Prediction about severity and outcome of sepsis by pro-atrial natriuretic peptide and pro-adrenomedullin

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【Abstract】Objective: Measurement of biomarkers is a potential approach to early prediction of the risk of mortality in patients with sepsis. The aim of the present study was to evaluate the prognostic value of pro-atrial natriuretic peptide (pro-ANP) and pro-adrenomedullin (pro-ADM) levels in a cohort of medical intensive care patients and to compare it with that of other known biomarkers and physiological scores.

Methods: Blood samples of 51 consecutive critically ill patients admitted to the intensive care unit and 53 age-matched healthy control people were evaluated in this prospective study. The prognostic value of pro-ANP and pro-ADM levels was compared with that of acute physiology and chronic health evaluation (APACHE) II scores and various biomarkers such as C-reactive protein, interleukin-6 and procalcitonin. Pro-ANP and pro-ADM were detected by a new sandwich immunoassay.

Results: On admission, 25 patients had systemic inflammatory response syndrome (SIRS), 12 sepsis, 9 severe sepsis and 5 septic shock. At that time, the median levels (ng/ml) of pro-ANP and pro-ADM were 87.22 and 0.34 respectively in patients with SIRS, 1533.30 and 2.23 in those with sepsis, 1098.73 and 4.57 in those with severe sepsis, and 1933.94 and 8.21 in those with septic shock. With the increasing severity of disease, the levels of pro-ANP and pro-ADM were gradually increased. On admission, the circulating levels of pro-ANP and pro-ADM in patients with sepsis, severe sepsis, or septic shock were significantly higher in non-survivors than in survivors (P<0.05). In a receiver operating characteristic curve analysis for the survival of patients with sepsis, the areas under the curve (AUCs) for pro-ANP and pro-ADM were 0.89 and 0.87 respectively, which was similar to the AUCs for procalcitonin and APACHE II scores.

Conclusion: Pro-ANP and pro-ADM are valuable biomarkers for prediction of severity of septic patients.

Key words: N-terminal proatrial natriuretic peptide; Proadrenomedullin; Sepsis

Sepsis is one of the most frequent causes of death in intensive care unit (ICU) patients worldwide. According to recent reports, the incidence of sepsis has been rising at a rate of 1.5%-8% per year.1 About 9% of the sepsis develops to serious sepsis and 3% to septic shock. Although great progress has been made in the treatment of sepsis, the mortality of patients with severe sepsis is still as high as 30%-70%.2-4 Moreover, the high expenditure of sepsis management has resulted in a heavy financial burden to the government and patients.1 Therefore, how to estimate the severity of sepsis early and apply targeted therapies timely is very important in the treatment of sepsis.

Blood culture is a gold standard for diagnosis of infection. However, it takes too long and the rate of false negative results is relatively high. The specificity of white blood cell count, C-reactive protein (CRP), interleukin (IL)-6 and their specific antibody complements are not significantly high enough for an early diagnosis of sepsis. There are some disputes about the distinction between infection and non-infection by procalcitonin (PCT).5 Especially in the early stage of sepsis when symptoms are not obvious, though the patient’s conditions have deteriorated, there are no positive results in the conventional laboratory test until the chance for prompt treatment has gone.

Members of the natriuretic peptide family have been recognized as markers for congestive heart failure. Defending against hypertension and salt-water retention, these markers antagonize the renin–angiotensin–aldosterone system, exerting influence on sodium reabsorp-
tion in the renal tubule, vascular tone and cell growth. Atrial natriuretic peptide (ANP) is predominantly produced in the atrium and comprises 98% of natriuretic peptides in circulation. Most sepsis patients are accompanied with cardiovascular dysfunction, mainly presenting as cardiac muscle inhibition, with dysfunction of contraction and relaxation of the left ventricle particularly obvious. Adrenomedullin (ADM), a peptide with 52 amino acids, has the effect of immune regulation, metabolic and vascular activities. It is a potent vasodilator, and its widespread production in tissues helps to maintain blood supply for individual organs. Interestingly, ADM also has bactericidal activity which can be further enhanced by its regulation and modulation of complement activities. Serum levels of ANP and ADM are shown to be increased in patients with sepsis, reflecting their involvement in the pathophysiological process of disease. However, the measurement of ANP and ADM is technically challenging and reliable measurement is almost impossible because ANP and ADM are cleared out rapidly in the circulation. But their prohormones, pro-atrial natriuretic peptide (pro-ANP) and pro-adrenomedullin (pro-ADM), which are secreted at the same molar ratio as ANP and ADM, have a much longer half-life compared to the mature ANP and ADM. It has been suggested that pro-ANP and pro-ADM are more reliable analytes and they have a positive correlation with the disease severity and may become new biomarkers for early diagnosis of sepsis. The updated laboratory methods can detect the indexes above, thus to set a foundation for their clinical application.

In this study, we aimed to further evaluate the prognostic value of pro-ANP and pro-ADM levels in a cohort of well defined medical ICU patients by comparing with other biomarkers like IL-6, CRP and PCT and a disease severity index—acute physiology and chronic health evaluation (APACHE) II scores.

**METHODS**

**Research design and subjects selection**

In the present study, we evaluated the pro-ANP and pro-ADM levels of 51 consecutive critically ill patients admitted from June to November 2007 to the medical ICU, Affiliated First People’s Hospital of Shanghai Jiaotong University, Shanghai, China. All the medical and surgical patients were included except those with neurologic trauma or cardiac operations.

Data on admission day (during the first 24 hours), the second day, and the day either discharge from ICU or death were collected. For patients who died within 24 hours after admission, only data on admission day were collected. Vital signs, clinical status and severity of disease, and laboratory parameters, including pro-ANP and pro-ADM levels, were assessed and calculated each day. The APACHE II scores were calculated by means of maximal daily deviations of 12 physiological variables and corrected with consideration of ages and various chronic diseases.

Patients were divided into four groups: SIRS, sepsis, severe sepsis and septic shock, as defined according to the well known consensus criteria. SIRS was characterized by the presence of at least two of the following clinical manifestations: fever or hypothermia (>38°C or <36°C); tachycardia (>90 beats/min); tachypnoea (>20 breaths/min, PaO₂<32 mm Hg or need for mechanical ventilation); white blood cell count>12 000 cells/µl or<4 000 cells/µl, or >10% band forms. Sepsis was defined as SIRS with an infection which was defined based on the Society of Critical Care Medicine/European Society of Intensive Care Medicine/American College of Chest Physicians/American Thoracic Society/Surgical Infection Society (SCCM/ESICM/ACCP/ATS/SIS) consensus criteria. Severe sepsis was defined as the presence of sepsis and at least one of the following manifestations of organ failure: hypoxaemia (arterial oxygen tension<75 mm Hg); metabolic acidos(s (pH<7.30); oliguria (output<30 ml/h); lactic acidosis (serum lactate>2 mmol/L); and an acute alteration in mental status without sedation (reduction by ≥ 3 points from the baseline value in Glasgow coma scale score). Septic shock was defined as the presence of sepsis accompanied with a sustained decrease in systolic blood pressure (<90 mm Hg or a drop of 40 mm Hg from the baseline) despite fluid resuscitation and a need for vasopressor agent to maintain adequate blood pressure.

Informed consents were obtained from conscious patients or their next of kins before enrolment. The protocol had been approved by the hospital ethics committee.

For comparison, pro-ANP and pro-ADM levels were
also measured in 53 cases of age-matched healthy blood donors.

Methods of measurement

Blood samples were obtained intravenously from the femoral vein or ulnar vein. Results of routine blood analyses such as complete blood count, serum chemistry including CRP and blood gas analyses were collected. Plasma was separated at the moment of blood sampling and frozen at -70°C until further assay. We conducted blind measurement and a batch analysis.

The levels of pro-ANP and pro-ADM were measured in all patients by an immunoluminometric assay (MR-proANP LIA; MR-proADM LIA; BRAHMS, AG, Hennigsdorf, Germany). Briefly, this assay employed two polyclonal antibodies specific to pro-ANP (amino acids 53–90) and pro-ADM (amino acids 45–92). The minimum detectable concentration in the assay was 0.01 ng/ml. Intraassay imprecision was less than 10% over the entire measuring range, and the functional assay (interassay coefficient of variation<20%) could detect a concentration as low as 0.05 ng/ml. The assay exhibited linear dilution and pooling of samples or addition of synthetic analyte had no impact on recovery of the analytes. Stability of the analytes (<20% loss of recovery) in ethylene diamine tetraacetic acid (EDTA) plasma was demonstrated for at least 3 days at room temperature, 14 days at 4°C and 1 year at -20°C.

PCT was measured by a rapid sensitive immunoassay (PCT LIA, BRAHMS, AG, Hennigsdorf, Germany). The minimum measurable concentration in the assay was 0.01 ng/ml and the functional assay (interassay coefficient of variation<20%) could detect a concentration as low as 0.05 ng/ml. The assay exhibited linear dilution and pooling of samples or addition of synthetic analyte had no impact on recovery of the analytes. Stability of the analytes (<20% loss of recovery) in ethylene diamine tetraacetic acid (EDTA) plasma was demonstrated for at least 3 days at room temperature, 14 days at 4°C and 1 year at -20°C.

CRP was determined by a routine enzyme immunoassay (hs-CRP, Shanghai Technology Co. Ltd, Shanghai, China). A serum level greater than 8 mg/L was considered abnormally elevated. IL-6 was measured by radioimmunoassay (IL-6 RIA kit (HY-078), Huaying Technology Co. Ltd, Beijing, China), and the minimum detectable concentration was 0.6 pg/ml.

Data analysis

Data were expressed as mean±standard deviation in the study. Comparison of frequencies was done by the χ² test. Comparisons between two groups were performed by the non-parametric Mann-Whitney U test and among multiple groups were conducted by one-way analysis of variance with least square difference post-hoc evaluation. A receiver operating characteristic (ROC) curves analysis was constructed by SPSS 15.0 statistical software. Undetectable levels were assigned equal to the minimum detectable concentration in the assay. Correlation analyses were performed by Spearman rank correlation. To estimate the potential clinical benefit of pro-ANP and pro-ADM levels, we used the likelihood ratio test to determine whether logistic regression models including the measurement of pro-ANP, pro-ADM and routine clinical parameters such as APACHE II score could provide a greater significance than logistic regression models limited to APACHE II score alone. All statistical tests were two-tailed and P<0.05 was considered statistically significant.

RESULTS

Characteristics of study subjects

Blood samples of 51 patients were collected. The mean age of these 51 patients was (54.3±20.7) years with a range of 17 to 90 years. The percentage was 53% for male patients and 47% for female patients. Thirty-four patients had other medical problems, and 17 had surgeries before. Detailed baseline characteristics of the study population are summarized in Table 1.

Correlation between pro-ANP, pro-ADM and severity of disease

Comparisons of po-ANP and pro-ADM concentrations on admission among each patient group and the control group are showed in Figure 1. The concentrations (ng/ml, range) of pro-ANP and pro-ADM in the healthy control group were 32.21 (4.35-67.46) and 0.14 (0.01-0.63) respectively. In patients with SIRS, sepsis, severe sepsis or septic shock, the pro-ANP concentrations were 87.22 (34.35-184.21), 533.30 (76.02-1419.23), 1061.70 (135.65-2822.00) and 1933.95 (171.93-3822.60) respectively. And corresponding values for pro-ADM concentrations were 0.35 (0.01-0.83), 2.08 (1.09-3.83), 4.23 (2.01-8.34) and 7.63 (3.08-13.80) respectively. According to the clinical severity of infection from SIRS to septic shock, pro-ANP and pro-ADM levels gradually increased (P<0.05). Post-test analyses revealed a significant difference (P<0.05) in patients with SIRS, sepsis or severe sepsis as compared with patients with septic shock.
Relation between pro-ANP, pro-ADM levels and outcomes in patients with sepsis, severe sepsis or septic shock

All pro-ANP and pro-ADM values of survivors and non-survivors with sepsis, severe sepsis or septic shock measured during hospitalization in the ICU are shown in Figure 2. The median pro-ANP and pro-ADM values in non-survivors were significantly higher than in survivors: 82.92 (25.65-137.09) vs 104.10 (41.97-167.41), P=0.22 for all time points and 101.34 (37.52-149.84) vs 128.96 (38.30-169.04), P=0.56 on admission. Similarly, pro-ADM values of patients without infections were not higher in non-survivors than in survivors: 0.35 (0.07-0.57) vs 0.52 (0.18-1.39), P=0.11 for all time points and 0.36 (0.11-0.97) vs 0.60 (0.24-1.83), P=0.11 on admission.

To define an optimal decision threshold for pro-ANP and pro-ADM values in septic patients, we performed ROC plot analysis on data from patients with sepsis, severe sepsis, or septic shock obtained within the first 48 hours after admission to the ICU. Sensitivity was calculated among non-survivors, and specificity was assessed among those discharged from ICU. For comparison, the same ROC plot analysis was performed on CRP, PCT, IL-6 and APACHE II scores. The areas under the curve (AUCs) were 0.89 for pro-ANP and 0.87 for pro-ADM, significantly higher than those for IL-6 (0.71) and CRP (0.53), but similar to those for PCT (0.81) and APACHE II score (0.81, Figure 3). The AUC for pro-ANP combined with pro-ADM was 0.92, which exceeded that of either peptide alone. The optimal threshold concentration of pro-ANP was 150.0 ng/ml. At this cutoff, the sensitivity for correct prediction of death in the ICU was 87% and the specificity was 86%. The optimal threshold concentration of pro-ADM was 0.80 ng/ml. At this cutoff, the sensitivity was 91% and specificity was 73%, respectively. The PCT and APACHE II scores were also predictive for prognosis but they had much lower values as compared with pro-ANP and pro-ADM. At a PCT threshold of 0.55 ng/ml, the sensitivity was 76% and specificity was 81%. At an APACHE II threshold of 23.5, the sensitivity was 80% and specificity was 76%. At a PCT threshold of 25 ng/ml, which was recommended by the US Food and Drug Administration for the use of activated protein C, the sensitivity was 63% and specificity was 78%. When the level of pro-ANP combined with pro-ADM was 151.0 ng/ml, the sensitivity was 90% and specificity was 89%.

DISCUSSION

We found a significant increase of plasma pro-ANP and pro-ADM concentrations in septic patients, espe-
As generally recommended, sepsis, severe sepsis and septic shock are diagnosed based on the consensus criteria defined by ACCP/SCCM in 1991. However, many clinicians believe that the criteria are not sensitive and can easily lead to misdiagnosis because they cannot show the pathophysiological process, state and course of disease. Thus, a new sepsis diagnostic criterion was defined at an international conference held in Washington in 2001. However, perfect gold standards for diagnosis of infections still do not exist, and clinical classification of critically ill patients is not 100% definite despite the use of these guidelines. An ideal sepsis marker should be able to guarantee an early diagnosis, predict the course of disease, and help to differentiate bacterial causes from noninfectious and viral causes of systemic inflammation, not only in sepsis trials but also in routine clinical application.

During infection, the increase of bacterial endotoxin and proinflammatory cytokines leads to a higher incidence of congestive heart failure, meanwhile, because of inadequate organ perfusion, the clearance of ANP and pro-ANP is decreased, which results in an obvious increase of ANP and pro-ANP levels in septic patients. There might be two mechanisms responsible for the marked increase of pro-ADM and mature ADM levels in circulation in sepsis. (1) As a member of calcitonin receptor-like receptor gene family, pro-ADM is widely expressed and extensively synthesized during sepsis.

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Sepsis is a complex syndrome including coagulation disorder, inflammatory response, cellular disorder and metabolic alteration. The ambiguity of clinical diagnosis and risk stratification are major problems in
sepsis management. In the past, almost all intervention trials failed to show any benefit from therapies for sepsis, and thus sepsis intervention has been referred to “graveyard for pharmaceutical companies”.\textsuperscript{19,20} Therefore, to find a better biomarker to direct the clinical treatment of sepsis is urgent. APACHE II scores, which synthesize 12 acute physiological and chronic health status parameters, serve to assess the severity of disease and associate closely with prognosis. In the present study, the prognostic value of pro-ANP and pro-ADM levels is comparable to that of PCT and APACHE II scores. More importantly, pro-ANP and pro-ADM levels are easier to determine and furthermore can reflect the significant pathophysiological changes in sepsis.

The limitation of our study is that only 51 patients are included in this trial. We consider that our results are prospective but need to be validated in more patients. If our findings can be further confirmed, pro-ANP and pro-ADM may become the new promising biomarkers for prognosis and individual risk stratification of sepsis.

REFERENCES