# Elevated serum levels of lipoprotein-associated phospholipase A2 predict mortality rates in patients with sepsis

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**Abstract.** Sepsis remains one of the leading contributors to mortality rates in the intensive care unit (ICU) and emergency intensive care unit (EICU). Therefore, any treatments against the agents which produce sepsis in a medical emergency, are welcome. Elevated serum levels of lipoprotein-associated phospholipase A2 (Lp-PLA2) have been reported in a small cohort of patients with inflammation. The present study evaluated serum levels of Lp-PLA2 in patients with sepsis and investigated the role of Lp-PLA2 in sepsis. The investigation involved the selection of 151 patients with sepsis admitted to the emergency department of the Affiliated Hospital of Nantong University (Nantong, China) and 30 healthy controls. All patients (39 with sepsis, 55 with severe sepsis and 57 with septic shock) were examined on admission to the EICU. A complete blood count was performed, and serum levels of Lp-PLA2, C-reactive protein, procalcitonin, and interleukin 6, sequential organ failure (SOFA) scores and Acute Physiology and Chronic Health Evaluation II (APACHE II) scores were determined on hospital admission. The EICU and overall mortality rates were evaluated at baseline. The present study also assessed various laboratory parameters, clinical data and inflammatory cytokines. The patient follow up duration was 90 days. The data suggested that the serum levels of Lp-PLA2 on admission to the EICU in patients with sepsis were elevated, compared with those in healthy controls. The concentrations of Lp-PLA2 were correlated with the severity of disease, and were significantly associated with experimental markers of inflammation and established prognostic scores. In the total cohort, persistently elevated levels of Lp-PLA2 on admission for EICU treatment was a predictor of poor prognosis, and provided superior diagnostic use, compared with the prognostic scoring systems, including SOFA or APACHE II scores. Taken together, the results suggested that Lp-PLA2, with respect to other markers of inflammation, may have a role as a prognostic marker in sepsis, and provide background evidence for further trials to evaluate the clinical and pathophysiologic roles of Lp-PLA2 in sepsis. Persistently elevated serum concentrations of Lp-PLA2 indicated an unfavorable outcome in patients with sepsis. In addition, the results indicated the potential role of Lp-PLA2 as a prognostic biomarker in patients with sepsis during the early course of EICU treatment.

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## Introduction

Sepsis, in the context of infection, is defined as a systemic inflammatory response syndrome (SIRS). For numerous individuals, it is a life-threatening and profoundly damaging condition. The consequent increase in hospitalizations and resource utilization in providing care to patients with sepsis leads to the increased incidence of sepsis (1). It is one of the major causes of admissions to the intensive care unit (ICU) and the emergency intensive care unit (EICU), and is associated with high mortality and morbidity rates (2), in addition to multiple organ dysfunction or injury, for example in the lungs, kidneys and bone marrow (3-5). Sepsis is a systemic inflammatory response, which occurs during infection (6), and its pathogenesis is a result of immune-inflammatory and anti-inflammatory processes triggered by the infection agent (7). As reported frequently, the effects of sepsis on the individual persists following the period of critical illness, and has an effect on mortality and morbidity rates (8,9). In addition, the inflammation present during EICU admission may cause subsequent deleterious effects (10). Following discharge from hospital, there is an increased risk of cardiovascular disease in patients who survive sepsis, which suggests that an acute episode of systemic inflammation has a long-term effect (11).

Phospholipase A2 (PLA2) is an enzyme, which is involved in lipid metabolism, hydrolyzes phospholipids, and liberates arachidonic acid and lysophospholipids (12). PLA2 enzymes are a diverse class of esterases, at the *sn*-2 position, which preferentially cleave glycerophospholipids to liberate a fatty acid and a lysophospholipid (13). Phospholipases of

mammalian species are critical in transducing cellular signals into biologically active lipid second messengers, including lysophospholipids and arachidonic acid (12). An enzyme from human plasma was previously isolated by investigators from the University of Utah (14), which hydrolyzes platelet-activating factor (PAF) and, in 1995, Tjoelker *et al* (15) reported its cloning. PAF acetyl hydrolase (PAF-AH) enzyme were later recognized as PLA2s, one of which for oxidized lipids in plasma was independently termed lipoprotein-associated phospholipase A2 (Lp-PLA2). In previous years, several reviews have examined the family of PLA2s or specific types, including secretory PLA2, cytosolic PLA2 (cPLA2), lysosomal PLA2, PAF-AH, calcium-independent PLA2 and adipose-specific PLA2 (16-19). In total, >30 isoforms and associated enzymes have been identified, which are involved in inflammation and several neoplastic conditions. Based on their localization, catalytic mechanism, structure and evolutionary associations, the isoforms are divided into the above six families (13). These enzymes differ in size, function, location, substrate specificity and calcium requirements. The classes of PLA2 inhibitors and their potential role in the treatment of inflammatory diseases have been summarized in several review articles (19-21).

Lp-PLA2 is a unique member of the PLA2 superfamily, also known as PAF-AH. In humans, it circulates in an active form as a complex with high- and low-density lipoproteins (LDLs). PLA2 has been used as a novel biomarker in cardiac diseases, predicting the prevalence and prognosis of chronic and acute congestive heart failure and pulmonary hypertension (22). In Japanese cohorts, genetic analyses have shown that loss of Lp-PLA2 function is a risk factor for vascular and inflammatory conditions in humans harboring an inactivating mutation at this locus (23,24). The overexpression of Lp-PLA2 has consistently shown anti-atherogenic and anti-inflammatory properties in certain animal models (23,25). It is an indicator or marker of inflammation in the vessel wall, measured in the plasma (26,27). Primarily secreted by macrophages, Lp-PLA2 binds to the apolipoprotein B moiety on LDL particles following release, and remains latent until the LDL is oxidized. Following LDL oxidization, oxidized phosphatidylcholine is released, which acts as a requisite substrate for Lp-PLA2. Lp-PLA2 then breaks the oxidized phosphatidylcholine into two bioactive compounds, lysophosphatidylcholine and oxidized nonesterified free fatty acids (27), which are considered to act as proinflammatory factors (26). Previous studies have shown that Lp-PLA2, which is considered to be a candidate inflammatory factor, is often upregulated in a number of diseases, including pancreatitis (28) and myocarditis (29), suggesting that circulating Lp-PLA2 may offer potential as a biomarker for inflammatory and infectious diseases.

Despite the novel roles of Lp-PLA2 in the regulation of inflammation, its functional involvement in systemic infections remains to be elucidated. In addition, whether Lp-PLA2 has diagnostic and prognostic value in patients with sepsis is currently unclear. Therefore, the present study examined patients with sepsis at an EICU and performed several measurements of serum concentrations of Lp-PLA2 during the first days of EICU treatment. The aim of the study was to examine the regulation and diagnostic value of serum concentrations of Lp-PLA2 in sepsis. The investigation focused on the expression of Lp-PLA2 and its potential contribution in predicting prognosis in sepsis, and whether serum levels of Lp-PLA2 can serve as a prognostic predictor for EICU and long-term survival rates.

## Materials and methods

*Study design and patient information*. The present prospective, observational study was based in the EICU at the Affiliated Hospital of Nantong University (Nantong, China). Between January 2008 and December 2012, patients with sepsis admitted to the EICU were screened. Specifically, patients with a diagnosis of sepsis according to the Surviving Sepsis Campaign criteria for sepsis were included. Patients who met the criteria set by the American College of Chest Physicians and the Society of Critical Care Medicine Consensus Conference Committee for sepsis, severe sepsis and septic shock were classified as patients with sepsis. A total of 151 consecutive patients (107 men and 44 women; median age 66 years, range 20-87 years) admitted to the EICU at the Affiliated Hospital of Nantong University were enrolled in the present study (Table I). The exclusion criteria were as follows: i) absence of informed consent; ii) patients <18 years old; iii) patients undergoing continuous renal replacement therapy prior to sampling; iv) patients receiving steroid therapy or immunosuppressants; v) patients who were expected to have short-term (<72 h) intensive care treatment due to post-interventional observation or acute intoxication; vi) patients with infections, including those induced by virus, chlamydia, mycoplasma and tubercle bacillus. The medium length of admission at the EICU was 15 days (range 1-34 days).

The patients' data, blood samples and clinical information were collected prospectively. The clinical course was observed in a follow-up period by directly contacting the patients, the relatives or their primary care physicians. In addition, 30 healthy blood donors were analyzed as a control population, with normal values for blood counts, liver enzymes and C-reactive protein (CRP). All blood samples were obtained with the consent of the patient, his or her spouse, or the appointed legal guardian. The study protocol followed the guidelines set in the Declaration of Helsinki and was approved by the Ethics Committee (Institutional Review Board) at the Affiliated Hospital of Nantong University.

*Definitions and determination of relevant parameters in patients with sepsis*. Serum was obtained on admission to the EICU prior to therapeutic intervention. For the 151 patients, follow-up measurements were available during EICU treatment. All samples were immediately placed on ice and centrifuged for 5 min at 2000 rpm and 0˚C, and serum samples were stored at -80˚C. CRP, procalcitonin (PCT) and interleukin 6 (IL-6) were measured. If a patient succumbed to mortality within 90 days following the onset of sepsis, this was defined as sepsis-associated mortality. EICU-associated mortality was defined as a patient succumbing to mortality admission in the EICU; the overall mortality included that within the EICU and during the observation period following discharge from the EICU and hospital. Generally, sepsis was diagnosed by an identifiable or suspected infection site, in addition to evidence of SIRS manifested by at least two of the following criteria: i) body temperature <36˚C or >38˚C; ii) respiratory rate

Table I. Baseline characteristics of patients with sepsis and healthy controls.

| Characteristic | Control (n=30) | Sepsis (n=39) | Severe sepsis (n=55) | Septic shock (n=57) |
|---|---|---|---|---|
| Age (years); mean (range) | 61 (30-82) | 65 (49-85) | 66 (26 to 87) | 67 (20-85) |
| Gender, n (%) | | | | |
| Male | 17 (56.7) | 27 (69.2) | 39 (70.9) | 41 (71.9) |
| Female | 13 (43.3) | 12 (30.8) | 16 (29.1) | 16 (28.1) |
| Site of infection, n (%) | | | | |
| Lung | - | 32 (82.1) | 31 (56.3) | 33 (57.8) |
| Urinary tract | - | - | 5 (9.1) | 3 (5.3) |
| Abdominal | - | 7 (17.9) | 14 (25.5) | 11 (19.3) |
| Skin | - | - | 2 (3.6) | 4 (7.0) |
| Heart | - | - | - | 1 (1.8) |
| Blood | - | - | 3(5.5) | 3 (5.3) |
| Other | - | - | - | 2 (3.5) |
| Laboratory measurements | | | | |
| White blood cells ($10^9$/l) | - | 16.9±2.5 | 16.3±3.4 | 14.7±3.1 |
| Platelets ($10^9$/l) | - | 305±20 | 236±27 | 191±14 |
| Bilirubin ($\mu$mol/l) | - | 35±5.7 | 50±7.9 | 62±13.6 |
| Creatinine (mg/dl) | - | 1.2±0.2 | 1.4±0.3 | 2.3±0.5 |
| C-reactive protein (mg/l) | - | 163±32 | 181±23 | 196±15 |
| Procalcitonin (ng/ml) | - | 4.7±2.1 | 7.2±1.9 | 19.7±3.1 |
| Interleukin 6, (pg/ml) | - | 179±69 | 1,296±723 | 21,234±12,705 |
| Positive blood cultures, n (%) | - | 5 (12.8) | 11 (20.0) | 29 (50.9) |
| EICU parameters | | | | |
| EICU (days) | - | 8±3 | 11±3 | 19±6 |
| Ventilation (days) | - | 4±2 | 7±3 | 11±4 |
| Catecholamine (days) | - | 0±0 | 2±2 | 6±2 |
| Renal replacement therapy (days) | - | 0±0 | 1±0.4 | 3±1 |
| APACHE II score | - | 19±4 | 22±3 | 27±1 |
| SOFA score | - | 6.3±1.9 | 6.4±2.1 | 12.1±1.9 |
| All-cause mortality, n (%) | | | | |
| EICU | | | | |
| Succumbed to mortality | 0 (0) | 2 (5.1) | 5 (9.1) | 11 (19.3) |
| Alive | 30 (100) | 37 (94.2) | 50 (90.9) | 46 (80.7) |
| Ovarall | | | | |
| Succumbed to mortality | 0 (0) | 4 (10.3) | 8 (14.5) | 17 (29.8) |
| Alive | 30 (100) | 35 (89.7) | 47 (85.5) | 40 (70.1) |

The laboratory values, EICU parameters, APACHE II and SOFA scores are presented as the mean ± standard error of the mean. APACHE II, Acute Physiology and Chronic Health Evaluation II; SOFA, Sequential Organ Failure Assessment; EICU, Emergency Intensive Care Unit.

>20 breaths/min; iii) heart rate >90 beats/min; iv) white blood cell count <4,000/$mm^3$ or >12,000/$mm^3$. When a patient with sepsis suffered from dysfunction of at least one organ within 24 h h following admission, severe sepsis was diagnosed. In the present study, patients with sepsis, but not severe sepsis, were designated as the sepsis group. Septic shock was defined as the beginning of vasopressor therapy by persisting hypotension despite fluid resuscitation and requiring vasopressor therapy, an identifiable site of infection or evidence of a systemic inflammatory response manifested by at least two of the following criteria: i) temperature <36˚C or >38˚C; ii) respiratory rate >20 breaths/min; iii) heart rate >90 beats/min; iv) white blood cell count <4,000/$mm^3$ or >12,000/$mm^3$. The severity and the development of organ dysfunction were assessed using the Sequential Organ Failure Assessment (SOFA) score (30) (range 0-24) measured 24 h into the EICU admission. According to the guidelines of the Surviving Sepsis Campaign (31), patients with septic shock were immediately treated with empiric broad-spectrum antibiotherapy following admission. Several characteristics were determined on the first day of admission, including disease severity, underlying comorbidities, demographic characteristics, the presence of concomitant organ

dysfunction and the Acute Physiology and Chronic Health Evaluation II (APACHE II) score (32). The SOFA score was also used to determine the severity of multi-organ dysfunction.

*Determination of serum concentrations of Lp-PLA2 using ELISA*. The plasma concentrations of Lp-PLA2 (ng/ml) were measured using an ELISA kit (second-generation PLAC test; diaDexus, Inc., South San Francisco, CA, USA). All samples were analyzed in duplicate, and the analytical coefficient of variation was 6.3%.

*Patient follow-up*. The patients were included in the survival model until they succumbed to mortality, there was censoring due to loss of follow up, or until the end of the follow-up period (90 days). If clinically indicated, more frequent examinations were scheduled. The mortality rates of patients with sepsis were measured as overall survival rates. The overall survival rate was defined as either the proportion of patients with sepsis remaining alive at a certain time point following their sepsis episode or the occurrence of non-sepsis-associated mortality, ensuring that the overall survival rate did not measure excess sepsis-associated mortality. Indication that a patient had succumbed to mortality was ascertained by reports from family and telephone conversations, and verified by a review of public records. The Kaplan-Meier method was used to calculate the overall survival analysis.

*Statistical analysis*. All statistical analyses were performed with SPSS 19.0 statistical software (IBM SPSS, Armonk, NY, USA) as previously described. The t-test was used for data comparison between two groups and one-way analysis of variance followed by Tukey's post hoc test was used for data comparisons among three groups. Kaplan-Meier curves and log-rank test calculations were performed to determine the effect on survival rates. The Kaplan-Meier method was used to compute survival analyses and the log-rank test was used to assess significant levels. All data were included for statistical analyses. The results are expressed as the mean ± standard error of the mean of at least three independent experiments. Spearman's correlation tests were used to analyze the correlations between variables. P<0.05 was considered to indicate a statistically significant difference.

## Results

*Study population*. The clinical characteristics of the patients with sepsis, severe sepsis and septic shock, and healthy controls are presented in Table I. In total, 151 patients were included in the present study: 25.8% of the patients (n=39) suffered from sepsis, 36.5% of the patients (n=55) suffered from severe sepsis and 37.7% of the patients (n=57) suffered from septic shock at the start of the investigation, with an EICU-associated mortality rate of 11.9% and an overall mortality rate of 19.2%. The primary sources of infection were the lung (96 patients; 63.5%; pneumonia), the abdomen (32 patients; 21.2%; peritonitis), the urinary tract (eight patients; 5.3%; UTI), the skin (six patients; 4.0%; dermatophyte infection), the heart (one patient; 0.7%; infectious endocarditis), blood (six patients; 4.0%; leukemia and aplastic anemia) and others (two patients; 1.3%; neutropenia).

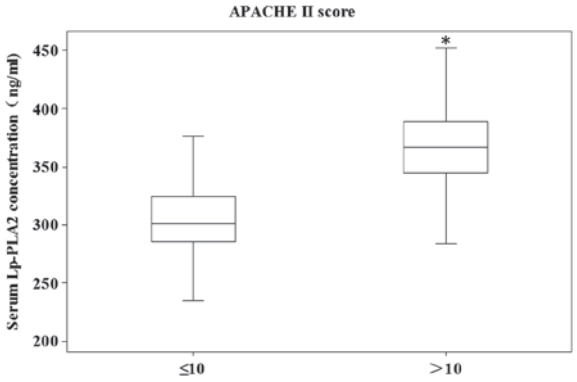


Figure 1. Serum concentrations of Lp-PLA2 in patients with sepsis and healthy controls on EICU admission. Serum concentrations of Lp-PLA2 on admission to the EICU were significantly higher in patients with sepsis, compared with healthy controls (*P<0.001). Lp-PLA2, lipoprotein-associated phospholipase A2; EICU, Emergency Intensive Care Unit.

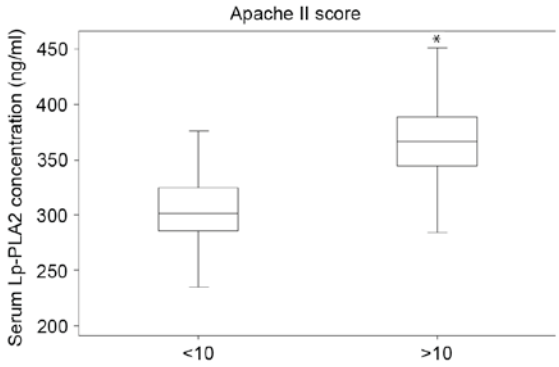


Figure 2. Serum levels of Lp-PLA2 on admission to the EICU. Serum levels of Lp-PLA2 on admission to the EICU were significantly elevated in patients with sepsis exhibiting high initial APACHE II scores (>10), compared with those exhibiting low APACHE II scores (≤10). (*P<0.001). Lp-PLA2, lipoprotein-associated phospholipase A2; EICU, Emergency Intensive Care Unit; APACHE, Acute Physiology and Chronic Health Evaluation.

*Serum concentrations of Lp-PLA2 are elevated in patients with sepsis*. The serum samples from the patients were analyzed on admission to the EICU, prior to specific therapeutic intervention. As shown in Fig. 1, patients had significantly higher serum concentrations of Lp-PLA2 on EICU admission (338 ng/ml, range 206-452 ng/ml), compared with healthy controls (123.9 ng/ml; range 12-202 ng/ml; P<0.001).

*Association of serum concentrations of Lp-PLA2 with disease severity in patients with sepsis*. High serum levels of Lp-PLA2 were associated with the severity of disease; patients with high APACHE II scores (>10) exhibited a further increase in Lp-PLA2 levels (median 360.8 ng/ml; range 264-452 ng/ml), compared with patients with low APACHE II scores (≤10; median 305.8 ng/ml; range 206-390 ng/ml; P=0.005), as shown in Fig. 2. These data indicated that elevated levels of Lp-PLA2 were primarily associated with disease severity in patients with sepsis.

All patients with sepsis were divided into three groups: Sepsis (median 297 ng/ml; range 206-388 ng/ml), severe sepsis (median 336.6 ng/ml; range 247-410 ng/ml) and septic shock (median 366.3 ng/ml; range 258-452 ng/ml). The

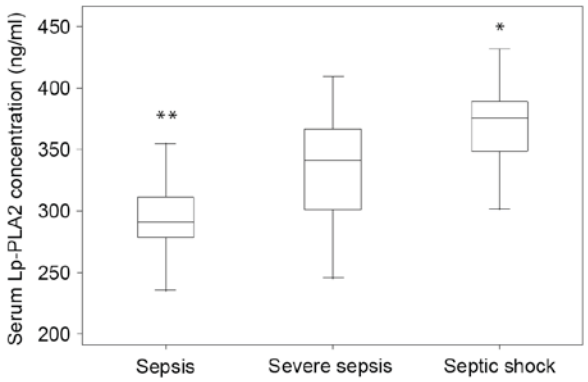


Figure 3. Serum levels of Lp-PLA2 in sepsis/severe sepsis/septic shock. Serum levels of Lp-PLA2 in septic shock were significantly higher, compared with those in severe sepsis (*P<0.05), whereas levels in severe sepsis were significantly higher, compared with those in sepsis (**P<0.001). Lp-PLA2, lipoprotein-associated phospholipase A2.

Table II. Correlations between serum concentrations of Lp-PLA2 and other laboratory markers in patients with sepsis.

| | Lp-PLA2, vs. marker/score on admission | |
|---|---|---|
| Marker/score | r | P-value |
| C-reactive protein | 0.37 | 0.006 |
| Procalcitonin | 0.55 | <0.0001 |
| Interleukin 6 | 0.39 | 0.001 |
| APACHE II | 0.27 | 0.004 |
| SOFA | 0.23 | 0.007 |

Values were measured on the day of admission to the Emergency Intensive Care Unit. Lp-PLA2, lipoprotein-associated phospholipase A2; APACHE II, Acute Physiology and Chronic Health Evaluation II; SOFA, Sequential Organ Failure Assessment.

serum concentration of Lp-PLA2 in the septic shock group was significantly higher, compared with that in the severe sepsis group (P<0.05; Fig. 3), and the serum concentration of Lp-PLA2 in the severe sepsis group was significantly higher, compared with that in the sepsis group (P<0.001; Fig. 3).

*Correlation between serum levels of Lp-PLA2 in sepsis and other laboratory markers*. To determine which factors may have promoted the elevated concentrations of Lp-PLA2 in patients with sepsis, correlation analyses were performed with extensive sets of laboratory parameters, using Spearman's rank correlation test. As shown in Table II, the serum concentrations of Lp-PLA2 were correlated with markers of systemic inflammation in patients with sepsis, including CRP (P=0.006), PCT (P<0.0001) and IL-6 (P=0.001). Compared with CRP and IL-6, PCT was more relevant to serum concentrations of Lp-PLA2 (r=0.55), and higher than CRP (r=0.37) and IL-6 (r=0.39). Consequently, an association was identified between serum concentrations of Lp-PLA2 on EICU admission with established clinical scores, including APACHE II (r=0.27; P=0.004) and SOFA (r=0.23; P=0.007) scores (Table II).

*Correlation of serum levels of Lp-PLA2 with EICU and overall mortality rates*. Based on the associations between serum concentrations of Lp-PLA2, inflammatory markers and prognostic clinical scores, the present study hypothesized that Lp-PLA2 measurements can predict the risk of mortality in patients with sepsis. Therefore, the concentrations of Lp-PLA2 on admission in patients that succumbed to mortality during EICU treatment were compared with those of survivors at overall follow-up. In the cohort of patients with sepsis, 11.9% (18 cases) succumbed to mortality in the EICU, whereas the overall mortality rate increased to 19.2% (29 cases) during the follow-up period. During the time spent in EICU, those who succumbed to mortality exhibited a further increase in levels of Lp-PLA2 (median 391.6 ng/ml; range 305-452 ng/ml), compared with the survivors (median 330.2 ng/ml; range 206-401 ng/ml; P=0.002) as shown in Fig. 4. During the overall follow-up, the non-survivors had higher levels of Lp-PLA2 (median 376.5 ng/ml; range 293-452 ng/ml), compared with the survivors (median 328 ng/ml; range 206-401 ng/ml; P=0.03; Fig. 5), which showed that a high level of Lp-PLA2 was a prognostic predictor for mortality rates.

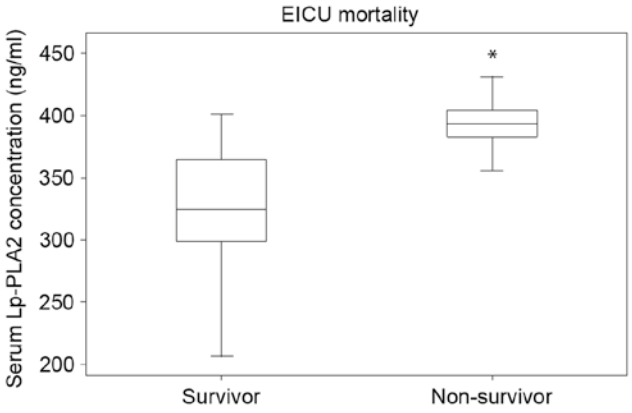


Figure 4. Elevated serum levels of Lp-PLA2 correlate with EICU mortality rates. Serum levels of Lp-PLA2 in patients discharged from the EICU (n=135) were compared with those in patients who succumbed to mortality during EICU admission (n=16). Data are presented as geometric means with 95% confidence intervals (*P<0.001). Lp-PLA2, lipoprotein-associated phospholipase A2; EICU, Emergency Intensive Care Unit.

*Serum concentrations of Lp-PLA2 are associated with survival rates of patients with sepsis*. To further substantiate the results in terms of the potential prognostic value of Lp-PLA2 measurements, Kaplan-Meier survival curves analysis was performed. The prognostic role of Lp-PLA2 on the overall survival rate of patients with sepsis was investigated by comparing the 90-day-survival rate of patients with high or low serum concentrations of Lp-PLA2 in sepsis using Kaplan-Meier survival curves and the log-rank test. Using the quartile limits of serum expression of Lp-PLA2 to divide patient the population into low and high levels allowed the interquartile range to be set as a cut-off, and a significant correlation between the serum expression of Lp-PLA2 and survival rates was established. The median serum expression of Lp-PLA2 was 346 ng/ml, dividing the samples into two groups: Low concentration (≤346 ng/ml) and high

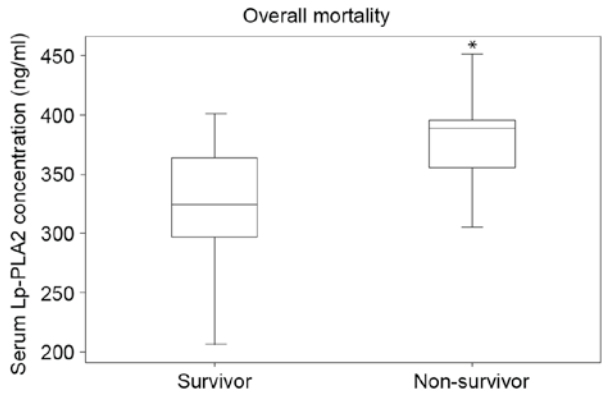


Figure 5. Elevated serum levels of Lp-PLA2 correlate with overall mortality rates. Serum levels of Lp-PLA2 in patients discharged from the EICU (n=125) were compared with patients who succumbed to mortality during EICU admission (n=26). Data are presented as geometric means with 95% confidence intervals (*P<0.001). Lp-PLA2, lipoprotein-associated phospholipase A2; EICU, Emergency Intensive Care Unit.

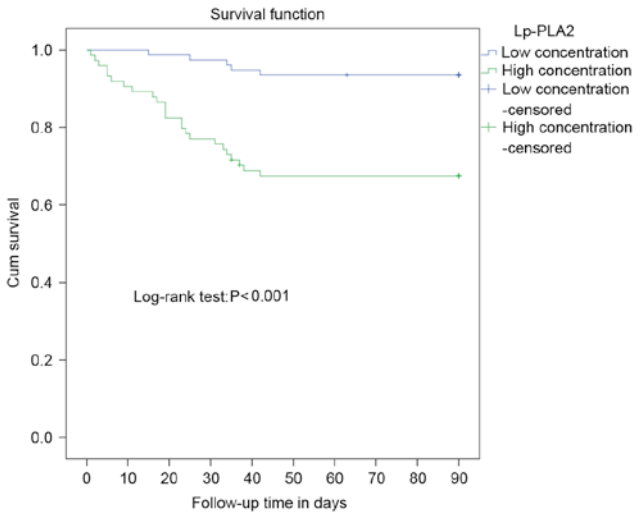


Figure 6. Overall mortality rates are correlated with levels of Lp-PLA2. Kaplan-Meier survival curves of patients in the EICU showed that patients with high concentrations of Lp-PLA2 (>346 ng/ml) had increased overall mortality rates, compared with patients with low concentrations of Lp-PLA2 (≤346 ng/ml). P-values from the log rank test are shown. P<0.001. Lp-PLA2, lipoprotein-associated phospholipase A2; EICU, Emergency Intensive Care Unit.

concentration (>346 ng/ml). There were 74 cases in the high concentration group, 24 of which succumbed to mortality and two cases were lost to follow-up, and the 90-day-overall survival rate was 64.8%. In the low concentration group, there were 77 cases, five of which succumbed to mortality and one was lost during follow-up. The 90-day-overall survival rate for the negative group was 92.2%. The overall survival rate of the low concentration group was significantly higher, compared with that of the high concentration group (P<0.001; Fig. 6).

Thus, the data obtained indicated that measuring the levels of Lp-PLA2 in a medical EICU environment may be valuable for evaluating the short-term and long-term prognoses of a patient with sepsis.

## Discussion

The present study focused on the expression of serum Lp-PLA2 in sepsis. The concentrations of Lp-PLA2 were examined on admission to the EICU, prior to specific therapeutic interventions, in a well-characterized cohort of patients with sepsis. The primary finding of the present study was that Lp-PLA2 provided as a marker of inflammation and severity of illness, which correlated with long-term prognosis (up to 90 days) following the episode of sepsis. From the serum measurements, there was a significant difference in the concentration of Lp-PLA2 between the patients with sepsis and the healthy controls. Subsequently, the association between serum concentrations of Lp-PLA2 and the severity of disease was determined. As expected, serum levels of Lp-PLA2 on admission to EICU were significantly elevated in patients with sepsis with high initial APACHE II scores, compared with those with low APACHE II scores. Therefore, the serum concentrations of Lp-PLA2 were positively correlated with the severity of disease. The present study then analyzed the associations between serum concentrations of Lp-PLA2, inflammatory markers and prognostic clinical scores, which showed that serum concentrations of Lp-PLA2 were associated with inflammatory markers and prognostic clinical scores. Therefore, the present study compared concentrations of Lp-PLA2 on admission in patients who succumbed to mortality during EICU treatment and in survivors at overall follow-up. The results showed that Lp-PLA2 measurements predicted the mortality rates in patients with sepsis. In addition, the results of Kaplan-Meier survival curves and the log-rank test showed that the overall survival rate of the low concentration group was significantly higher compared with that of the high concentration group. In these patients, serum concentrations of Lp-PLA2 were found to have a close association with the severity of disease, mortality rates and prognosis.

Sepsis is one of the most common contributors to mortality rates worldwide. Unfortunately, the prognosis of sepsis has improved only gradually despite advances in intensive care medicine. Evidence that PLA2 and inflammation are tightly coupled has accumulated in previous years (23). In previous reports, all six types of PLA2 have been discussed in relation to subgroups, terms of groups, mechanism of action, structure and interaction with membranes, role in disease, the various forms, biological activities and development in selective inhibitors (15-21). The secreted enzymes may occur in various intracellular vesicles. The PAF-AH and certain cPLA2 enzymes are $Ca^{2+}$-independent (33). As reported in a previous study, Lp-PLA2, which is upregulated by oxidized phospholipids in oxidized LDL, acts on oxidized phospholipids to promote it to produce the two pro-inflammatory mediators, lysophosphatidylcholines and oxidized non-esterified fatty acids (34). In a previous study of acute pancreatitis, important pathophysiological roles of PLA2 have been shown; the group II A secretory phospholipase A2 (PLA2-II) is considered to be important in cell injury and inflammation (35). PLA2-II appears to be the major enzyme in acute pancreatitis responsible for the systemic inflammatory process (36). A previous study also showed that the expression of PLA2-II was increased in the pancreas, induced by 4% sodium taurocholate injected into the pancreatic duct, whereas knockdown of the PLA2-II gene

mediated by small interfering (si)RNA relieved the severity of pancreatitis (35). This suggests that PLA2-II is important in inflammation. PLA2 also correlates with the appearance of SIRS in severe acute pancreatitis (37). In addition to these findings, the results of the present study, showed that Lp-PLA2 was associated with the severity, and the mortality and survival rates of sepsis. Of note, PCT exhibited higher correlation with the serum concentrations of Lp-PLA2, compared with CRP and IL-6, however the underlying mechanism remains to be elucidated. It was hypothesized that this may be caused by calcium ions, as mentioned above, as certain forms of PLA2 have been recognized as cytosolic $Ca^{2+}$-independent. Procalcitonin, a precursor of calcitonin manufactured in the thyroid, is significantly upregulated in several types of bacterial infection (38,39). Future investigations aim to focus on the underlying mechanism of this. Future investigations also aim to focus on the role of the downregulated expression of Lp-PLA2, including siRNA-mediated gene knockdown.

Although the present study obtained valuable results regarding Lp-PLA2 in sepsis, there were several limitations. The study did not record all causes of mortality, which makes it difficult to adequately establish a link between inflammation and the cause of mortality. In addition, no data were collected on terminal disease status, which may have an effect on results. In future investigations of Lp-PLA2, the potential prognostic value of Lp-PLA2 measurements requires substantiation using multivariate Cox regression analysis, with the markers of infection/inflammation, includingCRP, white blood cell count, renal (creatinine) and hepatic (bilirubin, INR) function, to determine whether Lp-PLA2 is an independent significant prognostic parameter to predict ICU survival rates.

In conclusion, the data obtained in the present study suggested a possible role for Lp-PLA2 as a prognostic marker in sepsis, and provided background evidence for larger trials to evaluate the clinical and pathophysiologic role of Lp-PLA2 in sepsis, compared with other markers of inflammation and hypoxia. Persistently elevated serum concentrations of Lp-PLA2 were associated with an unfavorable outcome in patients with sepsis. In addition to a possible pathogenic role of Lp-PLA2 in sepsis, the present study indicated the potential value for Lp-PLA2 as a prognostic biomarker in patients with sepsis during the early course of EICU treatment.

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