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Serum microtubule-associated protein light chain 3 type II levels correlate with aggravation and multi-organ dysfunction in septic patients

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Sepsis involves the early activation of both pro- and anti-inflammatory responses, but is also accompanied by major changes in nonimmunologic pathways.[1] Autophagy is the lysosome-dependent process of removing damaged proteins and organelles.[2] Accumulating evidence from *in vitro* and *in vivo* studies reveals that autophagy may have a protective effect on sepsis, and the late-stage suppression of autophagy is associated with a poor outcome.[3] Thus, reversal of late-stage autophagy may be a good therapeutic strategy for sepsis. Accordingly, the identification of biomarker, which could inform the clinical diagnosis of autophagic impairment, will be essential for guiding the management of septic patients. Microtubule-associated protein light chain 3 type II (LC3β) is a key protein for autophagy.[2] A previous study demonstrated that patients with sepsis-induced myocardial dysfunction (SIMD) had significantly lower serum levels of LC3β than patients without SIMD,[4] suggesting the potential use of this molecule as a pathophysiological biomarker in the course of sepsis. In light of these prior findings, the present study aimed to investigate the potential role of LC3β protein as an autophagy-related biomarker in a cohort of septic patients stratified according to disease progression. Moreover, in the same patient sample, serum levels of interleukin 1 β (IL-1β) and interleukin 18 (IL-18) were measured as indicators of proinflammatory cytokines.

This was a prospective cohort study conducted from June 2019 to August 2020 at Peking Union Medical College Hospital (PUMCH). Patients were enrolled within 24 h of intensive care unit (ICU) admission. All consecutive patients with a diagnosis of sepsis were assessed for possible inclusion. Diagnosis of sepsis/septic shock was performed according to the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis 3.0).[1] The exclusion criteria were as follows: age < 18 years, massive bleeding or pulmonary embolism, heart attack or acute exacerbation of previous heart disease in the previous week, heart surgery in the previous week, or a lack of informed consent from the patient or their family/guardian.

Patients were divided into three groups, including the infection group ($n = 29$), septic nonshock group ($n = 40$), and septic shock group ($n = 94$). The infection group comprised of patients admitted to the adult ICU with an active or suspected infection. The sepsis group comprised of patients who showed an acute change in total sequential organ failure assessment (SOFA) score of ≥ 2 points consequent to the infection. Inclusion in the septic shock group required receipt of vasopressors, as well as a lactate level of >2 mmol/L on the day of ICU admission. Septic patients who did not meet these criteria were then assigned to the septic nonshock group. The study was approved by the PUMCH institutional review board (approval number JS-2421). Written informed consent was obtained from all patients or their family/guardians prior to inclusion in this study.

Laboratory tests and clinical history evaluations were performed for all consecutive patients after obtaining informed consent. Clinical data were obtained from medical records or anamnesis with the patient, whenever possible, or their relatives by the research team trained in the study protocol. Acute Physiology, Age, Chronic Health Evaluation (APACHE) II, and SOFA scores were obtained from medical records or routine ICU exams. The main outcome measure was mortality or discharge from the ICU.
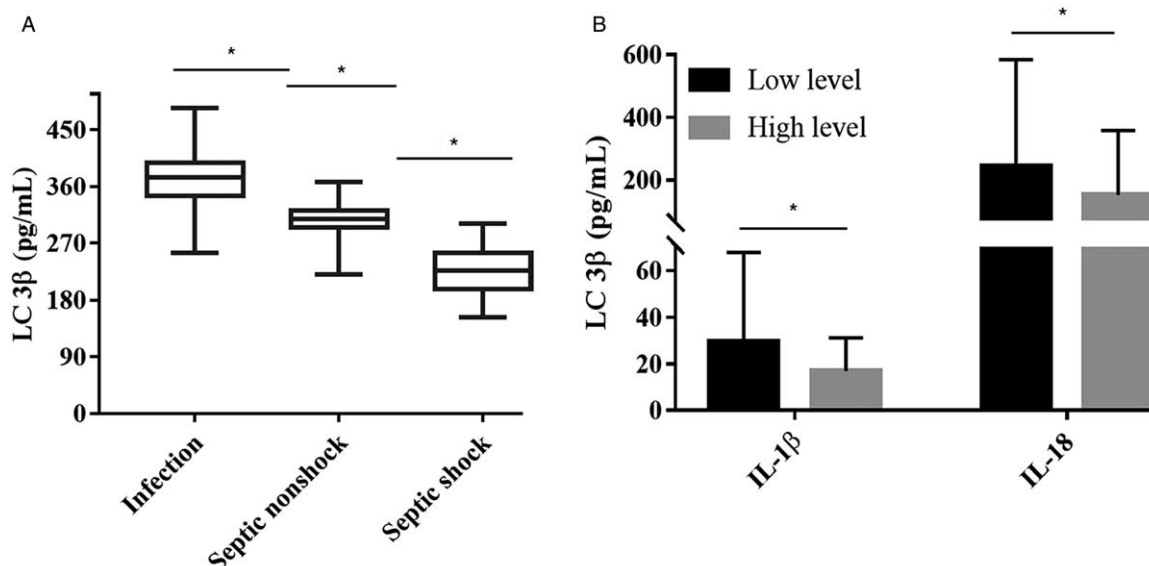
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Figure 1: Changes in serum LC3β levels in different cohorts of patients. (A) Comparative analysis of serum LC3β levels across an infection, septic nonshock and septic shock groups. (B) Levels of IL-1β and IL-18 in the low (<263.92 pg/mL) and high (≥263.92 pg/mL) level LC3β groups. * indicates $P < 0.001$ between two groups. IL-1β: Interleukin 1β; IL-18: Interleukin 18; LC3β: Light chain 3 type II. Variables are present as the mean and standard deviation or median and interquartile interval.

Peripheral blood samples were obtained within 24 h after admission. Serum was separated by centrifugation at 1000 g for 10 min and then stored at −80°C until further use. Serum levels of LC3β, IL-1β, and IL-18 were determined by enzyme-linked immunosorbent assay (ELISA), according to the manufacturer's instructions, using the Human LC 3β ELISA kit (ELISAGenie, Catalog No. SKU: HUFI01901, Dublin, Ireland), Human IL-1β ELISA kit (MULTI SCIENCES, Catalog No. EK101B-01, Hangzhou, China), and Human IL-18 ELISA kit (MULTI SCIENCES, Catalog No. EK118–01, Hangzhou, China).

The normality of the data was evaluated using the Kolmogorov–Smirnov test. Variables with normal distributions are reported as the mean and standard deviation, whereas the non-parametric data were reported as the median and interquartile interval. Differences between groups were evaluated using a one-way analysis of variance (ANOVA) or the Kruskal–Wallis test. $P < 0.050$ was considered to be statistically significant. All statistical analyses were performed using SPSS 22.0 software (IBM Inc., Chicago, USA).

Thus, the final sample comprised 163 patients. The three groups were as follows: (1) infection group ($n = 29$); (2) septic nonshock group ($n = 40$); and (3) septic shock group ($n = 94$). The septic shock group had a significantly higher SOFA score, higher norepinephrine dosage, greater inotrope usage, and higher lactic acid (Lac) and procalcitonin (PCT) levels compared with the septic nonshock group (all $P < 0.050$). The septic shock group also had significantly higher 30-day mortality compared with the septic nonshock group ($P < 0.050$). There were no significant differences in age, sex, APACHE II score, mechanical ventilation usage time, continuous renal replacement therapy (CRRT) usage, corticoid usage, heart rate (HR), mean arterial blood pressure (MAP), or ICU stay between the septic nonshock and septic shock groups.

Serum levels of LC3β differed significantly between the three groups ($P < 0.001$; Figure 1A). *Post-hoc* tests showed that the LC3β level was dramatically increased in the infection group. Among septic patients, the LC3β level progressively decreased in the septic nonshock and septic shock groups. Among patients with sepsis, the level of circulating LC3β at ICU admission was lower in non-survivors than that in survivors, although this difference was not statistically significant (non-survivors: median and interquartile interval; 227.74, 201.60–270.90 *vs.* survivors: 253.83, 212.40–298.13, $P = 0.069$). To investigate the relationship between autophagy and proinflammatory cytokines, we also measured serum IL-1β and IL-18 levels. We used the median LC3β level (263.92 pg/mL) to divide the ICU patients into two groups: a low-level group (LC3β < 263.92 pg/mL) and a high-level group (LC3β ≥ 263.92 pg/mL). Additionally, a higher serum level of LC3β was associated with significantly lower levels of IL-1β and IL-18 [Figure 1B]. Moreover, there was no significant difference observed with regard to the identified pathogens (gram-negative *vs.* gram-positive) (median and interquartile interval; 253.83, 219.99–300.80 *vs.* 297.32, 240.24–392.12 pg/mL, $P = 0.055$).

To determine whether serum LC3β levels reflect organ function, disease severity, and degree of infection, we explored its correlations with SOFA score, APACHE II score, and PCT. Serum LC3β levels were significantly correlated with SOFA score ($r = -0.609$, $P < 0.001$), APACHE II score ($r = -0.260$, $P < 0.010$), and PCT ($r = -0.311$, $P < 0.001$).

In this study, we aimed to investigate the possibility of using serum LC3β levels as an autophagy-related biomarker to evaluate autophagic activity during different stages of sepsis. Further, we divided patients into three groups to represent different stages of disease development. The infection group, septic nonshock group, and

septic shock group represented poor progression after infection. We found that, in the initial stage of infection, LC3β levels peaked and then declined as the disease became further aggravated. Autophagy is an innate immune response to intracellular pathogens during sepsis, as it can help eliminate pathogens.[5] A study explored the kinetics of autophagy in sepsis. They established a septic mouse model using cecal ligation and puncture (CLP).[6] In the liver, autophagosome formation peaked at 6 h and declined by 24 h, and the same tendency was observed in the heart and spleen.[6] Accumulating studies showed increased autophagy during the early phase of sepsis, but a decrease during the late phase. This proves that activation of autophagy is beneficial. As described above, the trend in serum LC3β levels that we observed in infected patients to septic shock patients is in accordance with changes in autophagic activity during sepsis. Of note, late-stage suppression of autophagy shows a detrimental effect in septic animal models, indicating that late-stage autophagy may be a decisive factor in predicting the outcome.[6] A multicenter prospective cohort study also revealed that polymorphisms in the autophagy-related gene are associated with excess mortality in sepsis.[7] Both experimental and clinical investigations support that autophagy has a protective role in sepsis. Furthermore, survivors tended to show higher serum LC3β levels compared with non-survivors. However, this difference was not significant, which maybe because of the relatively small sample size. In summary, our findings offer further evidence that, during the beginning phase of sepsis or mild sepsis, autophagy is adaptively augmented, which can sustain normal organ function. However, as sepsis progresses to a severe condition, autophagic responses become decompensated, leading to poor outcomes.

Autophagy can also provide control over inflammation by removing inflammasome activators and/or components and by reducing mitochondrial danger-associated molecular patterns (DAMPs).[8] In our study, we observed that a higher serum LC3β level was associated with significantly lower levels of IL-1β and IL-18, which is consistent with autophagy control of over-release of cytokines. Given that autophagy plays an important role in sepsis-induced pathology of organ dysfunction, it is notable that serum LC3β showed a significant negative correlation with SOFA score, indicating the ability of serum LC3β level to reflect organ function.

This study is subject to several limitations. First, this study was conducted at a single center with relatively small sample size. As a result, there may be bias in patient selection. Second, the use of LC3β to monitor the progression of sepsis or response to treatment interven-tions requires further investigation. Third, a larger, multi-center study is needed to adjust the confounders of LC3β.

Our results have shed new light on the role of autophagy-related biomarkers in the management of septic patients. We found that serum LC3β level accurately reflects aggravation of sepsis and is closely correlated with organ function. Further study is needed to elucidate the relationship of LC3β to sepsis.

Funding

Conflicts of interest

None.

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