# Circulating mitochondrial DNA levels are associated with early diagnosis and prognosis in patients with sepsis

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*To the Editor:* Both pathogen-associated molecular patterns and damage-associated molecular patterns (DAMPs) are known to enhance levels of inflammation and tissue injury during sepsis. Mitochondria are a major source of DAMPs because they release mitochondrial (mt) DNA fragments during sepsis. Similar to bacterial DNA, circulating mtDNA can activate signaling pathways and promulgate inflammation. Previously, Puskarich et al[1] found no significant elevation in the levels of plasma mtDNA in patients presenting to the emergency department with sepsis and septic shock. However, Schäfer et al[2] found that serum mtDNA significantly increased in septic patients compared with controls. Moreover, our previous study showed that circulating mtDNA levels within 24 h after admission were significantly increased in the group of septic patients with acute lung injury.[3] Thus, it remains unclear whether changes in mtDNA during sepsis are of clinical relevance. This study aimed to determine whether circulating mtDNA within 24 h of intensive care unit (ICU) admission can be used as a marker for the early diagnosis and prognosis of sepsis.

The study was carried out according to the principles of the *Declaration of Helsinki* and was approved by the Ethics Committee of the Peking Union Medical College Hospital (No. JS-3283). Informed consent was obtained from all participants or their relatives before inclusion in the study. The study was performed at the Peking Union Medical College Hospital between September 1, 2020 and October 31, 2021. Patients were enrolled within 24 h of entry into the ICU. All consecutive patients admitted with sepsis diagnosis in the ICU were assessed for possible inclusion.[4] Exclusion criteria were: age <18 years, ICU stay <24 h, pulmonary embolism, having experienced a heart attack or acute exacerbation of previous heart disease during the last week, and undergoing heart surgery during the last week. Additionally, non-infected patients after surgery within 24 h were enrolled as the control group over the same time period. Blood samples were obtained within 24 h after ICU admission. Serum was separated by centrifugation at 3000 × *g* for 10 min and stored immediately at –80°C until assay.

Serum mtDNA was extracted using a QIAamp DNA Blood Mini Kit (Qiagen, Catalog No. 51185, Beijing, China), quantified using polymerase chain reaction for the mitochondrial *ND1* gene, and measured in triplicate as reported previously.[3] The primers for the mt*ND1* gene were forward 5′-ACACTAGCAGAGACCAACCG-3′ and reverse 5′-GAAGAATAGGGCGAA GGGGC-3′; and β-globin (forward, 5′-GTG CACCTG ACT CCT GAG GAG A-3′; reverse, 5′-CCT TGA TAC CAA CCT GCC CAG-3′) was used as the control. The fold-change was calculated by the $2^{-\Delta\Delta Cq}$ method compared with standards in the kit (TaKaRa, Dalian, Liaoning, China) and showed using log-transformed copy number/μl to ensure normality.

Statistical analysis was performed using SPSS 23.0 (IBM, Armonk, NY, USA) and GraphPad Prism 8.0.2 (GraphPad Software Inc., San Diego, CA, USA). The normality of the data was evaluated using the Kolmogorov-Smirnov test. Variables with normal distribution are reported as mean and standard deviation, whereas the non-normal distribution data were reported as median ($Q_1$, $Q_3$). Differences between groups were evaluated using a one-way analysis of variance or the Kruskal-Wallis *H* test. Receiver operating characteristic curves were used to analyze the diagnostic efficacy of various indicators. $P < 0.05$ was considered to be statistically significant.

In total, 99 patients were enrolled in our study and were divided into two groups: the control group ($n = 32$) and the study group ($n = 67$). The study group was further divided into the septic non-shock subgroup ($n = 40$) and

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the septic shock subgroup ($n = 27$) according to Sepsis-3 guidelines. Moreover, according to the survival status at 28 days post-admission, the study group was divided into survival ($n = 55$) and non-survival ($n = 12$) groups. There were no significant differences in age, sex, body mass index, or Acute Physiology, and Chronic Health Evaluation II (APACHE II) score between the control and the study groups ($P > 0.05$). However, the study group had a significantly higher Sequential Organ Failure Assessment score, lactate, and procalcitonin levels than the control group.

Circulating mtDNA concentrations within 24 h of ICU admission were significantly increased in the study group compared with the control group (213.85 [144.18, 357.17] ng/ml *vs*. 44.53 (35.61, 56.70) ng/ml, $Z = 8.01$, $P < 0.0001$). Further subgroup analysis shows that serum mtDNA levels in the septic shock group were remarkably elevated compared with those in the septic non-shock group (297.58 [207.48, 525.49] ng/ml *vs*. 191.06 [126.71, 245.77] ng/ml, $Z = 2.95$, $P = 0.003$). Moreover, serum mtDNA levels in the non-survival group were remarkably elevated compared with those in the survival group (533.29 [370.84, 773.30] ng/ml *vs*. 192.82 (137.24, 245.171) ng/ml, $Z = 4.68$, $P = 0.003$).

Receiver operating characteristic curve analysis was performed to evaluate the diagnostic and prognostic value of mtDNA. mtDNA was found to have a good diagnostic value for septic shock (AUC 0.714 [0.586, 0.842], $P = 0.0031$, cutoff value 222.4 ng/ml, sensitivity 70.37%, specificity 72.50%), as well as a good prognostic value for the 28-day mortality for sepsis (AUC 0.930 [0.871, 0.989], $P < 0.0001$, cutoff value 318.3 ng/ml, sensitivity 99.99%, specificity 85.45%). We have also performed Kaplan–Meier survival analysis by using the mtDNA cut-off (318.3 ng/ml) in patients with sepsis [Supplementary Figure 1, http://links.lww.com/CM9/B329]. Results indicate that patients with serum mtDNA level above the cut-off values presented a significant decrease (log-rank [Mantel-Cox], $\chi^2 = 32.0$, $P < 0.0001$) in the survival rate in patients with sepsis [Supplementary Figure 2, http://links.lww.com/CM9/B329].

mtDNA is released into the extracellular space during different types of cell death and tissue injury in sepsis. A previous study[5] studies showed that circulating mtDNA levels were closely associated with multiple organ failure in pediatric sepsis. The present study found that circulating mtDNA levels within 24 h of admission to the ICU were significantly higher in patients with sepsis compared with controls, and serum mtDNA levels in the septic shock group were also significantly higher compared with those in the septic non-shock group. This differs from the findings of Puskarich et al[1] described above, which could reflect the fact that the two studies showed variations in sepsis inclusion criteria. For example, the previous study used the 1992 Surviving Sepsis Campaign Guidelines[6] as the reference standard. Alternatively, our study may have measured mtDNA at different time points and under different treatment settings compared to the study of Puskarich et al.[1]

Moreover, multiple mitochondrial functional characteristics are known to influence organ function in many disease states, including sepsis, ICU-acquired skeletal muscle dysfunction, acute lung injury, acute renal failure, and critical illness-related immune function dysregulation. Because mitochondrial damage is closely associated with many critical diseases, it lacks specificity. Therefore, many factors can affect the circulating mtDNA content, but the main one is patient heterogeneity. Although inclusion and exclusion criteria were uniformly applied across our studies, the inclusion of patients with varying underlying diseases and different interventions could confound the results. Thus, in the present study, circulating mtDNA was shown to detect septic shock, resulting in only a sensitivity of 70.37% and a specificity of 72.50% because of the reasons outlined above. Nevertheless, our findings support the potential value of circulating mitochondrial damage markers in clinical applications.

Besides, our results indicated that serum mtDNA levels in the non-survival group were remarkably elevated compared with those in the survival group. Further subgroup analysis showed that mtDNA could predict the 28-day mortality in sepsis, with both a high sensitivity and specificity. Multiple organ failure is the main cause of death from sepsis, and mitochondrial dysfunction has been suggested to contribute to the development of organ dysfunction and failure in sepsis. mtDNA acts as an alarmin or DAMP when released from injured cells into the circulation with functionally important immune consequences. Emerging evidence has revealed that mtDNA fragments from damaged mitochondria are released into the circulation after traumatic injury, and contribute to the development of sepsis and multiple organ dysfunction syndromes. Previous studies also suggest that elevated mtDNA levels are associated with increased ICU mortality.[7]

Our study has a number of limitations. First, we enrolled only a small number of patients. Moreover, the single-center setting, heterogeneity of sepsis, and complexity of the interventions used may limit the generalizability of our findings. Therefore, caution should be taken when interpreting our results.

In conclusion, our study indicated that circulating mtDNA levels within 24 h after admission to the ICU have an early diagnostic value for septic shock, and are also a valuable marker to predict the outcome of patients with sepsis. However, there is a long way to go before large-scale clinical application, and large prospective multicentre studies are required to further elucidate the value of mtDNA.

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## Conflicts of interest

None.

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