Acute respiratory distress syndrome (ARDS) is an acute, diffuse, inflammatory

lung injury that leads to increased pulmonary vascular permeability, increased lung weight, and a loss of aerated tissue. (1) Clinical hallmarks of ARDS are hypoxemia and bilateral radiographic opacities, while the pathological hallmark is diffuse alveolar damage (i.e., alveolar edema with or without focal hemorrhage, acute inflammation of the alveolar walls, and hyaline membranes); without mechanical ventilation most patients would die. (2) It is well known that mediators of inflammation have a significant role in ARDS. (3-5) Many of them, such as interferon- γ , interleukin- 1β (IL- 1β), and tumor necrosis factor- α , lead to increased production of nitric oxide (NO) which causes oxidative injury. (6,7) Nitric oxide is a reactive molecule produced by nitric oxide synthase (NOS) enzymes in a variety of cells. (8) Three distinct forms of NOS have been identified; endothelial NOS (eNOS), neuronal NOS (nNOS), and inducible NOS (iNOS). (9) Both eNOS and nNOS are constitutive forms of NOS (cNOS) that produce small amounts of NO for short periods of time when appropriately stimulated. The cNOS-derived NO is involved in maintaining physiologic functions such as regulating vascular tone and acting as a neurotransmitter. In contrast, iNOS produces a large amount of NO for sustained periods of time. The NO derived from iNOS is thought to be involved in inflammatory responses and host defense against infection. (6) Oxidant-mediated tissue injury is likely to be important in the pathogenesis of ARDS. (10,11) Important markers of oxidative injury in lungs include nitrogen oxide species (NOx), particularly NO, nitrite (NO₂⁻), and nitrate (NO₃⁻). (12) Nitric oxide readily reacts with superoxide ion to form a highly reactive intermediate known as peroxynitrite. (12-14) Peroxynitrite rapidly oxidizes and nitrates proteins such as α_1 -antitrypsin and surfactant protein A, respectively, thereby inhibiting their function. (15) Inhibition of these proteins may contribute to the proinflammatory environment believed to be pathogenic in ARDS. (16-18) Peroxynitrite cannot be measured directly because of its short half-life, but its presence can be inferred by measuring metabolites such as NO₂⁻ and NO₃⁻. (15) There is a small number of studies which investigated serum levels of NO in patients with ARDS caused by H1N1 pneumonia and in relation to survival. In accordance with this, we tested two hypotheses. The first hypothesis was that NO serum levels would be higher in ARDS patients compared to the healthy population. And the second hypothesis was that NO serum levels would be higher in ARDS survivors than in non-survivors.

Materials and methods

A prospective observational study was conducted in the Medical Intensive Care Unit (MICU) of University-affiliated hospital Banja Luka (Bosnia-Herzegovina) between December 1, 2009 and July 1, 2011. This MICU was established five years ago with the support of critical care specialists trained in the United States and Europe, members of the European Society of Intensive Care Medicine, and the Society of Critical Care Medicine. (19) The study included all mechanically ventilated patients with a diagnosis of H1N1 novel pneumonia complicated by ARDS. During the H1N1 epidemic, most patients admitted with ARDS were H1N1 positive. That was 90% of all ARDS admitted patients in MICU in a defined period. The diagnosis of novel Influenza A (H1N1) infection in all patients was confirmed by real-time reverse transcriptase polymerase chain reaction (RT-PCR) of nasopharyngeal swab specimens and respiratory secretions at the time of hospital admission. Data were extracted from hospital charts including demographic data, vital signs, laboratory parameters, radiological data, mechanical ventilation details, and chronic medical conditions: chronic obstructive pulmonary disease, asthma, congestive heart failure, neoplasm, chronic liver or renal diseases, diabetes mellitus, and the use of immunosuppressant medications. Severity of illness was assessed according to the Simplified Acute Physiology Score (SAPS II). Standardized definitions were used to determine the presence of ARDS. (20) All patients with ARDS caused by H1N1 pneumonia were treated using the same principle of mechanical ventilation, volume control ventilation with appropriated positive end-expiratory pressure (PEEP) according to the fraction of inspired oxygen (F_{iO_2}) (lung protective ventilation strategies).

Exclusion's criteria

The patients were excluded from the study if they met one or more of the following criteria: not diagnosed with ARDS; not diagnosed with H1N1 influenza; age younger than 18 yrs; unsupportable hypoxemia (partial pressure of oxygen – $PaO_2 < 80$ mm Hg with $F_{iO_2} 1.0$), these patients were transferred to a different MICU with possibilities of high frequency mechanical ventilation; evidence of acute myocardial infarction; cardiac arrhythmias (supraventricular tachycardia > 140 beats/min or complex ventricular ectopy).

Patients with other causes of ARDS were excluded from the study since we wanted to concentrate on H1N1-ARDS patients due to the lack of data within this specific group.

Blood samples from all observed patients were taken only once from the jugular vein via a central venous catheter within 24 hours of ARDS diagnosis.

Measurement of NO serum levels

The NO level in whole blood is determined by measuring nitrite and nitrate (NO_3^{2-} u NO_2^{2-}) production using the classical colorimetric reaction (Griess). Blood samples for the determination of NO concentration were diluted 1:1 (vol/vol) with 0.9% saline, protein-precipitated using 30% ZnSO_4 , 0.05 ml per ml of blood and centrifuged at 700 g for 10 minutes and frozen at -20°C . Conversion of NO_3^{2-} into NO_2^{2-} was done with nitrate reductase elementary zinc. NO_2^{2-} concentration in serum was determined by classic colorimetric Griess reaction. Briefly, equal volumes of samples and Griess reagent (sulfanilamide and naphthalene-ethylene diamine dihydrochloride) were mixed at room temperature. After 5 min, the absorbance was measured at 546 nm using spectrophotometer. The concentration of nitrite was determined by a standard curve prepared with sodium nitrite.

Statistical analysis

The results were processed using a standard statistical method (one way ANOVA post hoc multiple comparison technique Bonferroni and t-test) shown as mean \pm standard mean error ($X \pm S_X$). We tested significance of the difference in mean values between studied groups with an aim to monitor changes in serum NO levels. We considered the value of $p < 0.05$ statistically significant.

Results

Demographic and clinical characteristics

A total of 29 patients, 16 men (55%), mean age 52.72 ± 18 years, were mechanically ventilated in MICU Banja Luka. All observed patients presented with bilateral

infiltrates on chest radiographs on admission. The median P/F ratio (arterial oxygen concentration to the fraction of inspired oxygen) on day 1 was 86 ± 28.59 mm Hg. The proportion of patients with bilateral infiltrate was similar among survivors and non survivors. However the P/F ratio was significantly lower among non survivors (109.08 vs 67.25 , $P < 0.05$) (table 1). All healthy volunteers were nonsmokers with mean age 49.3 ± 24.8 years.

Serum levels of NO in studied patients and healthy volunteers

The study included 29 ARDS patients (13 ARDS survivors, 16 ARDS non-survivors) and 33 healthy volunteers (mean age 49.3 ± 24.8 years) as the control group. Figure 1 presents the comparison of mean serum levels of NO in the healthy control group ($5.03 \mu\text{mol/L} \pm 2.53$), ARDS survivor group of patients ($6.98 \mu\text{mol/L} \pm 2.58$) and ARDS non-survivor group of patients ($3.45 \mu\text{mol/L} \pm 0.83$). The ANOVA test showed a statistical difference between the three tested groups. Statistical analysis with t-test showed a significantly higher serum level of NO in the ARDS survivor group of patients in comparison to the ARDS non-survivor group of patients [$t(27)=5.17$, $p < 0.05$]. This test showed significantly higher serum levels of NO in the ARDS survivor group of patients in comparison to the healthy group [$t(44)=-5.19$, $p < 0.05$]. Serum levels of NO were lower in ARDS nonsurvivors compared to healthy subjects but without statistical significance [$t(47)=3.67$, $p > 0.05$]. Our study was 80% powered to detect a significant difference in serum NO levels between tested groups.

Discussion

Extended analysis of NO serum levels in patients with ARDS caused by H1N1 pneumonia showed the following results: H1N1-ARDS survivor patients had significantly higher serum NO levels compared to H1N1-ARDS non-survivor patients. (1) The main finding of this study is that H1N1-ARDS survivors have significantly higher serum levels of NO (metabolites NO_2^- and NO_3^-). Results of our study generate a very important question: can serum levels of NO (metabolites NO_2^- and NO_3^-) be a predictor of survival in H1N1-ARDS patients? Several physiological mechanisms of NO can help answer this question. One of the NO

effects on microcirculation is vasodilatation which consequently increases tissue perfusion. (13,21,22) Prior research has demonstrated an elevation in pulmonary dead space in patients with ARDS, in part because some alveoli are being ventilated but not perfused. (23) An increase in perfusion in these parts of the lungs leads to better ventilation to perfusion matching. Regarding the alveolar epithelium, NO has been shown to protect type II alveolar cells from stretch injury. (24) An alternate hypothesis to explain our findings is that endogenous NO has a beneficial effect in organs other than the lungs during ARDS.

Higher NO levels could help prevent further tissue damage by improving oxygen and nutrient delivery to the tissues while helping decrease the amount of toxic oxygen species. NO may also protect endothelial tissue by decreasing platelet and leukocyte adhesion to the endothelium. (21) NO would thereby decrease multiorgan failure, which contributes to mortality in ARDS. Finally, NO and NO_x have antibacterial effects that may be important in infectious conditions that predispose patients to ARDS. (21)

Healthy alveolar epithelium, alveolar macrophagi and endothelium cells produce NO, so elevated NO serum levels can be an indicator of a greater percentage of intact lung endothelium and epithelium as a result of a less severe initial injury. (25) Some studies in which NO levels in bronchoalveolar lavate (BAL) were observed, show high levels of NO in ARDS non-survivors. The reason for that can be found in the fact that NO found in BAL is produced exclusively by the lungs, while NO serum levels represent the whole body production of NO.

Prior research on animal models demonstrated a worse outcome in ARDS patients in the presence of elevated NO levels. The reason for this can be explained by the difference in human and animal models. (24,26-28) There were no significant difference in age and in preexisting comorbidities in ARDS survivors and ARDS nonsurvivors in this study. This might be the reason for the significantly higher serum NO levels in H₁N₁-ARDS survivors compared to healthy controls. On the other hand, similar levels of serum NO in healthy controls and H₁N₁ non-survivors implicate that the protective effects of NO were not activated in this group of patients.

From our results we conclude that higher serum levels of NO are strongly associated with better clinical outcomes, leading to increased survival. Our findings suggest that endogenous NO may be protective during ARDS or may serve as a marker of less severe organ injury or both.

Figure 1. Mean concentrations ($\mu\text{mol/L}$) of nitric oxide (NO) in all observed groups.

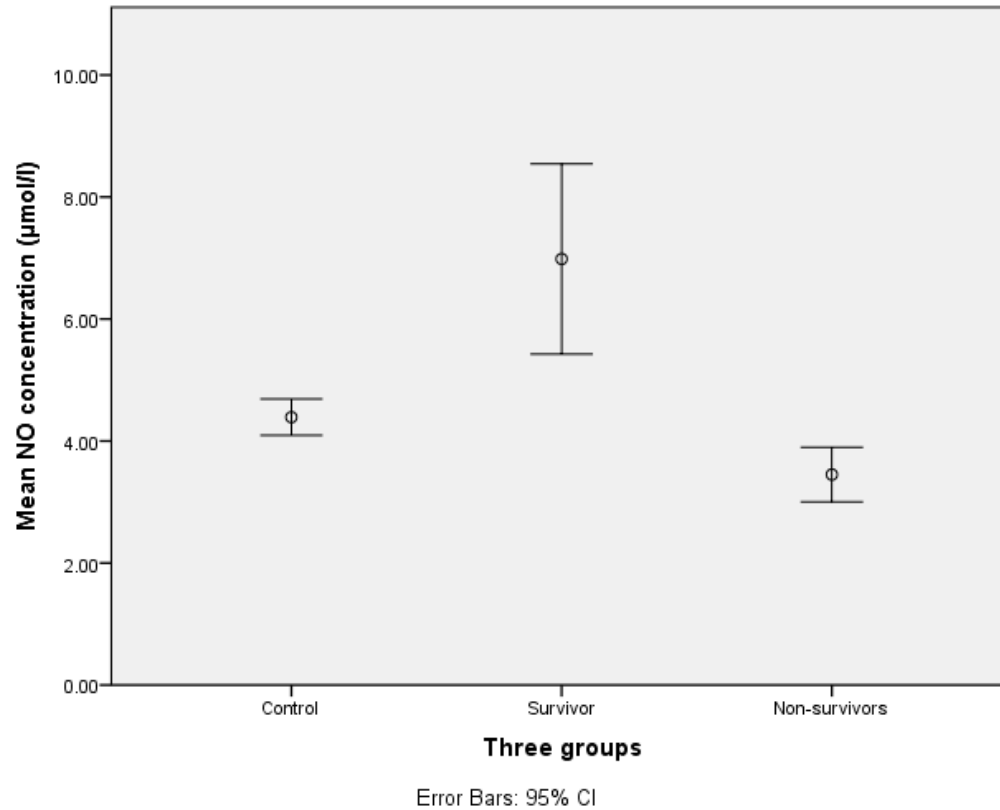


Table 1. Characteristics of Acute Respiratory Distress Syndrome (ARDS) survivors and ARDS non-survivors (*t-test was used).

	ARDS Survivors (n=13)	ARDS Non-survivors (n=16)	p*
Age	53 \pm 14.14	52.5 \pm 13.67	> 0.05
Male sex, n (%)	7 (54)	8 (50)	> 0.05
Ever smoker, n (%)	5 (38)	6 (38)	> 0.05
Chronic pulmonary diseases, n (%)	2 (15)	3 (19)	> 0.05
SAPS II, (\pm SD)	39.31 \pm 6.92	51.44 \pm 10.84	< 0.05
PaO ₂ /FiO ₂ , (\pm SD)	109.08 \pm 27.58	67.25 \pm 8.97	< 0.05
Hct (median)	34	33	< 0.05

ARDS, Acute Respiratory Distress Syndrome; Hct, hematocrit; PaO₂/FiO₂ ratio, the ratio of arterial oxygen partial pressure to fractional inspired oxygen; SAPS, Simplified Acute Physiology Score.

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