Prognostic value of nitric oxide as a serum biomarker in patients with ARDS caused by Influenza A H1N1

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July 21, 2020

Abstract

Background Due to profound morbidity and a high rate of mortality in patients with acute respiratory distress syndrome (ARDS), identification of potential biomarkers such as nitric oxide (NO) is important to determine prognosis and guide clinical decision-making. Methods and results: In this study, we included twenty-nine patients admitted to the Medical Intensive Care Unit diagnosed with ARDS caused by influenza A (H1N1) whose serum samples were collected on day 1 for determination of NO levels by GRIESS method. Simplified Acute Physiology Score (SAPS II) and Acute Physiology and Chronic Health Evaluation II (APACHE II) scoring were performed on day 1 as well. The mortality in the observed patients was 55%. SAPS II and APACHE II scores were significantly higher ($p < 0.05$) in non-survivors as compared to survivors. There were no significant differences in gender, age, cigarette smoking and chronic pulmonary diseases between the survivors and non-survivors. As compared to non-survivors, the serum levels of NO were significantly higher in survivors ($p < 0.05$). Spearman's rank correlation analysis indicated a significant positive correlation of SAPS II and APACHE II with NO. By using serum levels of NO, the receiver operating characteristic curve was plotted and the provided predictable accuracy of mortality (outcome) was 96%. Conclusion: The present study showed that measuring serum levels of NO in patients with ARDS (influenza A-H1N1) might be useful in predicting the clinical outcome.

Introduction

Acute respiratory distress syndrome (ARDS) is not only a global health issue, but also a global health priority, as morbidity and mortality rates remain high. This syndrome (ARDS) was first recognized in the late nineteen-sixties [1]. It is characterized by hypoxemia and bilateral radiographic opacities, with diffuse alveolar damage as main pathological hallmark (i.e., alveolar edema with or without focal hemorrhage, acute inflammation of the alveolar walls, and hyaline membranes) [2]. Most patients who present with an acute, diffuse, inflammatory lung injury that leads to increased pulmonary vascular permeability, increased lung weight, and a loss of aerated tissue need mechanical ventilation as a rescue therapy [3]. ARDS represents a common clinical problem in critically ill patients, especially today in pandemic circumstances (COVID 19); this syndrome is associated with a short-term risk of mortality as well as significant long-term risk of morbidity [4-5]. It is well known that lung endothelial and alveolar epithelial damage in ARDS are often caused by oxidative injury [6]. Many biomarkers and mediators of oxidative injury and inflammation have a significant role in this process; some of them such as interferon-γ, interleukin-1β (IL-1β), and tumor necrosis factor-α, lead to increased production of nitrogen oxide species (NOx), particularly nitric oxide (NO), nitrite (NO_2^-), and nitrate (NO_3^-) which all cause oxidative injury [7-9]. Once declared "molecule of the year," NO is involved in multiple pathophysiological processes in ARDS. Nitric oxide readily reacts with superoxide ion to form a highly reactive oxidizing and nitrating intermediate product - peroxynitrite. Peroxynitrite performs rapid oxidation and nitration of proteins α1antitrypsin and surfactant protein A, thereby inhibiting their

function. Inhibition of these proteins may create the proinflammatory environment which can lead to development of ARDS. Peroxynitrite cannot be measured directly because of its short half-life, but its presence can be inferred by measuring metabolites such as NO_2^- and NO_3^- [10-13]. Therefore, for clinical decision-making and ARDS treatment, accurate prediction of ARDS severity in the early stage and administration of appropriate therapy are the keys to improving therapeutic success in these patients. However, at present an objective and effective clinical outcome predictor or prognosticator of ARDS (especially caused by influenza A H1N1) remains unknown. Many studies have been undertaken till date, and no single biomarker has been identified as a predictor for the outcome in ARDS (caused by influenza A H1N1), rather a combination of biomarkers and scoring systems was suggested to predict the patients' outcomes [14]. There is a small number of studies which investigated serum levels of NO in patients with ARDS caused by H1N1 pneumonia in relation to survival. Hence, the present study was created to investigate serum levels of NO, age, gender, and comorbid conditions including: Simplified Acute Physiology Score (SAPS II), Acute Physiology and Chronic Health Evaluation II (APACHE II) score, in patients with ARDS (caused by influenza A H1N1) and its ability to predict the patients' outcomes.

Material and methods

A retrospective observational study was conducted in the Medical Intensive Care Unit (MICU) of University-affiliated hospital during the pandemic period. Twenty-nine adult patients fulfilling the Berlin criteria of definition of ARDS [15] admitted to MICU were included in the study. The patients' clinical histories were taken and routine blood and urine investigations including complete hemogram, renal function tests, serum electrolytes, liver function tests, urine routine, chest X-ray, arterial blood gas analysis, and electrocardiogram were performed in every patient and were later repeated depending on clinical profile. SAPS II and APACHE II scoring were calculated on day 1. The study included all mechanically ventilated patients with a diagnosis of Influenza A (H1N1) pneumonia complicated by ARDS. During the Influenza A (H1N1) pandemic period, most patients admitted with ARDS were Influenza A (H1N1) positive ($> 90\%$). The diagnosis of 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. Based on death end-point within 28 days after diagnosis or admission, the patients were divided into survivor or non-survivor groups. All patients with ARDS caused by Influenza A (H1N1) pneumonia were treated using the same principle of mechanical ventilation - volume control ventilation with appropriate positive end-expiratory pressure (PEEP) according to the fraction of inspired oxygen (FiO2) (lung protective ventilation strategies). This study was approved by the Institutional Ethical Committee.

Exclusion's criteria

Patients were excluded from the study if they met one or more of the following criteria: not diagnosed with ARDS; not diagnosed with influenza A (H1N1); age younger than 18 years; evidence of acute myocardial infarction; cardiac arrhythmias (supraventricular tachycardia > 140 beats/min or complex ventricular ectopy) and heart failure. Patients with other causes of ARDS were excluded from the study since we wanted to investigate Influenza A (H1N1)-ARDS patients due to the lack of data within this specific group.

Measurement of NO serum levels

Blood samples for serum levels of NO were collected on day one, i.e., within 24 h of enrollment in the study. The NO level in whole blood is determined by measuring nitrite and nitrate (NO32- u NO22-) 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% ZnSO4, 0.05 ml per ml of blood and centrifuged at 700 g for 10 minutes and frozen at -20°C. Conversion of NO32- into NO22- was done with nitrate reductase elementary zinc. NO22-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

Statistical analysis was performed using IBM SPSS, version 26. We tested the correlation of NO serum to APACHE II and SAPS II scoring in predicting the patients' outcomes. Student's t test (continuous variable) and Fisher's exact test (categorical variable) were used for comparisons between survivors and non-survivors. Spearman rank correlation was used to establish interrelationships between the different variables. For each predictor variable, sensitivity and specificity were calculated using Fisher's exact test. Receiver operating characteristic curve (ROC) was plotted using 1-specificity on X-axis (false-positive fraction [FPF]) and sensitivity (true-positive fraction [TPF]) on Y-axis for all the predictor variables.

Results

Demographic and clinical characteristics

A total of 29 patients, 16 men (55%), mean age 52.72 +- 18 years, were mechanically ventilated in MICU. Among the 29 patients enrolled, 13 patients recovered and 16 patients died (55%). The mean age of 13 patients who survived was 53+-20.94 years, whereas in non-survivors, it was 52+-17.17 years. Out of 13 survivors, 9 were males and 4 were females. On the other hand, out of 16 non-survivors, 8 were males. All observed patients presented with bilateral infiltrates on chest radiographs on admission. The mean 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 (105.23 +-21.89 vs 66.63+-9.75, $P < 0.01$).

Demographic and clinical characteristics of survivors and non-survivors at baseline are shown in Table 1.

Table 1. Demographic and clinical variables

	survivors	non-survivors	p value
Male, n (%)	9/13 (69.2)	8/16 (50)	0.296*
Age (± Sd)	53±20.94	52±17.17	0.944&
Ventilator days, n (%)	8.46±1.56	15.63±2.63	< 0.01&
PaO_2/FiO_2 ratio (±SD)	105.23 ±21.89	66.63±9.75	< 0.01&
Vasoactive drug, n (%)	1/13 (7.7)	9/16 (56.3)	< 0.01&
SAPS II score (±Sd)	39.31 ± 6.92	51.44 ± 10.84	< 0.01&
APACHE II (±Sd)	14.38 ± 2.29	21.75 ± 3.23	< 0.01&
Ever smoker, n (%)	5/13 (38)	6/16 (38)	> 0.05*
Chronic pulmonary diseases, n (%)	2/13 (15)	3/16 (19)	> 0.05

* Pearson χ^2 test

& Student's t test

Serum levels of nitric oxide (NO)

Figure 1 presents the comparison of mean serum levels of NO in ARDS survivor group of patients (6.98 µmol/L ±2.58) and ARDS non-survivor group of patients (3.45 µmol/L ±0.83). Statistical analysis with t-test showed a significantly higher ($p < 0.01$) serum level of NO in the ARDS survivor group of patients in comparison to the ARDS non-survivor group of patients.

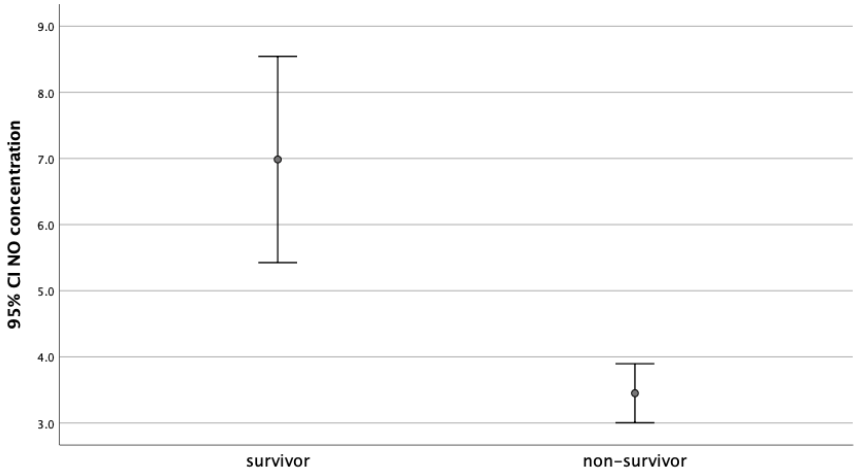

Figure 1. Mean concentrations (μmol/L) of nitric oxide (NO) in observed groups.

Correlation between Acute Physiology and Chronic Health Evaluation II (APACHE II) with serum levels of Nitric oxide

Spearman rank correlation was done to assess the association of the APACHE II with serum levels of nitric oxide (NO) [Figure 2]. Results indicate the significant negative correlation between NO serum levels and APACHE II score (r = -0.672; $P < 0.05$).

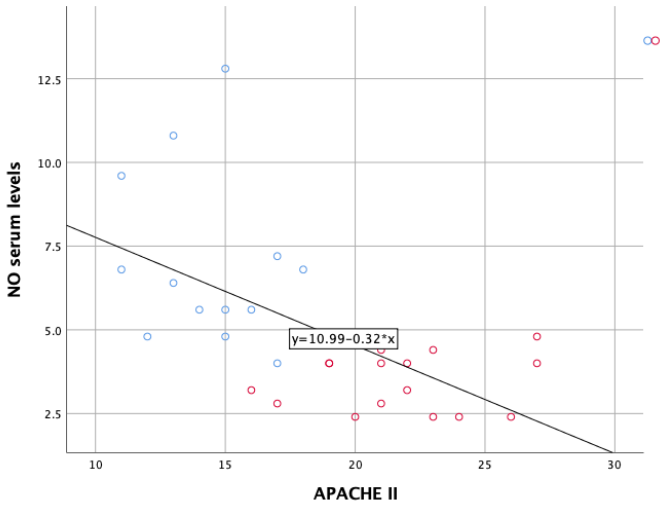

Figure 2. Spearman rank correlation between serum levels of NO and Acute Physiology and Chronic Health Evaluation II (APACHE II)

Correlation of Simplified Acute Physiology Score II (SAPS II) with serum levels of Nitric oxide

Spearman rank correlation was done to assess the association of the SAPS II with serum levels of nitric oxide (NO) [Figure 3]. Results indicate the significant negative correlation between NO serum levels and SAPS II score (r = -0.420; $P < 0.05$).

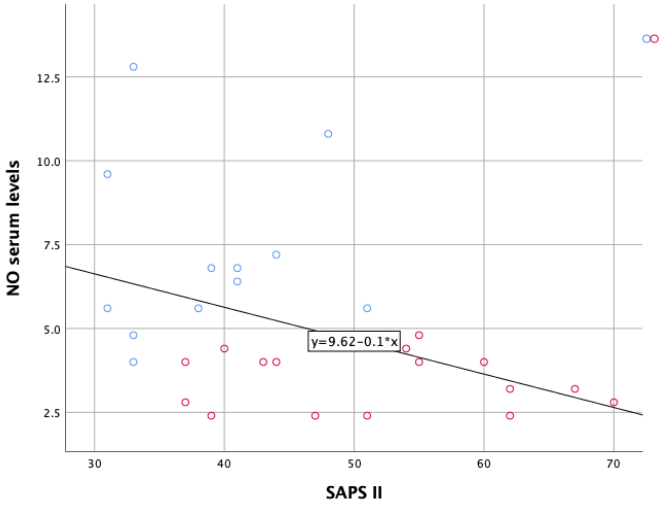

Figure 3. Spearman rank correlation between serum levels of NO and Simplified Acute Physiology Score II (SAPS II)

Receiver operating characteristic curve: Prediction of outcome (mortality) using serum levels of NO.

The predicted probability of mortality was assessed by using ROC curve. We used serum levels of NO as the predictive variable and mortality as the outcome variable. Sensitivity and specificity were calculated using Fisher's exact test. ROC was plotted using 1-specificity (FPF) on X-axis and sensitivity (TPF) on Y-axis for the predictor variable. A profile with area under the curve (AUC), (C) = 0.5 shows no predictable ability whereas (C) = 1 has a perfect predictable ability. As shown in Figure 3, the area under the ROC curve, C = 0.96 indicated a perfect predictive ability of mortality.

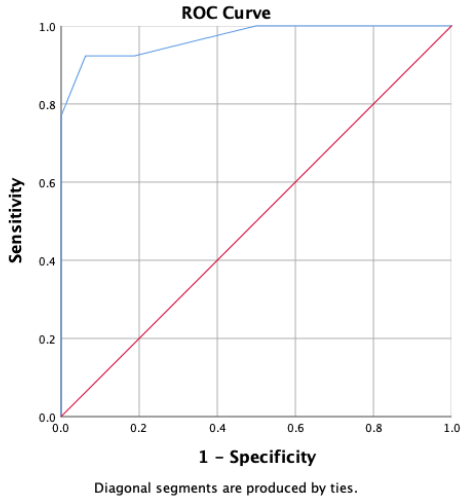

Figure 4. Receiver operating characteristic curve plotted to determine the NO serum levels prediction of mortality in patients with acute respiratory distress syndrome. AUC: 0.969.

Discussion

In our study, serum levels of NO, as well as SAPS II and APACHE II scores in patients with ARDS caused by

influenza A (H1N1) and the 28-day outcome were evaluated. The main finding of this study is that NO serum levels can serve as a biomarker of better clinical outcome in Influenza A (H1N1)-ARDS patients as shown by ROC curve analysis. We demonstrated that Influenza A (H1N1)-ARDS survivors had significantly higher serum levels of NO (metabolites NO2– and NO3–). SAPS II and APACHE II scores were higher among the non-survivors, and difference among survivors and non-survivors was statistically significant ($p<0.05$). In addition, serum levels of NO positively correlated with the SAPS II and APACHE II score. Several physiological mechanisms of NO might help in explaining our results. Well known effects of the NO on microcirculation is vasodilatation which consequently increases tissue perfusion [16, 17]. Elevation in dead space in ARDS patient is demonstrated in many studies performed in these patients and one of the reasons for this is because some alveoli are being ventilated but not perfused (shunt) [18]. 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 [19]. Additional explanation for our main findings is that endogenous NO has a beneficial effect in organs other than the lungs during ARDS. Higher serum levels of NO could help prevent further tissue damage by improving oxygen and nutrient delivery to the tissues while helping decrease the amount of toxic oxygen species [20]. Decreasing platelet and leukocyte adhesion to the endothelium caused by serum NO can protect endothelial tissue [21]. NO would thereby decrease multiorgan failure, which contributes to mortality in ARDS. Finally, NO and NOx have antibacterial effects that may be important in infectious conditions that predispose patients to ARDS [22]. Healthy alveolar epithelium, alveolar macrophage 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 [23]. Some studies in which NO levels in bronchoalveolar lavage (BAL) were observed, show high levels of NO in ARDS non-survivors [24]. 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. There was no significant difference in age and in preexisting comorbidities in ARDS survivors and ARDS non-survivors in this study.

There is a small number of studies which investigated serum levels of NO in patients with ARDS caused by H1N1 pneumonia in relation to survival. Study similar to ours was performed by *McClintock et al.*and they found that higher urine nitric oxide is associated with improved outcomes in patients with ALI/ARDS [25].

The limitations of the present study are small sample size; we analyze only one potential biomarker (serum levels of NO) in one time point only and this was a single center study.

In conclusion, we have demonstrated that alterations in serum levels of NO mirror the severity of ARDS caused by influenza A (H1N1) quite well and accurately predict clinical or treatment outcome of critically ill patients with this type of ARDS. This is clinically-relevant as it aids medical decision-making and informs therapeutic strategy in the ICU. Our study suggests that the monitoring of serum levels of NO alone or in combination with SAPS II and/or APACHE II scores in critically ill with ARDS (caused by influenza A H1N1) significantly improves the accuracy of predicting the severity of diseases by physicians and shortens the clinical decision-making time. Thus, this study highlights the potential role of serum levels of NO as specific and accurate biomarker for ARDS caused by influenza A (H1N1), and its correlation with predicting the better clinical outcome, including increased survival, in patients with ARDS caused by influenza A (H1N1).

Author Contributions : designed the research study: PK; performed the research SD, DM, MJ; analyzed the data: TK, BZ; wrote the paper: VDJ, RS

Funding: None

Declaration of interest: None

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