Original Article

Prognostic value of plasma mitochondrial DNA in acute respiratory distress syndrome (ARDS): a single-center observational study

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Background: Mitochondrial DNA (mtDNA) has been reported to play a critical role in the progression of systemic inflammatory response syndrome (SIRS). The pathophysiology of acute respiratory distress syndrome (ARDS) is mainly attributed to the diffuse injury of alveolar epithelial cells caused by dysregulated inflammation upon direct or indirect insults. We hypothesized that plasma mtDNA may serve as an early biomarker that can predict the outcome of patients with ARDS.

Methods: This study was conducted in the Department of Critical Care Medicine, Zhongda Hospital, Southeast University, a tertiary teaching hospital, from 1 May 2016 to 31 January 2017. Patients diagnosed with ARDS at admission were screened. The levels of plasma mtDNA on Day 1, Day 3 and Day 7 were detected by real-time quantitative PCR (RT-qPCR). The patients were followed-up, and all-cause mortality was recorded. The prognostic values of plasma mtDNA were evaluated in ARDS patients using receiver operating characteristic (ROC) analysis.

Results: In total, 136 patients with ARDS were prospectively screened, and 73 patients were finally enrolled, with a 28-day mortality of 39.7% (29 of 73 patients). The plasma mtDNA levels at Day 7 of mild, moderate and severe ARDS patients were 1,230 (588–22,387), 5,370 (628–13,052) and 15,792 (1,623–186,814), respectively (copies/μL, $P<0.05$) Compared with the survivors, the level of plasma mtDNA in the nonsurvivors was significantly higher on Day 7 [67,608 (19,498–346,736) *vs.* 7,585 (1,717–15,792) copies/μL; $P<0.05$]. The AUROC of plasma mtDNA on Day 7 for predictive mortality in patients with ARDS was 0.74, and the optimal cut-off value was 18,640 copies/μL.

Conclusions: Plasma mtDNA levels were positively associated with the severity of ARDS. Higher plasma mtDNA levels on Day 7 indicated a poor outcome in ARDS patients.

Keywords: Mitochondrial DNA (mtDNA); acute respiratory distress syndrome (ARDS); 28-day mortality; prognosis

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Introduction

Acute respiratory distress syndrome (ARDS) remains a great challenge in the intensive care unit (ICU). Despite improvements in therapeutic management, including lung-protective mechanical ventilation strategies (1-3), prone positioning and extracorporeal membrane oxygenation (4), the mortality of ARDS remains approximately 40% (5). The fundamental pathophysiology of ARDS is the diffuse injury of alveolar epithelial cells in the form of necrosis or apoptosis (6-8) caused by dysregulated inflammation in response to either infectious pathogens or noninfectious

irritants (9).

Microbial pathogen-associated molecular patterns (PAMPs) activate immune cells through pattern recognition receptors. Then, the injured cells release endogenous damage-associated molecular patterns (DAMPs) that also activate the host immune system (10). DAMPs are reported to play a critical role in the development of SIRS. A large number of studies have suggested that mitochondria are also a major source of DAMPs (11) by the release of mitochondria-associated molecules when cells are under various stress conditions. Mitochondria-derived DAMPs mainly include mtROS, mtDNA fragments, N-formy1 peptides, ATP, cytochrome C, cardiolipin and carbamoyl phosphate synthetase. Recent studies have shown that mitochondrial DAMPs could induce SIRS and organ dysfunction. Moreover, markedly increased levels of circulating free mtDNA fragments have been observed in both sepsis and trauma conditions (12-16).

Similar to bacterial DNA, extracellular mtDNA can activate signaling pathways and promulgate inflammation (11). A prospective study of adult patients with severe sepsis and septic shock revealed that an increase in the concentration of plasma mtDNA by 1 ng/mL is associated with an increase in the fatality rate by 0.7% (17). Another study reported that plasma mtDNA is a novel DAMP in pediatric sepsis and appears to be associated with multiple organ failure (MOF) (18). In addition, Nakahira *et al.* demonstrated that the 28-d mortality was high in medical ICU patients when the plasma mtDNA level was ≥3,200 copies/μL (19). However, it is unknown whether plasma mtDNA is a novel biomarker for patients with ARDS. Consequently, the prognostic role of mtDNA in ARDS was investigated in this study.

Methods

The present study was a single-centered prospective observational study conducted in a 60-bed ICU in Zhongda Hospital, a tertiary hospital, from 1 May 2016 to 31 January 2017. The study was approved by the Ethics Committees for Clinical Research of Zhongda Hospital (No. 2016ZDSYLL033.0) and registered in ClinicalTrials.gov (NCT02883231). Informed consent was obtained from the patients or their next of kin.

Patients

Patients diagnosed with ARDS according to the Berlin definition from May 2016 through January 2017 were screened. Patients were excluded if at least one of the following non-inclusion exclusion criteria was met: age <18 years old or pregnancy; death or discharge within 24 hours after admission; advanced malignant tumor; immune deficiency, such as from neutropenia, long-term use of corticoids or diseases such as HIV infection; chronic lung disease, such as rheumatic autoimmune disease or exacerbation of chronic obstructive pulmonary disease, active tuberculosis, bronchiectasis, bronchial asthma, interstitial lung disease, etc.

Clinical assessment and treatment

The pathological information of the patients was collected on Days 1, 3 and 7, including demographic data, Acute Physiology and Chronic Health Evaluation (APACHE) II score (20), number of organ failures included in the Sequential Organ Failure Assessment (SOFA) score (21) and Murray score. The levels of lactate and inflammatory mediators (i.e., plasma C-reactive protein and procalcitonin) were detected on Days 1, 3 and 7. All patients were followed up for 28 days, and all-cause mortality was recorded. The durations of mechanical ventilation and ICU stay were also recorded. The primary outcome was mortality on Day 28. Secondary outcomes included the ventilator-free days and ICU length of stay.

Sample collection and plasma mtDNA measurements

Peripheral blood samples (2 mL) were collected with EDTA-containing tubes on Day 1, Day 3 and Day 7 after admission to the ICU and centrifuged for 30 min at 3,000 rpm. Then, the supernatant plasma was collected and stored at –80 ℃ for the following analyses.

Plasma mitochondrial DNA was isolated using a DNeasy Blood and Tissue Kit (#69504; Qiagen), as previously reported (12). The concentration of plasma DNA was evaluated by RT-qPCR. The mitochondrial genome was amplified with primers MT-ND2-45F 5'-CGCAATGGCATTCCTAA-3' and MT-ND2-199R 5'-TAGATGTGGCGGGTTTT-3' by StepOne (7500). The amplicon was detected using the primer sequences and verified in the GenBank database (accession number NC012920.1). β-actin (forward, 5'-CGGGAAATCGTGCGTGACAT-3'; reverse, 5'-GAAGGAAGGCTGGAAGAGTG-3') was used as the control. The PCR parameters were 94 ℃ for 3 min, followed by 40 cycles for 10 s, 60 ℃ for 10 s, and 72 ℃ for 20 s.

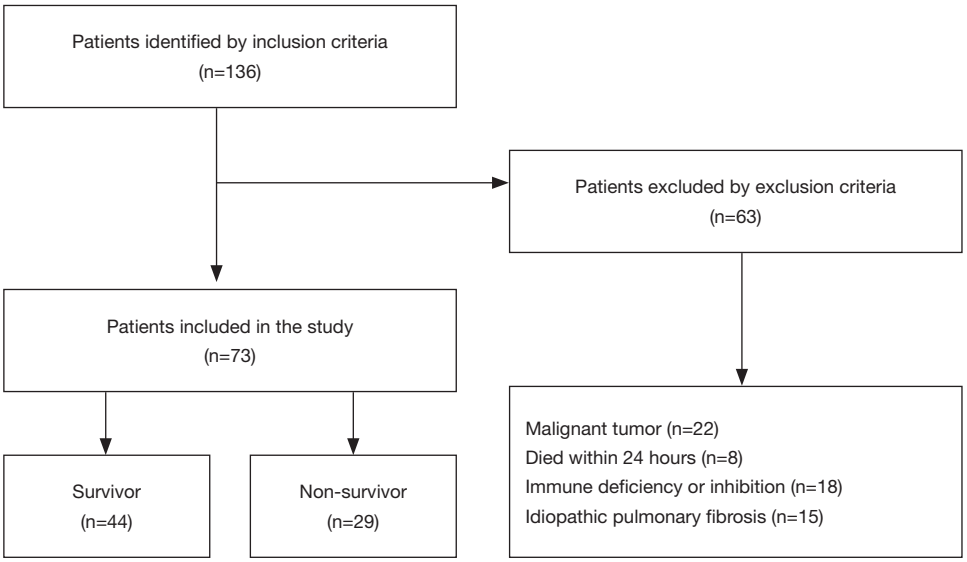

Figure 1 Selection of patients for inclusion in this analysis.

Statistical analysis

Continuous variables with normal distributions are shown as the means ± standard deviations (SDs) and were compared by *t* tests between two groups or one-way analysis of variance (ANOVA) for three or more groups; variables with skewed distribution are presented as the medians (interquartile ranges, IQRs) and were compared by Mann-Whitney *U* tests; *post hoc* tests were used for pairwise comparisons. The ROC curve was employed for estimating plasma mtDNA count in the prediction of 28-day mortality. The area under the curve (AUROC) was used to evaluate the predictive accuracy. The optimal cut-off values of different parameters for mortality was decided based on the maximum Youden index (sensitivity + specificity – 1). Comparison of the AUC between mtDNA and the PaO_2/FiO_2 ratio was adopted using the DeLong method. The Kaplan-Meier method was used to calculate the survival rate and generate the survival curve. Judgment of the prognostic value was determined with the log-rank test. A P value less than 0.05 was considered statistically significant. The statistical package IBM SPSS Statistics (ver. 23.0) was used for the computations and analysis.

Results

Baseline characteristics

A total of 136 patients who met the initial criteria were identified. After elimination for meeting the exclusion criteria, 73 ARDS patients were finally included. Among them, 29 died within the 28-day follow-up period (*Figure 1*), and 17 patients died or were discharged from the ICU before day 7. The baseline characteristics of the patient cohort are presented in *Table 1*. No significant differences were observed between survivors and non-survivors in age, gender, or the severity or etiology of ARDS. Compared with the survivors, the APACHE II scores of the non-survivors increased significantly [27 (22–30) *vs.* 23.5 (16–29), P<0.05]. The PaO_2/FiO_2 ratio of the non-survivors on Day 3 or Day 7 decreased significantly compared with the survivors (both P<0.05). Next, we adjusted the variables with logistic regression (i.e., age, gender, severity of ARDS, APACHE II score, SOFA score and Murray score on admission) (*Table S1*) and found that only age independently affected mortality [P=0.04, HR 0.97 (95% CI, 0.94–1.0)].

Plasma mtDNA levels associated with ARDS severity

Patients were divided into mild, moderate, and severe ARDS groups according to the Berlin definition based on the degree of hypoxemia. Interestingly, the three groups had different plasma mtDNA levels from one another on Day 7. Patients in the severe ARDS group had the highest plasma levels, followed by those in the moderate and mild ARDS groups [1,230 (588–22,387) *vs.* 5,370 (628–13,052) *vs.* 15,792 (1,623–186,814) copies/μL, respectively, P=0.03] (*Table S2*).

Table 1 Main characteristics of 73 patients with ARDS

Variable	Overall (n=73)	Survivors (n=44)	Non-survivors (n=29)	P value
Age (year)	64	59 [46–78.5]	75 [60–79]	0.05
Male	57 (78.08)	36 (81.8)	21 (72.4)	0.34
Height (cm)	169	170 [168–175]	170 [165–172]	0.25
Weight (kg)	69	70 [60–80]	70 [65–70]	0.78
Primary cause of ARDS				
Pneumonia	42 (57.53)	25 (56.8)	18 (62.1)	0.66
Aspiration	8 (10.96)	7 (15.9)	1 (3.4)	0.10
Severe trauma	5 (6.85)	4 (9.1)	1 (3.4)	0.35
Drowning	2 (2.74)	2 (4.5)	0	0.24
Sepsis	12 (16.44)	4 (9.1)	7 (24.1)	0.33
Others	4 (5.48)	2 (4.6)	2 (6.9)	0.56
ARDS severity				
Mild	13 (17.81)	8 (18.2)	5 (17.2)	0.92
Moderate	36 (49.32)	22 (50.0)	13 (44.8)	0.67
Severe	24 (32.88)	14 (31.8)	11 (38)	0.59
Mechanically ventilated	62 (84.9)	37 (84.1)	25 (86.2)	0.80
PaO_2/FiO_2 Day 1 (mmHg)	170 [130–229]	174 [126–231]	187 [140–227]	0.60
PaO_2/FiO_2 Day 3 (mmHg)	231 [183–310]	267 [202–341]	217 [148–274]	0.03
PaO_2/FiO_2 Day 7 (mmHg)	241 [180–323]	294 [213–357]	205 [78–260]	0.01
APACHE II score	25 [19–30]	23.5 [16–29]	27 [22–30]	0.04
SOFA score	13 [10–17]	13 [9–16]	14 [11–19]	0.14
Murray score	2 [1.6–2.7]	2 [1.55–2.67]	2.3 [1.67–3.0]	0.50
Ventilator days	7 [3–12.5]	6 [3–10.5]	8 [3–17]	0.34
ICU stay	13 [8.5–27]	14.5 [9.5–28]	11 [6–17]	0.09

Continuous variables are presented as mean ± SD or median [interquartile range] if not normally distributed; categorical variables are presented as No. (%). ARDS, acute respiratory distress syndrome; APACHE II, acute physiology and chronic health evaluation II; SOFA, sepsis-related organ failure assessment; FiO_2, fraction of inspired oxygen; ICU, intensive care unit; PaO_2, partial pressure of oxygen in arterial blood.

Plasma mtDNA levels and 28-day mortality in ARDS

Plasma mtDNA levels were higher in non-survivors than in survivors on Day 7 [67,608 (19,498–346,736) *vs.* 7,585 (1,717–15,792) copies/μL, P=0.005], and no significant differences were observed on Day 1 (P=0.24) or Day 3 (P=0.88) (*Table S3*). The plasma mtDNA levels of the survivors had decreased by Day 7, while the mtDNA levels of the non-survivors were still high on Day 7 (*Figure 2*). Patients with elevated mtDNA always had higher mortality than those with decreased mtDNA over time (*Table S4*).

Plasma mtDNA levels and 28-day mortality prediction

ROC analysis (*Figure 3*) showed that mtDNA level on Day 7 was a reliable predictor of 28-day mortality compared with the PaO_2/FiO_2 ratio in ARDS, with AUROCs of 0.74 (95% CI, 0.59–0.89) and 0.68 (95% CI, 0.54–0.82), respectively. Seventeen patients died or were discharged from the ICU before Day 7, so only the data from 56 patients on Day 7 were available. The optimal cut-off value for mtDNA for predicting 28-day mortality was 18,640 copies/μL (76.5% sensitivity and 76.9% specificity). Kaplan-Meier survival

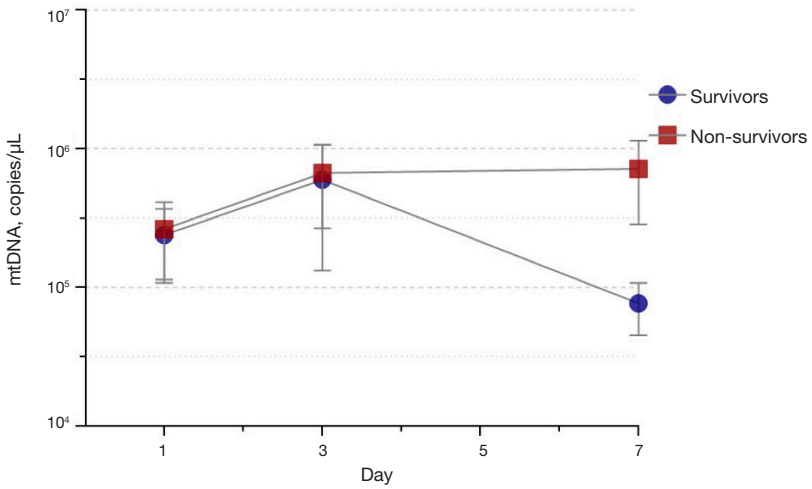

Figure 2 mtDNA levels in patients with ARDS during the first week in the ICU. mtDNA levels peaked by Day 3 and remained elevated in non-survivors but was reduced in survivors. n=29 for non-survivors, n=44 for survivors.

curves showed significant differences between patients with plasma mtDNA ≥18,640 copies/μL and patients with plasma mtDNA <18,640 copies/μL on Day 7 (P=0.002 by log-rank test) (*Figure 4*).

Plasma mtDNA levels were associated with ventilator-free days

Patients were grouped according to the cut-off value of plasma mtDNA, and the results revealed that patients with plasma mtDNA <18,640 copies/μL on Day 7 had longer ventilator-free days compared with those with plasma mtDNA ≥18,640 copies/μL (17±10 *vs.* 7±11 days, P=0.04) (*Table S5*). In addition, patients with increasing plasma mtDNA levels over time also had shorter ventilator-free days (18±8 *vs.* 6±9 days, P=0.03) (*Table S4*).

Plasma mtDNA levels were correlated modestly with SOFA

Plasma mtDNA levels demonstrated a slightly but obviously positive correlation with severity of illness as measured by SOFA score on Day 7 in non-surgical ARDS patients in the linear regression. Patients with higher SOFA scores had greater plasma levels of mtDNA (r=0.26, P=0.04) (*Figure S1*).

Plasma mtDNA levels correlate modestly with C-reactive protein (CRP)

Since mtDNA may activate immunity and initiate SIRS, it may be correlated with markers of inflammation. We therefore determined whether plasma mtDNA was associated with CRP. Plasma mtDNA levels were found to be significantly positively correlated with CRP on Day 3 (r=0.34, P=0.007) (*Figure S2A*) and Day 7 (r=0.31, P=0.03) (*Figure S2B*) in the linear regression.

Discussion

ARDS is a severe pulmonary disease. Although more than thirty clinical trials have been conducted in the past decade, the efficacy of treatments for this life-threatening disease is still unsatisfactory.

The severity of ARDS is often assessed using the PaO_2/FiO_2 ratio, even though the prognostic value of this variable remains low to moderate, with an AUC of only 0.58 (95% CI, 0.56–0.59) in a recent large study (22). Novel biomarkers for ARDS severity are needed. In this prospective study of patients with ARDS, higher levels of plasma mtDNA were associated with the severity of ARDS. This association was independent of age, gender, and risk

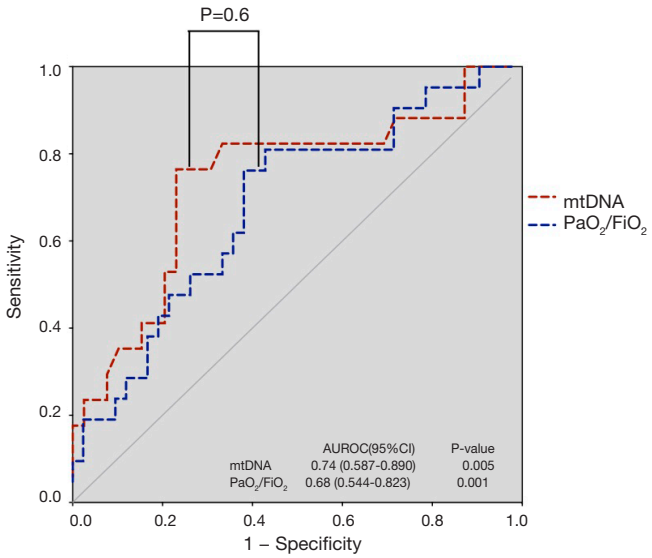

Figure 3 The ROC curves for both plasma mtDNA levels and PaO_2/FiO_2. The areas under the ROC curve on day 7 for the two measures were 0.74 and 0.68, respectively.

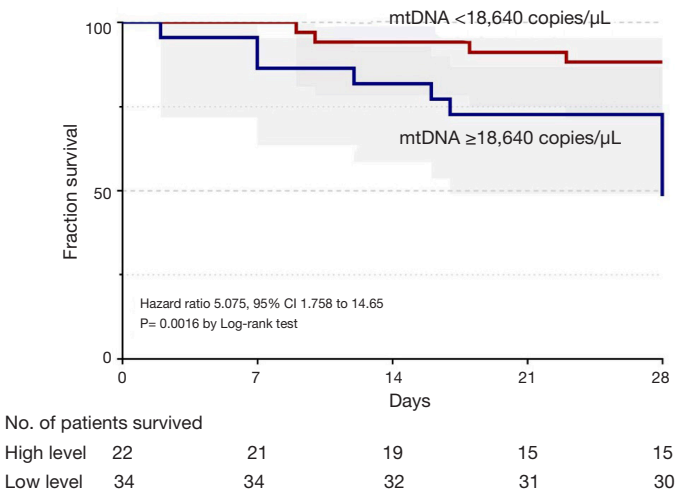

Figure 4 Kaplan-Meier plot of the 28-day mortality of ARDS patients stratified by plasma mtDNA level. Patients stratified with a ROC-determined cutoff point of 18,640 copies/μL of plasma mtDNA level. The Kaplan-Meier curves showed that survival in patients with plasma mtDNA levels above 18,640 copies/μL was significantly less than that of patients with plasma mtDNA levels less than 18,640 copies/μL ($P<0.01$).

factors for ARDS. We measured plasma mtDNA levels at three points during the early phase of ARDS (Day 1, Day 3, Day 7), and indicated that plasma mtDNA levels at Day 7 could significantly judge the severity of ARDS. On the other hand, plasma mtDNA levels on Days 1 and 3 seemed to lack utility. In studies of sepsis, we found that there was also no difference in mtDNA levels between control subjects and patients with sepsis on Day 1 (23,24). Furthermore, the trend of mtDNA seems more important in sepsis, as another study also demonstrated that mtDNA changes in sepsis were correlated with survival outcome (25), which was consistent with our study.

We also found that age may be an independent factor for evaluating patient mortality after adjustment. In

fact, mitochondrial function is closely associated with aging, partially due to the crucial role of mitochondria in modulating oxidative stress. For example, the number of mitochondria is lower in the liver or muscles in aged people than in young people (26,27). The level of mitochondrial protein is also reduced in older individuals (28). However, to date, the association between cell-free mtDNA level and age remains largely elusive.

In the ICU, the APACHE II score is widely used to evaluate disease severity, but it has limitations in predicting disease progression and prognosis in ARDS because it involves complex computations and subjective evaluation, which may introduce bias (29,30). Hence, it is urgent to develop novel molecular predictors for disease severity and patient outcome, especially plasma markers due to their high accessibility. In our study, we observed that plasma mtDNA levels could reflect disease severity, but the AUROC was no better than that obtained with the APACHE II score (0.74 *vs.* 0.63, P=0.27) (*Figure S3*). However, plasma mtDNA levels were associated with an increased risk of death in ARDS. These findings indicated that plasma mtDNA levels were superior to the APACHE II score for assessing severity and predicting patient outcome in ARDS.

The SOFA score is a scoring system used to assess disease severity and outcome prediction in critically ill patients (31,32). This score was designed to provide an objective approach to assess single or multiple organ failure (33). In our study, no correlation between plasma mtDNA level and SOFA score was observed on day 1 and day 3; however, a slightly but significant positive correlation was demonstrated between them when we excluded patients who underwent surgery. As with the APACHE II score, the plasma mtDNA level was no better a predictor for ARDS severity than the SOFA score in terms of the AUROC (0.74 *vs.* 0.71, P=0.36) (*Figure S3*). mtDNA is usually released from tissue or cells after surgery, so surgery may affect mtDNA levels in ARDS patients.

ARDS is a pulmonary inflammatory disorder that often occurs upon pulmonary or extrapulmonary injury. Recently, many researches (34-36) tried to clarify the role of plasma mediators and their relationship with the phenotype and outcome in ARDS patients. Inflammation and injury markers such as CRP (37,38) have been implicated in the prediction of the early onset and outcome of ARDS in cross-sectional studies. Many studies have shown that mtDNA derived from damaged cells functions as a DAMP and plays crucial roles in inflammation. In this study, we revealed that plasma mtDNA levels and CRP levels were significantly correlated on Day 3 and Day 7. A previous study showed that extracellular mtDNA could enhance endothelial permeability and promote neutrophil adhesion to the endothelium (39). Rats administered intravenous mitochondrial DAMPs showed marked evidence of lung injury and increased pulmonary albumin permeability (11). These findings may partly explain the pathological role of plasma mtDNA in ARDS. However, the molecular mechanisms of the effect of mtDNA need further investigation.

Our study has some limitations. First, this is a single-center study. The primary cause of ARDS in this study was pneumonia, and there were few patients with other sources of ARDS, so the results of this study may not represent all ARDS patients. Second, the number of patients enrolled in the present study was not large. Third, the proportion of patients with mild ARDS was small compared to the proportion of patients with moderate and severe ARDS, indicating that some ARDS patients may have received treatment for the disease before recruitment. This may have also influenced our study.

Conclusions

In summary, the mtDNA level in plasma was positively associated with the severity and could predict the outcome of ARDS. Therefore, plasma mtDNA levels may be applied in clinical practice as a new biomarker for ARDS.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/jtd.2020.02.49). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was approved by the Ethics Committees for Clinical Research of Zhongda Hospital (No. 2016ZDSYLL033.0). After discussing the study, parents who were eligible and interested in participating provided written informed consent.

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Supplementary

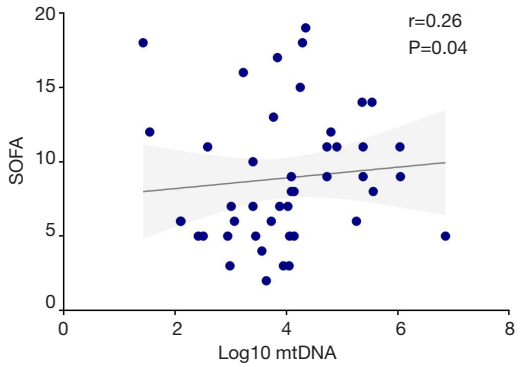

Figure S1 Log10 mtDNA level was correlated with SOFA score on Day 7 (Spearman rho=0.26, P<0.05). SOFA, sepsis-related organ failure assessment.

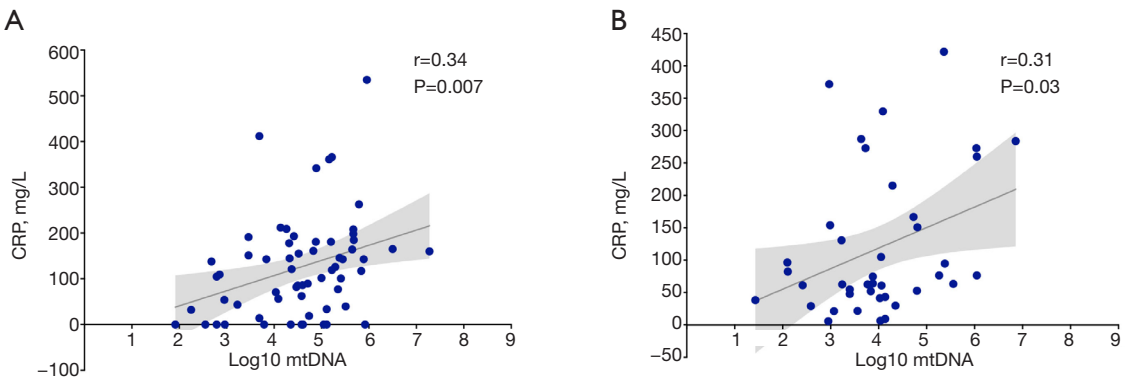

Figure S2 Log10 mtDNA level was correlated with CRP on Day 3 (Spearman rho=0.34, P<0.05) and Day 7 (Spearman rho=0.31, P<0.05).

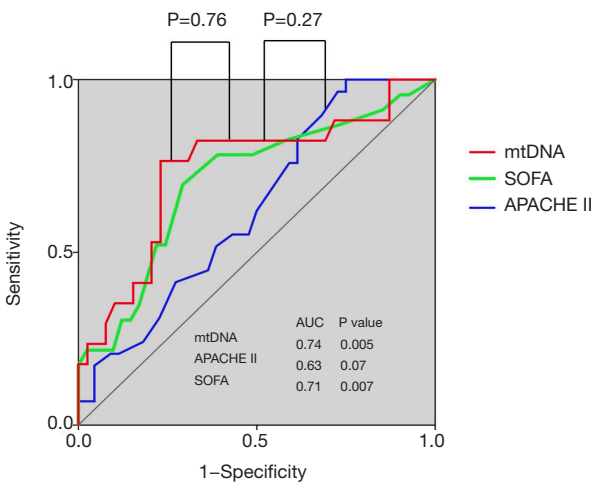

Figure S3 ROC curves for plasma mtDNA levels, SOFA scores and APACHE II scores. The areas under the ROC curve were 0.74, 0.70 and 0.63, respectively. SOFA, sepsis-related organ failure assessment; APACHE II, acute physiology and chronic health evaluation II.

Table S1 shows the adjusted analysis of covariates

Variable	Number (percent) or median (interquartile range)			P value	Adjusted P value	Adjusted HR (95% CI)
	Overall (n=73)	Survivors (n=44)	Non-survivors (n=29)			
Age (year)	64	59 [46–78.5]	75 [60–79]	0.05	0.04	0.97 [0.94–1.0]
Man (male)	57 (78.08)	36 (81.8)	21 (72.4)	0.34	0.30	
Sepsis	12 (16.44)	4 (9.1)	7 (24.1)	0.33	0.78	
Septic shock	4 (5.48)	2 (4.6)	2 (6.9)	0.56	0.28	
ARDS severity						
Mild	13 (17.81)	8 (18.2)	5 (17.2)	0.92	0.72	
Moderate	36 (49.32)	22 (50.0)	13 (44.8)	0.67	0.96	
Severe	24 (32.88)	14 (31.8)	11 (38)	0.59	0.50	
APACHE II score	25 [19–30]	23.5 [16–29]	27 [22–30]	0.04	0.11	
SOFA score	13 [10–17]	13 [9–16]	14 [11–19]	0.14	0.60	
Murray score	2 [1.6–2.7]	2 [1.55–2.67]	2.3 [1.67–3.0]	0.50	0.28	

Continuous variables are presented as mean ± SD or median [interquartile range] if not normally distributed; categorical variables are presented as No. (%). ARDS, acute respiratory distress syndrome; APACHE II, acute physiology and chronic health evaluation II; SOFA, sepsis-related organ failure assessment.

Table S2 The plasma mtDNA levels among patients with different degrees of ARDS

Time point	mtDNA (copies/μL)			P value
	Mild ARDS	Moderate ARDS	Severe ARDS	
Day 1	5,128 (1,659–10,719)	16,982 (5,744–48,977)	45,005 (4,692–298,557)	0.3
Day 3	18,197 (2,951–112,201)	31,622 (3,432–155,250)	85,678 (18,633–465,058)	0.2
Day 7	1,230 (588–22,387)	5,370 (628–13,052)	15,792 (1,623–186,814)	0.03

Data are presented as median (interquartile range) for variables that are not normally distributed. mtDNA, mitochondria DNA. See *Table 1* legend for expansion of other abbreviation. ARDS, acute respiratory distress syndrome.

Table S3 The plasma mtDNA levels between survivors and non-survivors

Time point	mtDNA (copies/μL)		P value
	Survivors	Non-survivors	
Day 1	15,135 (3,507–54,616)	18,197 (5,754–245,470)	0.24
Day 3	38,018 (4,897–161,497)	37,153 (6,918–239,883	0.88
Day 7	7,585 (1,717–15,792)	67,608 (19,498–346,736)	0.005

Data are presented as median (interquartile range) for variables that are not normally distributed. mtDNA, mitochondria DNA.

Table S4 shows the variation trend of plasma mtDNA associated with ARDS outcomes

Variable	Overall	Declined	Elevated	P value
Number	21	14	7	
Ventilator-free days	14±10	18±8	6±9	0.03
ICU stay	20±9	20±8	20±11	0.55
Mortality (%)	4 (19.0%)	1 (7.1%)	3 (42.9%)	0.09

Data are presented as mean ± SD or median (interquartile range) if not normally distributed. mtDNA, mitochondria DNA. Decrease Group means patients' mtDNA levels declined by time (Day 1, Day 3 and Day 7); Increase Group means patients' mtDNA levels rose by time (Day1, Day3 and Day7). See *Table 1* legend for expansion of other abbreviation.

Table S5 shows the plasma mtDNA levels associated with ventilator-free days and days of ICU stay

Variable	Overall	Plasma mtDNA level (copies/μL)		P value
		mtDNA ≥18,640	mtDNA <18,640	
Number	56	22	34	
Ventilator-free days	13±11	7±11	17±10	0.04
ICU stay	18±9	17±9	18±8	0.37

Data are presented as mean ± SD or median (interquartile range) if not normally distributed. mitochondria DNA. See *Table 1* legend for expansion of other abbreviation.