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Prognostic value of the biomarkers serum amyloid A and nitric oxide in patients with sepsis

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ABSTRACT

Objective: Sepsis is a major cause of mortality among critically ill patients in the intensive care unit (ICU). Alterations in serum amyloid A (SAA) and nitric oxide (NO) levels have been associated with mortality in critically ill patients. In the present study, we investigated the predictive value of SAA and/or NO compared to traditional predictive markers such as C-reactive protein (CRP) and Acute Physiology and Chronic Health Evaluation II (APACHE II) score.

Methods: 100 adult patients with sepsis and 25 without sepsis were enrolled in a prospective, randomized study in our ICU. The APACHE II score was calculated, and their peripheral venous blood SAA, NO and CRP levels were evaluated on days 1, 3, and 7 after sepsis was diagnosed. The patients were sorted based on incidence of septic shock into septic shock (A) and non-septic shock (B) groups. Comparative analyses of altered levels of these indicators between the two groups were performed, and correlations between SAA, NO, and the more traditional APACHE II score were probed. Patients were sorted based on survival status into death (D) and survival (S) groups based on death endpoint within 28 days after admission.

Results: We observed that the difference in APACHE II score, SAA and CRP levels were statistically significantly ($p < 0.05$) between groups A and B on days 1, 3 and 7 post-diagnosis, while inter-group NO level significantly differed ($p < 0.05$) on days 1 and 3 post-diagnosis, no apparent difference was observed on day 7 post-diagnosis. For groups D and S, SAA, CRP and NO levels significantly differed ($p < 0.05$) on days 3 and 7 post-diagnosis, with no apparent difference on day 1. APACHE II score was significantly different on day 7 ($p < 0.05$), however the difference on days 1 and 3 were non-significant. We also demonstrated a positive correlation between APACHE II scores, SAA levels on days 1, 3, and 7, as well as NO levels on days 1 and 3. In addition, for the D and S groups, SAA at all time points, NO on day 3 and CRP on day 7 positively correlated with increased death events.

Conclusion: The dynamic monitoring of SAA and NO serum levels with APACHE II scores better reflect the severity of sepsis than traditional indicators like CRP and may serve as independent prognosticators of sepsis in critically ill patients, shorten time to diagnosis confirmation and improve therapeutic decision-making.

1. Introduction

Sepsis is not only a global health issue, but also a global health priority, as morbidity and mortality rates remain high. Currently, there are approximately 30 million patients with sepsis, with an estimated 6 million sepsis-related deaths [1], and these numbers continue to increase by 1.5–8.0% each year [1–3]. The third international consensus definitions for Sepsis and Septic shock (Sepsis-3) released at the 45th critical care congress of the society of critical care medicine defined sepsis as a life-threatening organ dysfunction caused by dysregulated host response to infection [4]; Sepsis-3 mainly focused on the homeostatic imbalance of host caused by infection and organ dysfunction with potential risk of fatality [4]. With increased understanding of the pathogenesis and biology of sepsis and septic shock, the dysregulated reaction of the host to infection and organ dysfunction are emphasized [5–7]. Therefore, for clinical decision-making and sepsis treatment,

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accurate prediction of sepsis severity in the early stage and administration of appropriate therapy are the keys to improving therapeutic success in patients with sepsis. However, at present an objective and effective clinical outcome predictor or prognosticator of sepsis remains unknown.

SAA, mainly secreted by the salivary gland and pancreas, can digest polysaccharides such as starch and glycogen [8,9]. Once declared 'molecule of the year', NO is involved in multiple pathophysiological processes [10,11]. Recently, it was shown that SAA and NO play significant roles in the development of sepsis [12–15], thus informing our rationale for investigating the probable predictive and/or prognostic role of the duo in patients with sepsis.

In this study, the variations in the levels of SAA and NO in patients with sepsis were evaluated, and their probable clinical utility as sepsis-specific early disease indicators and disease-course predictors in patients with sepsis was examined in comparison with the conventional disease indicators, namely CRP and APACHE II score.

2. Materials and methods

2.1. General materials

Adult patients with signs of systemic inflammatory response syndrome (SIRS) and/or sepsis ($n = 100$; male = 65, female = 35; median age = 56 years old) admitted at Zhejiang Provincial People's Hospital between February 2014 and February 2017 and subjects without sepsis ($n = 25$; male = 15, female = 10; median age = 53 years old), were enrolled in the study after obtaining informed consent. The sites of infection were the lung ($n = 40$), abdomen ($n = 31$), blood ($n = 17$), urinary tract ($n = 7$), and soft tissues ($n = 5$). Subjects were enrolled based on the inclusion criteria: age $>$ 18 years old and $\leq$75 years old and presence of any 2 of the following - body temperature $>$ 38 °C or $<$ 36 °C, heart rate $>$ 90 per min, respiration rate $>$ 20 per min or hyperventilated with $PaCO_2 < 4.3$ kPa, leukocytosis (WBC $>$ 12,000 mm^3), leucopenia (WBC $<$ 4000 mm^3), $>$ 10% premature granulocytes and clinical signs or presentation of infection. All patients conformed to the diagnostic criteria in International Guidelines for Management of Sepsis and Septic Shock: 2012 [16]. Patients were excluded from the study based on the exclusion criteria: age $\leq$ 18 years old or $>$ 75 years old; patients who had received antibiotics during the 72 h preceding presentation or admission at the hospital; length of stay in ICU $<$ 7 days; patients with pregnancy, organ transplantation, liver cirrhosis, hematological disease, chronic organ dysfunction, tumor or immune suppressor administration, patients with end stage disease; non-compliance to treatment during the study. All the patients signed the informed consent and the study was approved by the Ethics Committee of our hospital.

2.2. Study methods

The general data of patients including gender, age, primary disease, infection site and length of stay in ICU were recorded prospectively. The patients were divided into septic shock group (A) and non-septic shock group (B); Septic shock was diagnosed when the patients met the following 2 diagnostic criteria [17] - systolic blood pressure (SBP) $<$ 90 mm Hg or SBP decrease $>$ 40 mm Hg and serum lactate level $>$ 3 mmol/L. Similarly, based on death end-point within 28 days after diagnosis or admission, the patients were divided into death (D) or survival (S) groups. Diagnosis and treatment of all patients were consistent with the International Guidelines for Management of Sepsis and Septic Shock: 2012 [16], including fluid resuscitation, antibiotics, administration of vasopressin, lung protective ventilation strategy, administration of glucocorticoid and surgical intervention et al. The APACHE II scores of all the patients on days 1, 3 and 7 post-diagnosis were evaluated and 5 mL fasting peripheral venous blood on same mornings were drawn into Ethylenediaminetetraacetic acid (EDTA) vacutainer blood collection tubes. The blood was centrifuged at 3500 rpm after 30 min at room temperature, and the plasma was collected and stored at −80 °C. Enzyme-linked immunosorbent assay (ELISA; Booster Biological Technology Co. Ltd. Wuhan, China) was used to detect and quantify SAA, NO and CRP following the manufacturer's protocol. ELISA data was further analyzed using the MJ182 automatic radioimmunoassay analyzer (Shanghai Institute of Applied Physics, Chinese Academy of Sciences, Shanghai, China).

2.3. Study outcome

The primary endpoint was death within 28 days after diagnosis/admission, where diagnosis is established just prior to admission to ICU. Secondary endpoints were disease exacerbation or complication including septic shock, and indications for mechanical ventilation or the initiation of dialysis. The occurrence of any of the endpoints was evaluated until day 28 after admission.

2.4. Statistical analysis

Statistical analyses were carried out using IBM SPSS Statistics for Windows version 20.0. (IBM Corp., Armonk, N.Y., USA) Normal distribution data were presented as mean ± standard deviation (mean ± SD), while skewed distribution data were presented as median (M) or interquartile range (IQR). Inter-group comparisons were made using students *t*-test (normal distribution) or nonparametric test (skewed distribution). Correlation analysis was performed by Spearman correlation analysis and the receiver operating characteristic (ROC) was used to determine the predictive significance of SAA, NO or CRP at different time-points within 28 days after admission of patients with sepsis. The correlation between APACHE II score and SAA or NO was determined by Spearman correlation analysis. $p < 0.05$ was considered as statistically significant.

3. Results

3.1. Comparison of indicators between groups A and B

Comparative analysis of SAA, NO, CRP and APACHE II score between the groups A and B showed that the SAA and CRP levels, as well as the APACHE II score significantly differed between groups on day 1, 3, and 7 post-diagnosis ($p < 0.05$), while the NO levels were significantly different on day 1 and 3 ($p < 0.05$), but non-significant on day 7 ($p > 0.05$) (Table 1).

3.2. Comparison of indicators between groups D and S

Results from comparing the SAA, NO, CRP and APACHE II score between groups D and S revealed that the serum SAA, CRP and NO levels were significantly different on day 3 and 7 ($p < 0.05$), but non-significant on day 1 ($p > 0.05$). APACHE II score was significantly different on day 7 ($p < 0.05$), however the difference was not significant on days 1 and 3 ($p > 0.05$) (Table 2).

3.3. Correlation of SAA or NO with APACHE II score

We also observed that on SAA levels on days 1, 3, and 7 post-diagnosis, as well as NO levels on days 1 and 3 positively correlated with the APACHE II scores ($p < 0.05$) (Table 3).

3.4. The predictive significance of SAA, NO or CRP

3.4.1. SAA levels, mortality risk and prediction of outcome

The area under the receiver operating characteristic curves (AUC) on days 1, 3, and 7 were 0.765 ($p = 0.000$), 0.996 ($p = 0.001$) and 1 (0.000), respectively, suggesting that the serum SAA level is an

Table 1
Comparison of indicators between group A and B.

Indicator	Day 1		t value	*p* value	Day 3		t value	*p* value	Day 7		t value	*p* value
	Group A	Group B			Group A	Group B			Group A	Group B		
SAA (mg/L)	51.04 ± 18.74	72.53 ± 12.07	−6.645	< 0.001	122.89 ± 83.84	196.33 ± 125.5	−3.493	0.001	104.18 ± 121.56	182.26 ± 147.13	−2.914	0.004
NO (μmol/L)	52.10 ± 8.59	55.88 ± 10.79	−1.946	< 0.001	90.49 ± 14.47	99.07 ± 19.11	−2.552	0.012	45.42 ± 8.76	41.04 ± 13.15	1.989	0.05
CRP (mg/L)	90.08 ± 35.01	162.09 ± 70.28	−6.661	< 0.001	83.75 ± 38.74	158.94 ± 90.58	−5.569	< 0.001	88.22 ± 64.76	182.23 ± 147.13	−4.265	< 0.001
APACHE II score	16.05 ± 2.95	22.04 ± 3.69	−9.015	< 0.001	16.09 ± 2.94	20.62 ± 4.05	−5.637	< 0.001	15.34 ± 6.69	21.18 ± 5.99	−4.540	< 0.001

Table 2
Comparison of indicators between groups D and S.

Indicator	Day 1		t value	*p* value	Day 3		t value	*p* value	Day 7		t value	*p* value
	Groups				Groups				Groups			
	S	D			S	D			S	D		
SAA (mg/L)	74.59 ± 11.27	69.96 ± 12.82	−1.289	0.204	112.42 ± 23.54	301.24 ± 122.16	7.57	< 0.001	68.08 ± 20.01	324.99 ± 102.83	12.23	< 0.001
NO (μmol/L)	55.36 ± 12.35	56.52 ± 8.74	0.353	0.726	93.61 ± 16.01	105.88 ± 20.84	2.236	0.031	51.05 ± 6.55	28.52 ± 7.06	−11.07	< 0.001
CRP (mg/L)	158.18 ± 62.99	166.98 ± 79.88	0.413	0.681	128.376 ± 49.43	197.14 ± 114.68	2.706	0.01	70.56 ± 37.27	321.83 ± 107.02	10.964	< 0.001
APACHE II score	20.9 2 ± 2.94	23.45 ± 4.12	2.4	0.121	19.76 ± 3.50	21.7 ± 4.49	1.628	0.111	16.96 ± 3.45	26.45 ± 3.94	8.614	< 0.001

Table 3
Correlation of SAA and NO with APACHE II score.

	Day 1		Day 3		Day 7	
	r value	*p* value	r value	*p* value	r value	*p* value
SAA and APACHE II score	0.501	< 0.001	0.386	< 0.001	0.798	< 0.001
NO and APACHE II score	0.590	< 0.001	0.339	0.001	0.139	0.169

Table 4
The predictive values of SAA, NO and CRP on the prognosis of septic patients.

	Cutoff value	AUC	95% CI	Sensitivity (%)	Specificity (%)
SAA day 1	58.7	0.765	0.675–0.856	0.861	0.609
SAA day 3	164.75	0.996	0.988–1.000	0.944	1
SAA day 7	146.1	1	1	1	1
NO day 1	49.55	0.660	0.551–0.770	0.835	0.453
NO day 3	90.75	0.781	0.687–0.875	0.806	0.656
NO day 7	17.9	0.038	0.002–0.073	1	0
CRP day 1	50.45	0.498	0.374–0.621	0.722	0.344
CRP day 3	87.35	0.745	0.646–0.845	0.889	0.516
CRP day 7	98.45	0.984	0.964–1.000	0.972	0.891

Note: AUC: area under the curve; 95% CI: 95% confidence interval.

independent predictor of sepsis-related death. We also showed that the SAA level (> 164.75 mg/L) on day 3 was highly sensitive and specific for sepsis as demonstrated by a sensitivity of 94.4% and specificity of 100%, using a cutoff point of 164.75 mg/L, as shown in Table 4.

3.4.2. NO levels, mortality risk and prediction of outcome

The AUC of the NO levels on days 1, 3, and 7 post-diagnosis was 0.66 ($p = 0.001$), 0.78 ($p = 0.002$) and 0.04 ($p = 0.002$), respectively, suggesting that serum NO level (> 90.75 μmol/L) on the day 3 is an independent predictor of sepsis-related death with sensitivity of 80.6% and specificity of 65.6%, using a cutoff point of 90.75 μmol/L, as shown in Table 4.

3.4.3. CRP levels, mortality risk and prediction of outcome

The AUC on days 1, 3, and 7 was 0.498 ($p = 0.968$), 0.745 ($p = 0.001$) and 0.984 ($p = 0.002$), respectively, indicating that the serum CRP level of > 98.45 mg/L on day 7 is a good predictor of mortality with a sensitivity of 97.2% and specificity of 89.1%, using 98.45 mg/L as the cutoff point, as shown in Table 4. Together, our data showing strong correlation between our proposed indicators of sepsis and the risk of 28-day sepsis-related mortality demonstrate that the discriminatory power of serum SAA, NO, or CRP levels for differentiation between survival and mortality is high and statistically significant. Thus, we present serum SAA and NO levels, as well as APACHE II score as novel accurate predictors of 28-day sepsis-related deaths.

4. Discussion

Sepsis remains common in critically ill patients in the Intensive Care Unit (ICU), may be complicated by multiple organ dysfunction syndrome (MODS), and often results in fatality despite advances made in diagnostic and therapeutic approaches in the last decade consistent with the International Guidelines for Management of Sepsis and Septic Shock: 2012 [16,18,19]. The clinical status of patients with sepsis changes rapidly with huge range of fluctuation in disease severity, thus the need for disease-specific and sensitive bio-indicators for real-time monitoring of disease severity and/or prediction of treatment outcome. Presently, CRP detection which is relatively easy and quick, as well as low-cost, is widely used in the clinics for determining sepsis severity and response to therapy [20–22]. CRP demonstrates high sensitivity to sepsis and has been shown to aid clinical evaluation of patients' status, especially in the early stage of inflammation [20–22]. However, as sepsis evolves and progresses, the CRP level becomes insufficient for clinical assessment of disease progression.

Similarly, APACHE II score, evaluated based on age, vital signs, Glasgow coma scale (GCS), routine blood test, blood gas analysis and blood biochemistry, is not only popular, but is one of the most important indicators for evaluation of disease severity and prognosis in critically ill patients [23–25]. In the present study, we corroborated the critical role of APACHE II score in evaluating the disease severity and predicting clinical outcome. However, because the APACHE II scoring system consists of many variables, including 12 acute physiologic variables, age, and chronic health status points [25], and the score calculation is complicated, with some variables limited by objective medical conditions, thus, limiting its clinical application for rapid and accurate evaluation of the severity and prognosis of patients with sepsis. These underscore the clinical relevance of this study in which we proffer novel accurate biomarkers for early, easy, rapid and cost-effective determination of disease severity in patients with sepsis, and for prediction of their clinical outcome.

Sepsis is a syndrome of dysregulated acute inflammatory response, characterized by excessive inflammation which is associated with systemic inflammatory response syndrome (SIRS) and the release of endogenous inflammatory mediators which induce compensatory anti-inflammatory response syndrome (CARS) [26,27]. The imbalance between SIRS and CARS results in aberrant inflammatory response, aggravates systemic injury, induces hemodynamic instability, metabolic disturbance and homeostatic imbalance, and causes injures to remote organs or even death [26,27]. It has been suggested that SAA may be associated with the pathogenesis of sepsis, and SAA level in patients with sepsis was shown to be ~1000 times higher than the normal level [28–30]. It is believed that SAA plays an important role in the promotion of acute inflammation, however, the molecular mechanism underlying SAA-mediated promotion of acute inflammation remains unclear [31], despite its implication in chronic inflammatory diseases such as atherosclerosis and diabetes [32–35]. SAA belongs to a highly conservative acute-phase protein family, and in acute inflammatory conditions, plasma SAA level has been shown to be increased 1000 folds [36]. In addition, it has been demonstrated that SAA is involved in important immune-associated activities by inducing the production of inflammatory mediators including TNF-α, NO and IL-6 by immune cells, such as mononuclear macrophages [37], as well as increase the chemotaxis of neutrophils and monocytes, thus it has potential pro-inflammatory function [38].

NO plays an important pathophysiological role in patients with septic shock. In physiology, NO exhibits significant bioactivities including regulating tissue oxygen consumption and blood flow [39]. NO has also been shown to regulate vascular tension in patients with septic shock [40]. Recently it was demonstrated that NO production regulated by berberine ameliorates lipopolysaccharide (LPS)-induced sepsis and prevents septic shock-related mortality, *in vivo* [41].

In our study, serum SAA, NO, and CRP levels, as well as APACHE II score in patients with sepsis, complicated by septic shock or not, and the 28-day outcome were evaluated. We demonstrated that serum SAA, and CRP levels, as well as APACHE II score in the septic shock group significantly differ from the non-septic shock group on days 1, 3, and 7 ($p < 0.05$), for NO level, the difference was significant on days 1 and 3 ($p < 0.05$), but non-significant on day 7 ($p > 0.05$). SAA level at all time-points and NO level on the day 1 positively correlated with APACHE II score ($p < 0.05$), suggesting a short early-stage release peak of SAA and NO in the serum of patients with sepsis, which is consistent with the findings from other studies [37,38]. We posit that in the early stages of sepsis, serum SAA and NO levels mirror the severity of the disease in patients with sepsis, such that elevated SAA, or NO level represents increased severity or disease exacerbation. Compared with serum NO, our study revealed that in the early stage of sepsis, a

stronger correlation exists between serum SAA level and the severity of sepsis, however, for intermediate (severe sepsis) or advance (septic shock) stages, a lower NO level suggests worsening severity. This is suggestive of the stage-dependent divergent functionality of serum NO in sepsis, and is corroborated by documented role of endogenous NO in the enhancement of injury-related inflammation, on the one hand, while on the other hand, it is known to block LPS-induced inflammatory signaling [42,43]. The biological role of NO is extensive and complex, as is also reflected in its divergent roles at various stages of septicopyemia [42,43].

We also demonstrated that the serum SAA level at all time-points, serum NO on day 3 and CRP level on day 7 were statistically significantly in predicting sepsis-related death. The AUC of serum SAA on day 3 was the largest, with corresponding highest sensitivity and specificity on the ROC. The AUC, sensitivity and specificity of CRP on day 7 was significantly higher than that of NO; besides, compared with NO, CRP and APACHE II score, SAA exhibited a higher predictive power, as it more accurately predicted the prognosis of sepsis patients based on the 28-day primary outcome, namely death. Serum SAA levels on days 1, 3, and 7 significantly differed between the death and survival groups, while NO, CRP and APACHE II score were only significantly different on day 7 ($p < 0.05$). Our results showed that although SAA and CRP are both acute-phase proteins, SAA exhibited better sensitivity and specificity than CRP in the patients with sepsis. We demonstrated that though serum SAA, NO and CPR levels to different degrees predict the prognosis of patients with sepsis, SAA displayed the best sensitivity and specificity, conversely, CRP had the lowest. The observed rise in serum SAA level was rapid and significant in patients with sepsis.

In conclusion, while this study is limited by the relative small cohort size, probable influence of treatment regimen on study results, and the hitherto evolving or limited understanding of the specific mechanism underlying the dynamism and disease-related changes in SAA and NO levels in patients with sepsis, we have demonstrated that alterations in serum SAA and NO levels better mirror the severity of sepsis and more accurately predict clinical or treatment outcome of critically ill patients with sepsis, compared to CRP. This is clinically-relevant as it aids medical decision-making and informs therapeutic strategy in the ICU. While abandoning the use of CRP and APACHE II in sepsis clinic for dynamic monitoring of patients' status is not foreseen in the nearest future, our study suggests that the dynamic monitoring of serum SAA or NO alone or in combination with APACHE II score in critically ill patients significantly improves the accuracy of predicting the severity of sepsis by physicians, shortens the clinical decision-making time and improves early-stage therapeutic efficiency. Thus, this study highlights the potential role of serum SAA or NO as specific and accurate biomarker for sepsis, and its correlation with septic shock and the death of patients with sepsis.

Authors' contributions

Conceived and designed the study: MHY, FH, YXT; Performed the experiments: MHY, FH, MHC, XL; Analyzed the data: MHY, RHS, YXT; Wrote the paper: MHY, FH, YXT; Provided reagents, materials, and experimental infrastructure: RHS, YXT; All authors read and approved the final version of the manuscript.

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

The datasets supporting the conclusions of this article will be available in a repository upon publication of the manuscript.

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