CHEST

Circulating Mitochondrial DNA as Predictor of Mortality in Critically Ill Patients

A Systematic Review of Clinical Studies

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BACKGROUND: Despite numerous publications on mitochondrial DNA (mtDNA) in the last decade it remains to be seen whether mtDNA can be used clinically. We conducted a systematic review to assess circulating cell-free mtDNA as a biomarker of mortality in critically ill patients.

METHODS: This systematic review was registered with PROSPERO (CRD42016046670). PubMed, CINAHL, the Cochrane Library, Embase, Scopus, and Web of Science, and reference lists of retrieved articles were searched. Studies measuring circulating cell-free mtDNA and reporting on all-cause mortality in critically ill adult and pediatric patients were included. The primary and secondary outcomes were mortality and morbidity, respectively.

RESULTS: Of the 1,566 initially retrieved publications, 40 studies were included, accounting for 3,450 critically ill patients. Substantial differences between studies were noted in how mtDNA was isolated and measured. Sixteen of the 40 included studies (40%) explored the association between mtDNA levels and mortality; of those 16 studies, 11 (68.8%) reported a statistically significant association. The area under the receiver operating characteristic (AUROC) curve for mtDNA and mortality was calculated for 10 studies and ranged from 0.61 to 0.95.

CONCLUSIONS: There is growing interest in mtDNA as a predictor of mortality in critically ill patients. Most studies are small, lack validation cohorts, and utilize different protocols to measure mtDNA. When reported, AUROC analysis usually suggests a statistically significant association between mtDNA and mortality. Standardization of mtDNA protocols and the completion of a large, prospective, multicenter trial may be warranted to firmly establish the clinical usefulness of mtDNA. CHEST 2019; 156(6):1120-1136

KEY WORDS: biomarkers; circulating cell-free DNA; critical illness; mitochondrial DNA; mortality

ABBREVIATIONS: APACHE = Acute Physiology and Chronic Health Evaluation; AUC = area under the curve; CSF = cerebrospinal fluid; DAMP = damage-associated molecular pattern; MELD = Model for End-Stage Liver Disease; mtDNA = mitochondrial DNA; QUIPS = Quality in Prognosis Studies; TLR-9 = Toll-like receptor 9

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The complex mechanisms by which mitochondria regulate innate immunity and whether extracellular mitochondrial DNA (mtDNA) influences the pathogenesis of disease are currently the subject of intense research. Thus far, murine models have linked mtDNA to the development of posttraumatic acute lung injury, ventilator-associated pneumonia, inflammatory arthritis, acute kidney injury, and nonalcoholic steatohepatitis.[1-5] There is also mounting evidence that extracellular mtDNA might be useful as a biomarker of human disease severity. One study of critically ill patients found that mtDNA was significantly associated with mortality, even after adjustment for procalcitonin and lactate.[6] Investigators have also found that mtDNA performed similarly to Model for End-Stage Liver Disease (MELD) scores in patients with acetaminophen-induced acute liver failure and outperformed troponin as a biomarker of mortality in patients with pulmonary embolism.[7,8] If mtDNA can be successfully developed as a biomarker, it could provide clinicians with timely insight into disease severity affecting treatment decisions.

The molecular biology accounting for these observations is deeply rooted in our understanding of mtDNA as a potent damage-associated molecular pattern (DAMP). When cells are exposed to pathogen-associated molecular patterns or DAMPs, mitochondria respond by increasing their production of reactive oxygen species (Fig 1).[9,10] The resulting oxidative stress causes fragmentation of the mitochondrial genome and disruption of the mitochondrial membrane, allowing mitochondrial DAMPs, such as fragmented mtDNA, to leak into the cytosol.[9,11-13] Because of the bacterial ancestry of mitochondria, mtDNA contains unmethylated CpG repeats that are recognized by pattern recognition receptors.[14,15] This allows cytosolic mtDNA to promote expression of IL-6 and tumor necrosis factor-α or IL-1β and IL-18 via activation of the Toll-like receptor 9 (TLR-9)/nuclear factor-κB pathway

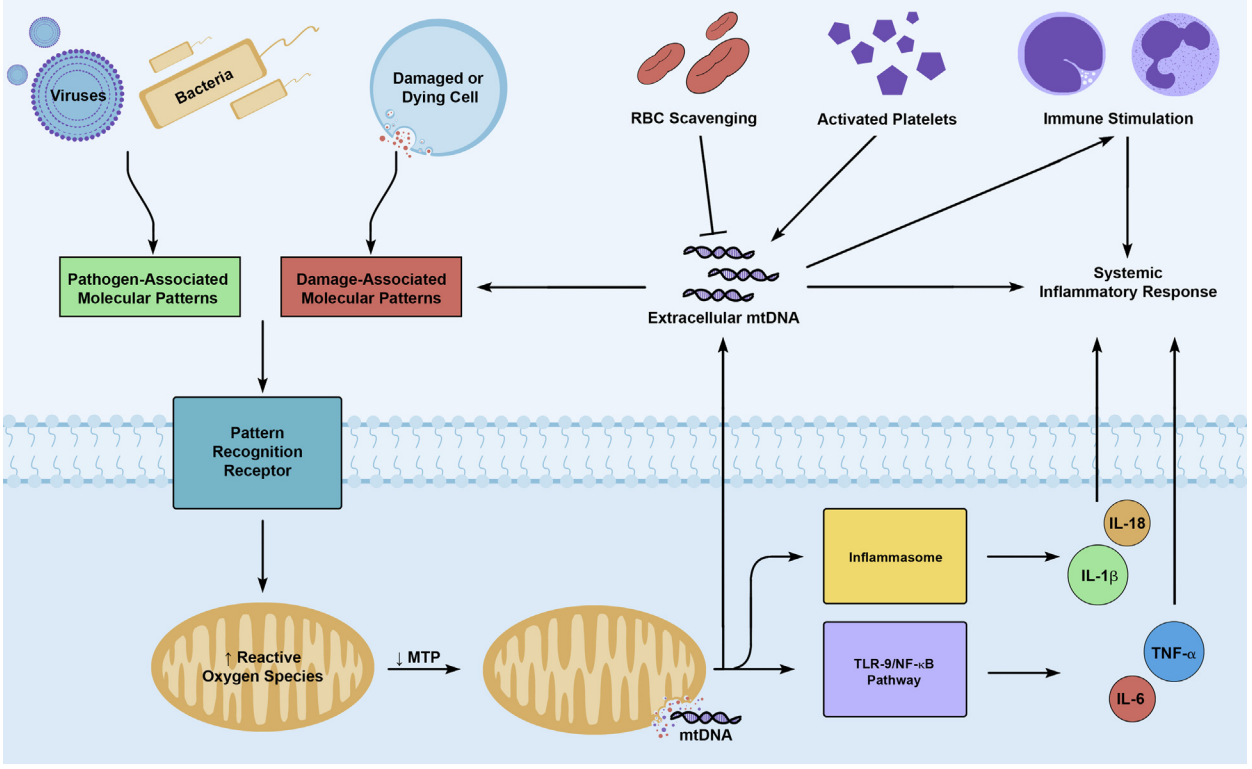

Figure 1 – *Production and release of extracellular mitochondrial DNA. Pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) are released in the setting of infection or cellular injury, respectively. Both PAMPs and DAMPs can stimulate pattern recognition receptors, leading to increased production of mitochondrial reactive oxygen species. This causes fragmentation of the mitochondrial genome and decreased mitochondrial membrane transition permeability, allowing mitochondrial DNA (mtDNA) to enter the cytosol. mtDNA is a potent DAMP; it can stimulate inflammasomes or the Toll-like receptor 9 (TLR-9)/nuclear factor-κB pathway, leading to the production of proinflammatory cytokines (IL-1β, IL-18, tumor necrosis factor-α, IL-6). Cytosolic mtDNA can also enter the extracellular environment via necroptosis, NETosis, or platelet activation, where it can propagate the initial inflammatory response through further recognition as a DAMP by immune and nonimmune cells expressing TLR-9. RBCs have been shown to scavenge extracellular mtDNA through binding to TLR-9. MTP = membrane transition permeability; NF-κB = nuclear factor-κB; NETosis = formation of neutrophil extracellular traps (NETs); TNF-α = tumor necrosis factor-α.*

or the NALP3 inflammasome, respectively.[9,16] Cytosolic mtDNA may also enter, via necroptosis or NETosis (neutrophil extracellular trap [NET] formation), the extracellular environment, where it is believed to propagate a systemic inflammatory response through recognition as a DAMP.[17,18]

Despite the strong biologic basis for the potential usefulness of mtDNA, its role as a biomarker of critical illness has yet to be firmly established. To address this, we set out to perform a systematic review to determine whether circulating mtDNA could be clinically useful as a predictor of mortality in critically ill patients.

Methods

Protocol and Registration

This systematic review was registered with PROSPERO (the International Prospective Register of Systematic Reviews) (www.crd.york.ac.uk/prospero; record No. CRD42016046670)[19] and conducted in accordance with the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement.[20]

Data Sources

A systematic review of the literature was performed by using the key phrase, "mitochondrial DNA AND (critically ill OR emergency department OR intensive care)," to search PubMed, CINAHL (the Cumulative Index to Nursing and Allied Health Literature), the Cochrane Library, Embase, Scopus, and the Web of Science from database inception to June 1, 2018. The reference lists of included studies were manually reviewed for additional publications.

Selection of Studies

Two authors (J. S. H. and I. I. S.) independently searched the databases and performed study selection, with disagreements resolved by consensus. Observational studies measuring circulating cell-free mtDNA in critically ill patients (adult or pediatric) while reporting all-cause mortality were considered eligible for inclusion. We defined "critically ill" as an admitting diagnosis that could result in admission to an ICU (further clarification provided in e-Appendix 1). No limitations were applied to the publications on the basis of time or language.

Data Extraction

Two authors (J. S. H. and I. I. S.) independently read and extracted data from the included studies according to a standard data extraction form (e-Table 1). Any disagreement between the authors was resolved by consensus. Corresponding authors of the selected studies were contacted by e-mail to request any missing data, which was then incorporated into our analysis.

Outcomes of This Systematic Review

All-cause mortality, assessed at any time point, was the primary outcome for this systematic review. Morbidity, as suggested by ICU length of stay, hospital length of stay, need for mechanical ventilation, and need for vasopressors, served as secondary outcomes.

Assessment of Risk of Bias of Included Studies

Studies in this review were assessed for bias according to a modified version of the Quality in Prognosis Studies (QUIPS) tool.[21] QUIPS assesses studies of prognostic factors by prompting reviewers to classify a study as having low, moderate, or high risk of bias according to the following domains: study participation, study attrition, prognostic factor measurement, outcome measurement, study confounding, and statistical analysis. Details are provided in e-Appendix 1.

Results

Characteristics of Selected Studies

The flow diagram illustrating the study selection process can be found in Figure 2. Our search of databases yielded 1,566 publications. Thirty-four of them met our inclusion criteria.[2,6-8,22-51] On reviewing the references of these publications, we found six additional studies that were deemed relevant.[52-57] Thus, 40 studies,[2,6-8,22-57] involving 41 cohorts and a total of 3,450 critically ill patients (range, 12 to 753 patients per study), were included in our systematic review.

Characteristics of the included studies can be found in Table 1. All of the studies were published in 2004 and later, with the majority (75%) published after 2012. Ten studies were conducted in North America, 15 studies in Europe, 13 studies in Asia, one study in Africa, and one study in Australia. All of the included studies were observational and five (12.5%) were multicenter.[6,7,22,28,46] Only one study had a validation cohort.[6] Patients were admitted to medicine in 17 studies (42.5%),[6-8,28-30,32,33,36,39,46-49,52,53,55] to surgery in 13 studies (32.5%),[2,23,25-27,31,34,37,40,43,51,54,57] and to neurology in five studies (12.5%).[41,44,45,50,56] Four studies[22,24,38,42] enrolled medical and surgical patients. Only one study enrolled pediatric patients[35]; all of these patients had sepsis. Severity of illness scores for the individual studies are presented in Table 1.

Measurement of mtDNA

Characteristics of the protocols used by investigators to measure mtDNA can be found in Table 2. Twenty-four studies (60%)[2,22,23,25-29,32-34,37,40-45,47,48,50-52,56] performed serial mtDNA measurements. The specific time points, including when mtDNA peaked for those studies, can be found in e-Table 2.

As shown in Table 2, heterogeneity was observed amongst studies regarding the preparation of cell-free mtDNA, specifically in terms of centrifugation settings,

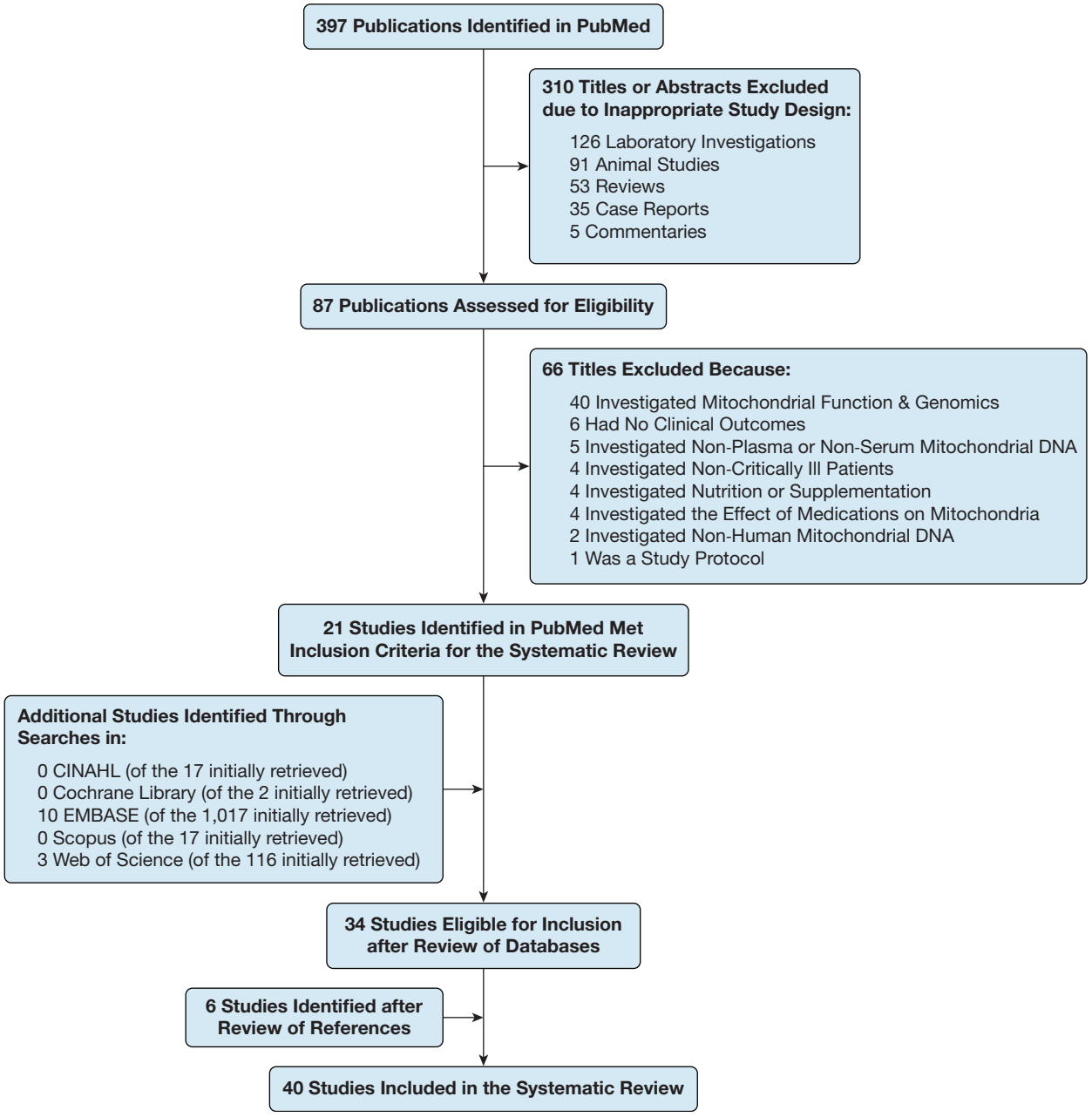

Figure 2 – *Flow diagram illustrating study selection process. CINAHL = Cumulative Index to Nursing and Allied Health Literature.*

the type of specimen (plasma or serum), and expression of mtDNA levels (concentration, a copy number, a fold change, genome equivalents, arbitrary units, or as a ratio to nuclear DNA). Heterogeneity was also observed regarding the primers used to recognize a single portion of the mitochondrial genome, with NADH dehydrogenase (or one of its subunits) being the most commonly used followed by cytochrome *c* oxidase III, cytochrome *b*, the D-loop, ATPase 8, cytochrome *c* oxidase I, cytochrome *c* oxidase II, ATPase 6, and $tRNA^{Leu}$.

Risk of Bias Within Studies

The results of the modified QUIPS for each individual study can be found in e-Table 3. A moderate risk of bias was common.

Mortality

All of the studies in this systematic review reported on mortality.[2,6-8,22-57] Mortality was assessed at variable time points, such as at ICU[22,31,35] or hospital discharge[2,25,28,30,34,39,40,45-47,51,52,54]; at 3,[55] 15,[8] 21,[7] 28,[6,23,24,32,37,42] and 30[33,36,38] days; and at 3,[24,50,56] 6,[44]

TABLE 1] Article Characteristics

Study/Year	Country	Study Design	Discipline	Population	Setting	No. of Patients	Severity of Illness[a]	Validation Cohort
Aslami et al[22]/ 2018	The Netherlands	Post hoc analysis of RCT with healthy control subjects	Medicine and surgery	Post-cardiac arrest	ICU	20	36°C group APACHE II: 23.8 ± 7.0	No
							33°C group APACHE II: 19.3 ± 8.9	
Leijte et al[23]/ 2018	The Netherlands	Pro cohort with healthy control subjects	Surgery	Post CRS-HIPEC	OR → ICU	20	ECOG performance score: 1 (1-1)	No
Jansen et al[24]/ 2018	The Netherlands	Pro case-control plus in vivo and in vitro	Medicine and surgery	Noninfectious SIRS	ICU	37	NR	No
Paunel-Görgülü et al[25]/2017	Germany	Pro cohort with healthy control subjects plus in vitro	Surgery	Cardiopulmonary bypass	OR → ICU	48	CPB < 100 SAPS II: 28.5 ± 8.9	No
							CPB > 100 SAPS II: 29.8 ± 7.4	
Hampson et al[26]/2017	United Kingdom	Pro cohort with healthy control plus ex vivo	Surgery	Burn	ICU vs floor	63	APACHE II: 26 (12-31)	No
Simmons et al[27]/2017	United States	Pro case control	Surgery	Trauma	ICU	14	No ARDS ISS: 24.5 ± 4.3	No
							ARDS ISS: 23.0 ± 6.3	
Donnino et al[28]/ 2017	United States	Pro cohort with healthy control subjects	Medicine	Post-cardiac arrest	ED → ICU	102	NR	No
Qin et al[29]/2017	China	Pro cohort with control subjects	Medicine	Myocardial infarction	NR	38	NR	No
Simmons et al[2]/ 2017	United States	Pro cohort with in vivo	Surgery	Ventilator-associated pneumonia	ICU	31	Non-VAP ISS: 27 ± 8	No
							VAP ISS 23 ± 8	

(Continued)

TABLE 1] *(Continued)*

Study/Year	Country	Study Design	Discipline	Population	Setting	No. of Patients	Severity of Illness[a]	Validation Cohort
Marenzi et al[30]/2016	Italy	Pro cohort	Medicine	Myocardial infarction	NR	753	Nondetectable cyt *c* TIMI score: 4.2 ± 2.2	No
							Detectable cyt *c* TIMI score: 4.3 ± 2.3	
Mohamed et al[31]/2016	Egypt	Pro cohort with healthy control subjects	Surgery	Trauma	ED → ICU	61	NR	No
Timmermans et al[32]/2016	The Netherlands	Pro cohort with healthy control subjects plus in vivo	Medicine	Sepsis	ICU	121	APACHE II: 23 (11-45)	No
Omura et al[33]/2016	Japan	Pro cohort	Medicine	Post-cardiac arrest	ED → ICU	21	"Favorable" APACHE II: 24 (15-29)	No
							"Unfavorable" APACHE II: 30 (26-37)	
Qin et al[34]/2016	China	Pro cohort	Surgery	Cardiopulmonary bypass	OR → ICU	46	NR	No
Di Caro et al[35]/2016	United States	Pro cohort with healthy control subjects plus in vitro	Pediatrics	Sepsis	ICU	28	PRISM score: 9.5 (1-32)	No
Schäfer et al[36]/2016	Germany	Pro cohort with healthy control subjects plus in vivo and in vitro	Medicine	Sepsis	ICU	165	SAPS II: 32 (23-42)	No
Timmermans et al[37]/2016	The Netherlands	Pro cohort with healthy control subjects plus ex vivo	Surgery	Trauma	Field → ED → ICU	166	ISS: 26 (17-37)	No
Krychtiuk et al[38]/2015[b]	Austria	Pro cohort with healthy control subjects	Medicine and surgery	Mixed	ICU	228	APACHE II: 20 (13-25)	No
Timmermans et al[52]/2015	The Netherlands	Pro cohort with healthy control subjects plus ex vivo	Medicine	Post-cardiac arrest	ICU	14	APACHE: 24 (15-39)	No

(Continued)

TABLE 1] *(Continued)*

Study/Year	Country	Study Design	Discipline	Population	Setting	No. of Patients	Severity of Illness[a]	Validation Cohort
Bhagirath et al[39]/2015	Canada	Pro cohort with healthy control subjects plus in vitro	Medicine	Sepsis	ICU	12	APACHE II: 24 (11-41)	No
McIlroy et a l[40]/ 2015	Australia	Pro cohort with healthy control subjects	Surgery	Trauma	ICU vs floor	35	ISS: 14 (9-22)	No
McGill et al[7]/ 2014	United States	Pro cohort with healthy control subjects	Medicine	Acetaminophen-induced acute liver failure	NR	69	NR	No
Wang et al[41]/ 2014	Taiwan	Pro cohort with healthy control subjects	Neurology	Traumatic brain injury	ED → ICU	88	GCS: 15 (13-15) ISS: 16 (11-20)	No
Fernández-Ruiz et al[53]/2014	Spain	Pro cohort with healthy control subjects plus in vitro	Medicine	Myocardial infarction	NR	75	NR	No
Nakahira et al[6]/ 2013[c]	United States	Pro cohort	Medicine	MICU	ICU	200	APACHE II: 24 (18-30)	Yes
				ARDS		243	APACHE II: 22 (18-26)	
Yamanouchi et al[42]/ 2013[d,e]	Japan	Pro cohort with healthy control subjects	Medicine and surgery	Sepsis	ED	23[d]	APACHE II: 14 (11-19)[d]	No
				Trauma		37[e]	APACHE II: 11 (6-15)[e]	
							ISS: 25 (16-34)[e]	
Simmons et al[43]/2013	United States	Pro cohort	Surgery	Trauma	ICU	13	ISS: 21.1 ± 1.7	No
Gu et al[54]/2013	China	Pro cohort with healthy control subjects	Surgery	Trauma	ICU	86	ISS: 18 (13-22)	No
Arnalich et al[8]/ 2013[f]	Spain	Pro cohort with healthy control subjects	Medicine	Pulmonary embolism	ED → ICU	74	PESI class III: 11, 0[f]	No
							PESI class IV: 20, 16[f]	
							PESI class V: 6, 21[f]	

(Continued)

TABLE 1] *(Continued)*

Study/Year	Country	Study Design	Discipline	Population	Setting	No. of Patients	Severity of Illness[a]	Validation Cohort
Wang et al[44]/ 2013	Taiwan	Pro cohort with healthy control subjects	Neurology	Subarachnoid hemorrhage	ED → ICU	21	"Good outcome" GCS: 15 (14.25-15)	No
							"Poor outcome" GCS 11 (6-13)	
Wang et al[45]/ 2012	Taiwan	Pro cohort with healthy control subjects	Neurology	Intracerebral hemorrhage	ICU	60	"Good outcome" GCS: 15 (15-15)	No
							"Poor outcome" GCS 14 (9-15)	
Arnalich et al[55]/ 2012	Spain	Retro cohort	Medicine	Post-cardiac arrest	ED	85	Survivors APACHE II: 35 (32-38)	No
							Nonsurvivors APACHE II: 38 (33-41)	
Puskarich et al[46]/2012	United States	Pro cohort with ED control subjects	Medicine	Sepsis	ED	69	Sepsis SOFA: 1 (0-3)	No
							Septic shock SOFA: 4 (1-8)	
Kung et al[47]/ 2012	Taiwan	Pro cohort with healthy control subjects	Medicine	Sepsis	ED → ICU vs floor	67	Survivors APACHE II: 18.9 ± 6.9	No
							Nonsurvivors APACHE II: 20.9 ± 3.8	
McGill et al[48]/ 2012	United States	Pro cohort with healthy control subjects plus in vivo	Medicine	Acetaminophen-induced acute liver failure	ED → ICU	40	NR	No
Garrabou et al[49]/2012	Spain	Pro cohort with healthy control subjects plus ex vivo	Medicine	Sepsis	NR	19	SAPS II 45.5 ± 13.3	No
Tsai et al[56]/ 2011	Taiwan	Pro cohort with at-risk control subjects	Neurology	Acute ischemic stroke	NR	50	NIHSS (day 1): 7 (1-24)	No

(Continued)

TABLE 1] *(Continued)*

Study/Year	Country	Study Design	Discipline	Population	Setting	No. of Patients	Severity of Illness[a]	Validation Cohort
Lu et al[50]/2010	Taiwan	Pro cohort with healthy control subjects	Neurology	Bacterial meningitis	NR	22	GCS: 11 ± 4.1	No
Chou et al[51]/2008	Taiwan	Pro cohort	Surgery	Corrosive ingestion	ED → NR	48	NR	No
Lam et al[57]/2004	Hong Kong	Pro cohort with healthy control subjects	Surgery	Trauma	ED	38	ISS < 16: 28 patients	No
							ISS > 16: 10 patients	

APACHE II = Acute Physiology and Chronic Health Evaluation II; CPB < 100 = cardiopulmonary bypass less than 100 minutes; CPB > 100 = cardiopulmonary bypass greater than 100 minutes; CRS-HIPEC = cytoreductive surgery with hyperthermic intraperitoneal chemotherapy; cyt *c* = cytochrome *c*; ECOG = Eastern Cooperative Oncology Group; GCS = Glasgow Coma Scale; ISS = Injury Severity Score; MICU = medical ICU; NIHSS = National Institutes of Health Stroke Scale; NR = not reported; OR = operating room; PESI = Pulmonary Embolism Severity Index; PRISM = Pediatric Risk of Mortality; Pro = prospective; RCT = randomized control trial; Retro = retrospective; SAPS II = Simplified Acute Physiology Score II; SIRS = systemic inflammatory response syndrome; SOFA = Sepsis-Related Organ Failure Assessment; TIMI = thrombolysis in myocardial infarction; VAP = ventilator-associated pneumonia.

[a]Severity of illness is preferentially reported for the entire cohort. However, some studies described severity of illness only according to subpopulations or "good vs bad outcome." When this occurred, severity of illness was reported for each individual group. Please see e-Appendix 1 for further clarification of the subpopulations or on how "good vs bad outcome" was defined.

[b]This study had a "mixed" population composed of medicine and post-cardiothoracic surgery patients.

[c]This study was composed of a derivation cohort and a validation cohort. The data presented at the top of the row represent characteristics of the derivation cohort, whereas the data at the bottom of the row represent characteristics of the validation cohort.

[d,e]This was a single study in which patients were analyzed within a sepsis or a trauma subgroup. Data denoted by a *d* represent characteristics of the sepsis subgroup whereas data denoted by an *e* represent characteristics of the trauma subgroup.

[f]Severity of illness was reported according to PESI classification. The first and second numbers, for a given class, correspond to the number of patients with submassive and massive pulmonary embolism, respectively.

TABLE 2] Description of mtDNA Assay

Study/Year	Blood Draw(s)[a]	Sample Processing[b]	Serum or Plasma	Primer	Standards	Units of Measurement	Cutoff[c]
Aslami et al[22]/ 2018	Serial, arterial, NR	WB 600*g* 10 4°C	Plasma	MT-ND1, MT-ND2, MT-CO3, MT-CYB	cDNA	AU	NR
Leijte et al[23]/ 2018	Serial, arterial, EDTA	WB 1,600*g* 10 4°C → 16,000*g* 10 4°C	Plasma	MT-ND1	Healthy volunteers	Fold change	—
Jansen et al[24]/ 2018	Single, arterial, heparin	WB 1,550*g* 20 RT → 1,550*g* 20 RT	Plasma	MT-ND1, MT-ND2, MT-CO3	NR	AU	NR
Paunel-Görgülü et al[25]/2017	Serial, NR, heparin	WB 3,000*g* 10	Plasma	MT-ATP8	Nuclear DNA	mtDNA:nDNA	NR
Hampson et al[26]/2017	Serial, NR, variable	WB 2,000*g* 20 RT → 13,000*g* 20	Plasma	MT-CYB	K562 mtDNA	ng/mL	NR
Simmons et al[27]/2017[d]	Serial, venous, citrate	WB 1,200*g* 25 21°C	Serum	MT-ND6	NR	ng/mL	NR
Donnino et al[28]/2017	Serial, NR, variable	WB NR → 16,000*g* 10	Plasma	D-loop, MT-tRNA^{Leu}	Whole blood DNA	Fold change	NR
Qin et al[29]/ 2017	Serial, NR, EDTA	WB 1,000 rpm 15 4°C → 16,000*g* 15 4°C	Plasma	MT-ND1	Plasmid DNA	Copies/μL	—
Simmons et al[2]/2017[e]	Serial, NR, NR	WB 1,200*g* 25 21°C	Serum	MT-ND6	cDNA	ng/mL	—
Marenzi et al[30]/ 2016	Single, venous, NR	P 700*g* 5 4°C → 18,000*g* 15 4°C	Plasma	MT-ND1	Plasmid DNA	Copies/μL	NR
Mohamed et al[31]/2016	Single, venous, EDTA	P 16,000*g* 5 4°C → 18,000*g* 15 4°C	Plasma	MT-ND1	Plasmid DNA	Copies/μL	NR
Timmermans et al[32]/2016[f]	Serial, arterial, EDTA	WB 1,600*g* 10 4°C → 16,000*g* 10 4°C	Plasma	MT-ND1	Healthy volunteers	Fold change	NR
Omura et al[33]/ 2016	Serial, NR, NR	WB 1,600 rpm 10 → 0.22-μm filter	Plasma	MT-ND1, MT-CO3, MT-CYB	A549 mtDNA	μg/mL	NR
Qin et al[34]/ 2016	Serial, NR, NR	NR	NR	For: 5′-CGAGCAGTAGCCCAAACAAT-3′ Rev: 5′-TGTGATAAGGGTGGAGAG GTT-3′	NR	Copies/μL	—
Di Caro et al[35]/ 2016	Single, NR, heparin	WB 1,914*g* 10	Plasma	MT-CO1	Plasmid DNA	Copies/mL	NR
Schäfer et al[36]/ 2016	Single, NR, NR	NR	Serum	MT-ATP6, D-loop	NR	fg/μL	NR

(Continued)

TABLE 2] *(Continued)*

Study/Year	Blood Draw(s)[a]	Sample Processing[b]	Serum or Plasma	Primer	Standards	Units of Measurement	Cutoff[c]
Timmermans et al[37]/2016[g]	Serial, venous, EDTA	WB 1,600*g* 10 4°C → 16,000*g* 10 4°C	Plasma	MT-ND1	Healthy volunteers	Fold change	NR
Krychtiuk et al[38]/2015	Single, arterial or venous, EDTA	WB 2,500*g* 30 4°C	Plasma	MT-ND2, MT-CO3, MT-CYB	Human smooth muscle cells	ng/mL	38.2 ng/mL
Timmermans et al[52]/2015	Serial, arterial, EDTA	WB 1,600*g* 10 4°C → 16,000*g* 10 4°C	Plasma	For: 5′-GCCCCAACGTTGTAGGCCCC-3′ Rev: 5′-AGCTAAGGTCGGGGCGGTGA-3′	Healthy volunteers	Fold change	NR
Bhagirath et al[39]/2015	Single, NR, citrate	WB 2,000*g* 10 → 3,000*g* 10	Plasma	MT-CYB, Mit 3130F, Mit 3301R	Purified mtDNA	μg/mL	NR
McIlroy et al[40]/2015	Serial, NR, NR	P 12,000*g* 10	Plasma	MT-ND3, MT-CO3	Purified mtDNA	ng/mL	—
McGill et al[7]/2014	Single, NR, NR	S 20,000*g* 10	Serum	MT-ND, MT-CO3	HepaRG mtDNA	ng/mL	14 ng/mL
Wang et al[41]/2014	Serial, venous, citrate	WB 3,000 rpm 10	Plasma	MT-ND2	Human gDNA	ng/mL	—
Fernández-Ruiz et al[53]/2014	Single, venous, NR	NR	Plasma	For: 5′-CCACGGGAAACAGCAGTGAT-3′ Rev: 5′-CTATTGACTTGGGTTAATCGTGTGA-3′	NR	Copies/μL	NR
Nakahira et al[6]/2013	Single, NR, EDTA	P 700*g* 5 4°C → 18,000*g* 15 4°C	Plasma	MT-ND1	Plasmid DNA	Copies/μL	3,200 copies/μL
Yamanouchi et al[42]/2013	Serial, NR, NR	WB 1,600 rpm 10 → 0.22-μm filter	Plasma	MT-ND1, MT-CO3, MT-CYB	A549 mtDNA	μg/mL	NR
Simmons et al[43]/2013	Serial, venous, citrate	WB 1,200*g* 25 21°C	Plasma	MT-ND1, MT-ND6, MT-CO1, D-loop	NR	Fold increase	NR
Gu et al[54]/2013	Single, venous, EDTA	WB 900*g* 10 → 9,600*g* 10	Plasma	MT-ND2	THP-1 mtDNA	pg/mL	1.3185 μg/mL
Arnalich et al[8]/2013	Single, NR, NR	WB 1,800*g* 10 4°C → 16,000*g* 10 4°C	Plasma	MT-ND2	Human gDNA	GE/mL	3,380 GE/mL or 22.308 ng/mL
Wang et al[44]/2013	Serial, venous, EDTA	WB 3,000 rpm 10 → 10,000 rpm 10	Plasma	MT-ND2	Human gDNA	ng/mL	NR
Wang et al[45]/2012	Serial, venous, citrate	WB 3,000*g* 10 4°C	Plasma	MT-ND2	Human gDNA	ng/mL	—
Arnalich et al[55]/2012	Single, venous, NR	WB 1,600*g* 10 4°C → 16,000*g* 10	Plasma	MT-ATP8	Human gDNA	GE/mL	3,495 GE/mL

(Continued)

TABLE 2] *(Continued)*

Study/Year	Blood Draw(s)[a]	Sample Processing[b]	Serum or Plasma	Primer	Standards	Units of Measurement	Cutoff[c]
Puskarich et al[46]/2012	Single, NR, NR	NR	Plasma	MT-ND1, MT-CO3, MT-CYB	Purified PCR products	μg/mL	NR
Kung et al[47]/2012	Serial, venous, EDTA	WB 3,000 rpm 10 → 10,000 rpm 10	Plasma	MT-ND2	Human gDNA	ng/mL	198 ng/mL
McGill et al[48]/2012	Serial, NR, NR	NR	Plasma	MT-ND, MT-CO3	HepaRG mtDNA	ng/mL	NR
Garrabou et al[49]/2012	Single, NR, EDTA	WB 1,500*g* 15 → 0.22-μm filter	Plasma	MT-ND2	NR	Gene/mL	NR
Tsai et al[56]/2011	Serial, venous, EDTA	WB 3,000 rpm 10 → 10,000 rpm 10	Plasma	MT-ND2	Human gDNA	Kilogenome equivalents/L	—
Lu et al[50]/2010	Serial, venous, EDTA	WB 3,000 rpm 10 → 10,000 rpm 10	Plasma	MT-ND2	gDNA	ng/mL	58.9 ng/mL
Chou et al[51]/2008	Serial, venous, EDTA	WB 1,500*g* 5	Plasma	Mit 3130F, Mit 3301R	Plasmid DNA	Kilogenome equivalents/L	NR
Lam et al[57]/2004	Single, venous, EDTA	WB 1,600*g* 10	Plasma	Mit 3130F, Mit 3301R	Plasmid DNA	Copies/mL	NR

AU = arbitrary unit; cDNA = complementary DNA; For = forward primer; *g* = force of gravity; gDNA = genomic DNA; GE = genome equivalents; MT-ATP8 = mitochondrially encoded ATP synthase 8; MT-CO1 = mitochondrially encoded cytochrome *c* oxidase I; MT-CO2 = mitochondrially encoded cytochrome *c* oxidase II; MT-CO3 = mitochondrially encoded cytochrome *c* oxidase III; MT-CYB = mitochondrially encoded cytochrome *b*; mtDNA = mitochondrial DNA; MT-ND = mitochondrially encoded NADH dehydrogenase; MT-ND1 = mitochondrially encoded NADH dehydrogenase I; MT-ND2 = mitochondrially encoded NADH dehydrogenase II; MT-ND6 = mitochondrially encoded NADH dehydrogenase VI; nDNA = nuclear DNA; P = plasma; Rev = reverse primer; RT = room temperature; S = serum; WB = whole blood. See Table 1 legend for expansion of other abbreviation.

[a]"Blood draw(s)" consists of three distinct elements: the first (single or serial) corresponds to whether the study consisted of a single or serial mtDNA measurements; the second (venous or arterial) defines the source of the whole blood specimen; and the third element (EDTA, heparin, citrate, or variable) describes the type of vacutainer used for whole blood collection. The term "variable" means a combination of tubes were used; these details can be found in e-Appendix 1.

[b]The notation for "sample processing" is as follows: WB, P, and S represent the initial specimen; these abbreviations correspond to whole blood, plasma, and serum, respectively. After the specimen designation there will be two to three numbers. The first number corresponds to the centrifugation speed, the second the centrifugation time in minutes, and the third the temperature in Celsius. If a third number is not present it means the study did not report this information. An "→" supplements the use of the word "then;" it means an additional step was performed. As an example, "WB 1,600*g* 10 4°C → 16,000*g* 10 4°C" reads as "whole blood was initially centrifuged at 1,600*g* for 10 min at 4°C and then the sample was centrifuged at 16,000*g* for 10 min at 4°C."

[c]The cutoffs presented correspond to different types of all-cause mortality; consequently, direct comparisons cannot be made. The two exceptions are Reference 54 (Gu et al, 2013) and Reference 50 (Lu et al, 2010), where the cutoffs correspond to the development of posttraumatic SIRS and "poor outcome" (including mortality), respectively. In addition, this field does not apply to studies where the mortality was zero, which is represented by a dash (—).

[d,e]There are two separate Simmons et al (2017) studies: References 2 and 27. Data represented by *d* correspond to their study on trauma patients receiving blood transfusions, while data represented by *e* correspond to their study on ventilator-associated pneumonia.

[f,g]There are two separate Timmermans et al (2016) studies: References 32 and 37. Data represented by *f* correspond to their study on sepsis while data represented by *g* correspond to their study in patients with trauma.

and 12[30,53] months. Eight studies (20%)[26-28,41,43,48,49,57] did not provide details on the timing of their mortality assessment. Eleven studies (27.5%)[6-8,31,36,38,43,47,51,55,57] reported cell-free mtDNA levels were higher in nonsurvivors than survivors; while four studies (10%)[26,28,30,46] showed there was no association between mtDNA and mortality. Interestingly, one study[42] showed mtDNA was significantly elevated in nonsurvivors of trauma, but not significantly elevated in nonsurvivors of sepsis. Two studies[44,50] found cell-free mtDNA to be significantly higher at one or more time points in patients with poor outcome (including mortality) than in patients with a good outcome from subarachnoid hemorrhage and bacterial meningitis. Another study found cell-free mtDNA to be significantly elevated on admission in post-cardiac arrest patients, but mtDNA did not correlate with unfavorable neurological outcome (including mortality).[33] For the remaining studies, the association between mortality and levels of mtDNA could not be assessed, either because the mortality was zero[2,23,29,34,40,41,45,54,56] or it was not discussed in the source material.[22,24,25,27,32,35,37,39,48,49,52,53] Of these 23 studies without a formal assessment between mtDNA and mortality, only two studies[23,45] did not find mtDNA to be significantly elevated in their population of interest. Mortality data for studies, stratified by patient population, can be found in e-Table 2.

Data on the receiver operating characteristic curves for mtDNA and mortality were reported in eight studies (20%).[6-8,31,38,47,51,55] Five investigators[7,30,35,38,42] furnished additional information on receiver operating characteristic curves from their studies on request. The data are presented in Figure 3. The area under the curve (AUC) ranged from 0.61 (95% CI, 0.52-0.70)[38] to 0.95 (95% CI, 0.88-1.01).[51] In three studies, the AUC for mtDNA and mortality was compared with a currently accepted biomarker/clinical model (namely, lactate, Acute Physiology and Chronic Health Evaluation [APACHE], troponin, and MELD).[6-8] These details can be found within the "Observation" column of e-Table 2.

Morbidity

The association between indicators of morbidity (ie, ICU length of stay) or specific organ failures (ie, acute kidney injury or acute respiratory distress syndrome) and mtDNA was not consistently reported. The available details can be found in e-Table 2.

Discussion

By systematically reviewing data from almost 3,500 subjects included in 40 clinical studies, we found that there is a growing international and multidisciplinary interest in mtDNA as a biomarker in critically ill patients. Over one-quarter of the studies in this systematic review found mtDNA to be significantly elevated in nonsurvivors relative to survivors. When reported, AUC analysis usually suggested a statistically significant association between the levels of mtDNA and mortality of critically ill patients.

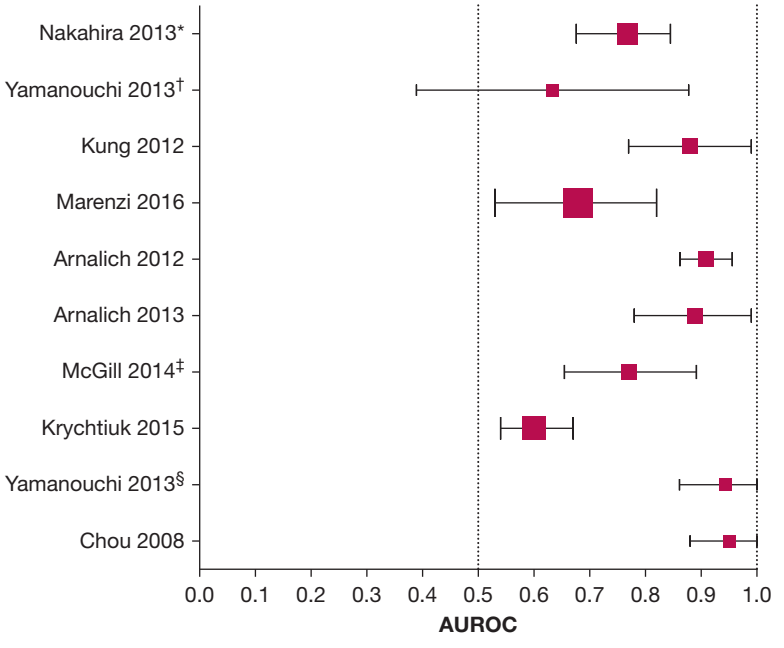

Figure 3 – *Forest plot illustrating the area under the receiver operating characteristic curve with CIs for mtDNA and all-cause mortality in critically ill patients. The data presented here were either readily available within the articles or made available by the authors on request. The studies are organized according to discipline, with medicine patients near the top and surgery patients near the bottom of the plot. The size of each square denotes the proportional size of each cohort. *Data derived from the Brigham and Women's Hospital cohort. †Data derived from the sepsis subgroup. ‡Data collected from use of NADH as a primer. §Data derived from the trauma subgroup.*

An important clinical question is whether mtDNA outperforms established biomarkers (eg, lactate) or prognostic tools (eg, APACHE). Nakahira et al[6] found mtDNA, procalcitonin, and lactate to be significantly associated with 28-day mortality in critically ill patients. They further found that mtDNA remained associated with mortality after adjustment for procalcitonin and lactate, but the odds ratio for lactate was significantly attenuated when adjusted for mtDNA. Although the above results from a single study imply that mtDNA might outperform lactate,[6] it should be emphasized that this is not a robust way to compare biomarkers and that further studies are warranted before drawing any conclusions. Most studies did not provide a detailed comparison between mtDNA and current prognostic tools. Of what was reported, three of five studies found no correlation between mtDNA and APACHE II[6,33,38,42,54] and two studies found that mtDNA did not correlate with Sepsis-Related Organ Failure Assessment (SOFA), while a third study actually found a slight negative association between mtDNA and SOFA in sepsis.[38,42,46] On the other hand, mtDNA was found to correlate with Injury Severity Score in trauma[42,54] and to have an AUC similar to MELD for 21-day mortality in acetaminophen-induced acute liver failure.[7]

Even though most studies reporting on the association between mtDNA and mortality found a positive association, a handful of studies found no association. Such discrepant results may be due to unreported differences in patient characteristics or management. Recently, Hotz et al[58] provided compelling data that RBCs scavenge circulating mtDNA through TLR-9. They theorized that mtDNA levels rise during critical illness because the inflammatory milieu either saturates or impairs RBC scavenging. Interestingly, TLR-9 is not ubiquitously expressed on the surface of RBCs, and while the factors influencing expression have not been elucidated there may be differences in RBC scavenging based on blood type. In contrast, other investigators have shown negative correlations between plasma mtDNA levels and the administration of intraoperative IV fluids or antibiotics.[40,47] We acknowledge that differences in ABO blood type, blood transfusion, resuscitation, and timing of antibiotics are unlikely to impact mtDNA measurements on a large scale, but such differences may become significant in small cohorts, a prominent feature of the studies in this review.

Apart from differences in patient characteristics, another explanation for discrepant results could be related to a significant amount of variation in the isolation and measurement of mtDNA. Care must be taken in the preparation of plasma to ensure adequate removal of mitochondria-containing cells, with two-step centrifugation, higher speeds, and filtration yielding platelet-poor plasma of higher quality.[59] Furthermore, while most investigators selected primers to subunits of NADH dehydrogenase, the actual sequence or size of the amplicon is seldom reported—thus, it should not be assumed that different investigators are measuring identical regions of the reported genes. Primer differences may lead to variation in the number of CpG repeats within measured mtDNA fragments, with CpG-dense fragments more likely to yield significant associations due to a stronger interaction with TLR-9.[16,17]

Another consideration is whether the prognostic ability of plasma mtDNA is affected by the timing of specimen retrieval. An interesting observation lies in the kinetics provided by Wang and colleagues[44] during their study of patients with aneurysmal subarachnoid hemorrhage. Cerebrospinal fluid (CSF) mtDNA, not plasma mtDNA, was significantly elevated in patients with poor outcome on day 1. As CSF mtDNA trended down, plasma mtDNA rose until it became significantly elevated on day 8, corresponding to when CSF mtDNA began to nadir. Similarly, in a study of patients with ventilator-associated pneumonia, Simmons et al[2] found mtDNA to be significantly elevated in bronchoalveolar lavage in patients on day 1, but 24 hours needed to transpire before mtDNA was significantly elevated in serum. Together, these findings suggest it may be prudent to trend mtDNA like troponin.[60]

This systematic review has limitations. Although our key phrase was broad, permitting a rigorous review of the literature, we recognize our search results may nonetheless be subject to publication bias. Also, while the generalizability of this study is increased by the diversity of the patient populations, we recognize that the small size and lack of external validation cohorts is limiting, especially when attempting to assess the prognostic value of a candidate biomarker.[61] The small size of included studies may also explain the considerable imprecision regarding the association between mtDNA levels and mortality, as indicated by the wide 95% CI of Figure 3. In addition, a meta-analysis could not be performed because of considerable clinical heterogeneity among the included studies, mainly in terms of measurement of mtDNA without risk of providing a summary treatment effect

that could be inaccurate or misleading.[62] Plus, six different units of measurement were used to report mtDNA levels, rendering it impossible to synthesize the data in a statistically meaningful manner. Given that clinical heterogeneity precluded carrying out a meta-analysis, we chose not to quantify statistical heterogeneity (using I^2) in Figure 3. Also, data were not consistently available to examine the association between mtDNA and specific organ failures, such as acute kidney injury or acute respiratory distress syndrome. Last, while there are encouraging data about the use of mtDNA as a biomarker for a variety of conditions, we remain hesitant to draw any firm conclusions since almost half of the studies had either a mortality of zero or the investigators did not comment on the relationship between mtDNA and mortality. With respect to the latter, we reached out to corresponding authors for additional mortality data and included it when available.

Conclusion

There is a growing international interest in evaluating the prognostic value of circulating cell-free mtDNA for predicting mortality of critically ill patients. Most of the published studies are relatively small, without validation cohorts, and vary considerably in terms of how mtDNA is measured. The results of most studies that performed AUC analysis suggest there is a statistically significant association between mtDNA levels and the mortality of critically ill patients. To move forward, the field needs to take steps to standardize how mtDNA is measured to facilitate large, prospective, multicenter trials to further define the ability of mtDNA to predict outcomes in critically ill patients.

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Additional information: The e-Appendix and e-Tables can be found in the Supplemental Materials section of the online article.

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