CHEST Online Supplement

Circulating Mitochondrial DNA as Predictor of Mortality in Critically Ill Patients

A Systematic Review of Clinical Studies

John S. Harrington, MD; Jin-Won Huh, MD; Edward J. Schenck, MD; Kiichi Nakahira, MD, PhD; Ilias I. Siempos, MD; and Augustine M.K. Choi, MD

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e-Appendix 1.

Discrepancies from PROSPERO – QUIPS

Although we initially intended to use a modified version of the Quality Assessment of Diagnostic Accuracy Studies (QUADAS) tool, after registering this systematic review with PROSERPO and prior to extracting data from any articles, we chose to use the Quality in Prognosis Study (QUIPS) tool, because QUIPS is a more appropriate tool than QUADAS to assess the prognostic, rather than the diagnostic, potential of mitochondrial DNA in critically ill patients.

Discrepancies from PROSPERO – Lack of a Sensitivity Analysis

As seen in Supplemental Table E3, all studies had a fairly moderate-to-high risk of bias, depending on the domain, and therefore a sensitivity analysis including only trials with low risk of bias was not feasible.

Discrepancies from PROSPERO – Decision to Not Proceed with a Meta-Analysis

Our intent was to perform a meta-analysis with three pre-planned sub-group analyses (medical versus surgical patients, adult versus pediatric populations, and size of clinical studies). However, after data extraction it became clear there were significant differences between how plasma was being collected and processed as well as how mtDNA was being measured and reported. Due to these differences in technique, the results were not felt to be comparable. Consequently, a meta-analysis was not performed.

"Critically Ill" Further Defined

For the purposes of this study, we defined "critically ill" as having a diagnosis that could result in admission to an ICU. Accordingly, we prioritized studies were investigators explicitly stated patients were being admitted to the ICU or provided ICU length of stay for the cohort. In circumstances were ICU admission was not clearly stated or when a portion of the cohort was admitted to the ICU, both JSH and IIS had to agree the cohort was critically ill enough to warrant enrollment. Studies not meeting this criterion included: one study of patients with AML receiving scheduled chemotherapy [1], one longitudinal study of patients with idiopathic pulmonary fibrosis [2], one study of patients with lipodystrophy from HIV on HAART [3], and one study of patients with obstructive sleep apnea [4].

Details on Assessment of Risk of Bias of Included Studies

In order for a study to have a low risk of bias for "Study Participation" the investigators had to cite consecutive enrollment or provide a flow diagram detailing enrollment to ensure adequate participation of eligible patients. Studies that did not meet one of these requirements were considered to at least be at a moderate risk of bias. Regarding "Study Attrition," studies that did not explicitly state their drop-out-rate or provide information on patients who were lost to follow-up were considered to at least be at a moderate risk of bias.

The "Prognostic Factor Measurement" domain is intended to assess how the prognostic factor was measured to screen for misclassification bias. As there is no universally accepted protocol for the measurement of mtDNA, such an assessment is inevitably arbitrary. Hence, the decision was made to examine whether or not investigators reported: centrifugation speed and time, DNA isolation kit, quantitative polymerase chain reaction (qPCR) primer, and qPCR standard. These four variables were selected to assess the validity of the technique of each investigator because they were considered to be the minimum amount of information needed to replicate a given protocol. Any article reporting information for all four variables was considered to have a low risk of bias for this domain. Articles missing information on one or more than one of these variables was considered to have a moderate or high risk of bias, respectively. Given this review's focus on the prognostic ability of mtDNA, studies had to cite either mortality or severity of illness as their primary outcome to be considered at low risk of bias for "Outcome Measurement." If mortality or severity of illness was the secondary outcome or incidentally reported, then the article was considered to be at moderate or high risk of bias due to study design.

For the "Study Confounding" domain, studies were appraised according to whether or not investigators took steps to address any confounding variables they deemed relevant. Thus, studies that performed a univariate analysis before a detailed multivariate analysis were at a low risk of bias, those reporting only a univariate analysis or a multivariate analysis without providing a rationale for the selection of covariates were considered to be at a moderate risk of bias, and studies that did not address confounding variables at all were at a high risk of bias. Finally, to be at low risk of bias for 'Statistical Analysis and Reporting' a study had to be pre-registered and provide a power analysis to account for how they arrived at their sample size. The absence of one of these details resulted in a moderate risk of bias and the absence of both a high risk of bias.

Table 1: Article Characteristics – Further Definition of Subpopulations and 'Good versus Bad Outcome'

Aslami 2018 measured mitochondrial DNA (mtDNA) in patients who were cooled to 33°C post-cardiac arrest and patients who were cooled to 36°C post-cardiac arrest [5].

Paunel-Görgülü 2017 investigated mtDNA in patients who were on cardiopulmonary bypass for less than 100-minutes (denoted by CPB < 100) and those who were on cardiopulmonary bypass for more than 100-minutes (denoted by CPB > 100) [6].

Simmons 2017 investigated mtDNA in trauma patients requiring blood transfusions. 'Severity of Illness' was reported on the basis of who would go on to develop acute respiratory distress syndrome (ARDS) [7].

Simmons 2017 investigated mtDNA in patients with suspicion for ventilator associated pneumonia (VAP). 'Severity of Illness' was reported according to final diagnosis of VAP as determined by the results of quantitative culture from bronchoalveolar lavage (a positive culture was defined as > 10,000 CFU/mL) [8].

Marenzi 2016 investigated the prognostic ability of cytochrome c in acute myocardial infarction. 'Severity of Illness' was reported according to whether or not there was a detectable level of cytochrome c (defined as a level > 0.08ng/mL) in

plasma. While there was both a derivation and a validation cohort for cytochrome c, mtDNA data was only measured in the cytochrome c validation cohort [9].

In *Omura 2016,* 'Severity of Illness' was reported according to whether patients had a 'Favorable' (cerebral performance category 1 and 2 at 30-days) or 'Unfavorable' (cerebral performance category ≥ 3 at 30-days) outcome [10].

In *Wang 2013* patients were analyzed according to whether they had a 'Good Outcome' (defined as a Modified Rankin Scale score ≤ 2 at 6-months) or a 'Poor Outcome' (defined as a Modified Rankin Scale score ≥ 3 at 6-months) [11].

In *Wang 2012* patients were analyzed according to whether they had a 'Good Outcome' (defined as a Modified Rankin Scale score ≤ 1) or a 'Poor Outcome' (defined as a Modified Rankin Scale score ≥ 2) [12].

Table 2: Description of mtDNA Assay – 'Variable' Further Defined

In *Hampson 2017*, 'variable' means blood was collected in vacutainers containing either heparin, EDTA, z-serum clotting activator, or citrate [13].

In *Donnino 2017,* 'variable' means blood was collected in vacutainers containing citrate or unspecified clotting tubes [14].

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e-Table 1: Standard Data Extraction Form with Definition of Variables

Variable	Definition of Variable
Author	Last name of the study's first author.
Year of Publication	The year of written publication.
Article	Full title of the article.
Country	The country patients lived in at the time of enrollment.
Single Center vs. Multi-Center	Whether it was a single or multi-center study.
Study Design	Description of how the observational study was designed (prospective vs. retrospective, cohort vs. case-control, etc.). Also extracted was whether healthy volunteers were used as a control. Besides the observational study, some investigators performed complementary *ex vivo*, *in vivo*, or *in vitro* studies. These additional details were extracted.
Validation Cohort	If the study contained a derivation and validation cohort for mitochondrial DNA as a biomarker.
Discipline	Broad description of the specialty caring for the enrolled patients (i.e. medicine, surgery, neurology, etc.).
Population	Admitting diagnosis.
Setting	Location of the patients and the blood draw(s) during the study. All reported locations were included. Post-cardiac arrest paints and acute aneurysmal subarachnoid hemorrhage patients were assumed to have been admitted to an intensive care unit. If the location was not reported it was documented as 'NR.'
Number of Patients	The total number of patients enrolled in the cohort of interest. Details on the size of relevant sub-populations, as defined by the investigators, was also extracted.
Age	The reported age for the cohort of interest. Similar to 'Number of Patients,' we prioritized data for the entire cohort, but also recorded details on relevant subpopulations. Data not provided in the final manuscript.
Cancer	If patients with malignancy were included in the study, the characteristics of this subpopulation was extracted. Appropriate documentation was also performed if these patients were excluded or their inclusion was not reported. These details were not provided in the final manuscript due to limited data points.
Lactate	Data pertaining to the measurement of lactate in the cohort(s) was extracted. There details were not provided in the final manuscript due to limited data points.
Severity of Illness	The severity of illness score for the cohort(s) of interest. Some investigators published multiple severity of illness scores for their patient population(s). When this occurred, we extracted all available data. However, we elected to report the most commonly used or relevant scores (i.e. APACHE II or ISS) to avoid making Table 1 unnecessarily convoluted.
Morbidity	Data related to ICU length of stay, hospital length of stay, etc. was extracted from the text, tables, and figures as appropriate. Special attention was paid to data related to the association between mitochondrial DNA and morbidity.
Mortality	Data related to all-cause mortality was extracted from the text, tables, and figures as appropriate. Special attention was paid to data related to the association between mitochondrial DNA and all-cause mortality.

AUC for mitochondrial DNA	When provided, the area under the receiver operating characteristic curve with confidence intervals for mitochondrial DNA and all-cause mortality was extracted.
Cut-Off	The cut-off values calculated for mtDNA and all-cause mortality from ROC curve analysis.
Blood Draw	How blood was drawn from patients. This included whether the source was arterial or venous, the type of tube used for specimen collection, and whether blood was drawn once or at multiple time points.
Time Points	When blood was drawn from patients.
Peak	If multiple blood draws were performed, the time mtDNA was observed to peak was recorded. Data was extracted from the text, tables, and figures as appropriate.
Sample Processing	How patient samples were centrifuged. Special attention was paid to the speed of centrifugation, length of centrifugation, temperature of centrifugation, and number of rounds of centrifugation.
Filtration	Whether a filter was used to isolate mitochondrial DNA. If a filter was used, the size of the filter was recorded.
Serum or Plasma	Whether mitochondrial DNA was being measured in serum or plasma.
Kit	The name of the kit used by the investigators to isolate mitochondrial DNA. Data not provided.
Primer	The primer(s) used by investigators to measure the level of mitochondrial DNA.
Standards	The type of standard investigators used to quantify the level of mitochondrial DNA.
Units of Measurement	The units of measurement used to report mitochondrial DNA levels.

e-Table 2: Kinetics, Observations, and Mortality

Author and Year of Publication	Time Points	Peak	Observations	Mortality
MEDICINE - SEPSIS				
Timmermans 2016 (32)	24h post-dx of septic shock (Day 1) → ICU Day 3, 5, 7, 9, 14, 21, 28	Day 1	- mtDNA was significantly ↑ in patients relative to controls from Day 1 to Day 21 (~ 5-fold inc.) - mtDNA did not correlate with levels of inflammatory cytokines (TNF-α, IL-6, IL-8, IL-10, IL-1RA, and WBC)	- *28-Day Mortality: 54/121* - Investigators did not comment upon the relationship between mtDNA and mortality
Schäfer 2016 (36)	Within 24h of diagnosis of sepsis	NA	- mtDNA was significantly ↑ in patients relative to controls - D-Loop: 76-fold inc.; $p < 0.0001$ - MT-ATP6: 123-fold inc.; $p < 0.0001$ - In a human monocyte model, mtDNA exposure significantly ↑ mRNA expression of proinflammatory cytokines (TNF-α, IL-1β, and HIF-1α)	- *30-Day Mortality: 59/165* - mtDNA was significantly ↑ in 30-day NS vs. S - D-Loop: 1.6 fg/µl ± 3.6 *v.* 0.4 fg/µl ± 1.2; $p = 0.003$ - MT-ATP6: 1.3 fg/µl ± 3.4 *v.* 0.55 fg/µl ± 2.3; $p = 0.005$
Bhagirath 2015 (40)	ICU Admission	NA	- mtDNA was significantly ↑ in patients relative to controls - MT-CYB: > 50-fold inc. - 0.43 µg/ml ± 0.25 *v.* 8.5×10^{-3} µg/ml ± 6.4×10^{-3}; $p < 0.05$ - Mit3153T: - 1.5 µg/mL ± 1.2 *v.* 6.1×10^{-5} µg/mL ± 9.0×10^{-5}; $p < 0.05$	- *Hospital Mortality: 4/12* - Investigators did not comment upon the relationship between mtDNA and mortality
Puskarich 2012 (50)	At Enrollment	NA	- mtDNA was not ↑ in patients relative to controls - MT-CYB and MT-CO3 had a significant negative association with SOFA score	- *Hospital Mortality: 11/69* - mtDNA was not associated with mortality
Kung 2012 (51)	ED Admission (Day 1) → Day 4, 7	Day 1	- mtDNA was significantly ↑ in patients with sepsis relative to controls - 436 ng/ml [IQR 216 – 1140] *v.* 149 ng/ml [IQR: 79 – 304]; $p < 0.001$ - mtDNA, at admission, was significantly ↑ in patients receiving mechanical ventilation in the ED *v.* those who were not - 380 ng/mL *v.* 183 ng/ml; $p = 0.047$ - mtDNA ↓ after initiation of antibiotics - mtDNA did not correlate with and other biomarkers (lactate, CRP, N-terminal BNP, and procalcitonin)	- *Hospital Mortality: 11/67* - mtDNA was significantly ↑ in NS vs. S on admission - mean: 723 ng/mL ± 830 vs. 161 ng/mL ± 128, $p < 0.001$ - mtDNA was significantly ↑ in NS vs. S on Day 4 - mean, 406 ng/mL ± 367 vs. 182 ng/mL ± 129, $p = 0.001$) - Fatality rate inc. by 0.7% per 1.0 ng/mL inc. in mtDNA
Garrabou 2011 (53)	NR	NA	- mtDNA was significantly ↑ in patients with sepsis relative to controls (316% inc.; $p < 0.05$)	- *Mortality, Unspecified: 6/19* - Investigators did not comment upon the relationship between mtDNA and mortality

Author and Year of Publication	Time Points	Peak	Observations	Mortality
MEDICINE - ACUTE MYOCARDIAL INFARCTION				
Qin 2017 (29)	Admission → 12h, 24h, 48h Post-PCI	Admission	- mtDNA, at admission, was significantly ↑ in patients relative to controls - 478 copies/µl ± 106 *v.* 157 copies/µl ± 97; $p < 0.01$ - mtDNA quickly ↓ from admission to 12h post-PCI - mtDNA gradually normalized from 12h to 48h post-PCI - mtDNA correlated positively with markers of inflammation (WBC, TNF-α, IL-6, and CRP)	- *Mortality, Unspecified: 0/38*
Marenzi 2016 (30)	Hospital Admission	NA	- mtDNA was significantly ↑ in patients with detectable levels of cytochrome c	- *Hospital Mortality: 20/753* - *1-Year Mortality: 47/753* - mtDNA was not associated with mortality
Fernández-Ruiz 2014 (43)	36h (± 6h) Post Hospital Admission	NA	- mtDNA was significantly ↑ in patients with STEMI relative to those with unstable angina or controls (~ 3.5 fold inc.; $p < 0.01$) - mtDNA was slightly ↑ in patients with NSTEMI - *in vivo* data suggests mtDNA is associated with M2 polarization	- *1-year Fatal Myocardial Infarction: 2/75* - Investigators did not comment upon the relationship between mtDNA and mortality

Author and Year of Publication	Time Points	Peak	Observations	Mortality
MEDICINE – POST-CARDIAC ARREST				
Donnino 2017 (28)	Within 6h of sustained ROSC → 12h, 24h, 36h, 48h Post-ROSC	No Peak	- No difference in $tRNA^{leu}$ levels in patients relative to controls - 1.0-fold change [IQR 0.7 – 1.3] *v.* 0.8-fold change [IQR 0.5 – 1.3]; p = 0.10 - D-Loop levels were significantly ↓ in patients relative to controls - 0.7-fold change [IQR 0.4 -1.1] *v.* 1.4-fold change [IQR 0.6 – 2.4]; p = 0.001	- *Hospital Mortality: 55/102* - mtDNA was not associated with mortality
Omura 2016 (33)	ED Admission → Day 2, 3, 5, 7 Post-ROSC	Admission	- mtDNA, on admission, was significantly ↑ relative to Day 2, 3, 5, 7 (p < 0.001) - mtDNA correlated with initial lactate (r 0.463, p = 0.034) - mtDNA did not correlate with time to ROSC, NH_3, myoglobin, APACHE II, or IL-6 - mtDNA was not significantly different between patients with a favorable *v.* unfavorable neurological outcome (p = 0.573)	- *30-Day Mortality: 7/21*
Timmermans 2015 (39)	Within 24h of ICU Admission (Day 0) → Day 1, 2	Day 2	- mtDNA was significantly ↑ in patients on Day 0 and Day 2 relative to controls	- *Hospital Mortality: 5/15* - Investigators did not comment upon the relationship between mtDNA and mortality
Arnalich 2012 (49)	Immediately Post-ROSC	NA	- mtDNA, in NS, correlated with sFas (r = 0.32; p < 0.01)	- *3-Day Mortality:* Represented by ROC Curve - *24-Hour Mortality: 30/85* - *Hospital Mortality: 56/85* - mtDNA was significantly ↑ in NS *v.* S - 6982 GE/mL ± 2102 *v.* 3504 GE/mL ± 1484; p < 0.01

Author and Year of Publication	Time Points	Peak	Observations	Mortality
MEDICINE – ACETAMINOPHEN OVERDOSE				
McGill 2014 (7)	Day 1, 2, 3, 4, 5	Day 1	- mtDNA was significantly ↑ in patients relative to controls - MT-ND: 30-fold inc. 1.0 ng/mL ± 0.3 *v.* 32 ng/mL ± 5; p < 0.05 - MT-CO3: 40-fold inc. 1.0 ng/mL ± 0.8 *v.* 40 ng/mL ± 6; p < 0.05	- *21-Day Mortality: 35/69* - mtDNA, at admission and at ALT peak, was significantly ↑ in NS *v.* S - ROCC for mtDNA and mortality was similar to the ROCC for MELD
McGill 2012 (52)	Day 1, 2, 3, 4, 5, 6	Coincided with ALT Peak	- mtDNA is significantly ↑ in patients relative to controls	- *Mortality, Unspecified: 1/40* - Investigators did not comment upon the relationship between mtDNA and mortality

Author and Year of Publication	Population	Time Points	Peak	Observations	Mortality
MEDICINE - MISCELLANEOUS					
Nakahira 2013 (6)	MICU (BWH RoCI Cohort)	Within 24h of enrollment → Day 7	NA	- mtDNA was significantly ↑ in patients with sepsis and ARDS than other ICU diagnoses - mtDNA was associated with acute kidney injury, the need for mechanical ventilation, use of vasopressors, and an underlying diagnosis of cancer - The odds ratio for mtDNA (≥ 3,200 copies/μL) and 28-day mortality was 6.6 [95% CI 3.2 – 13.4; p = $3x10^{-7}$] - The odds ratio for an elevated procalcitonin and 28-day mortality was 1.02 (95% CI 1.01 – 1.03; p = 0.004) - The odds ratio for lactate and 28-day mortality was 1.4 (95% CI 1.1 – 1.7; p = 0.002) -	- *28-Day Mortality: 60/200* - *Hospital Mortality: 51/200* - *Overall Mortality: 93/200* - mtDNA was significantly ↑ in NS *v.* S - median: 9,504 copies/μL *v.* 1,927 copies/μL; p = 2×10^{-8}
	ARDS (ME ARDS Cohort)	Within 48h of diagnosis	NA	- mtDNA was significantly ↑ in patients with sepsis and ARDS than other ICU diagnoses - mtDNA was associated with mortality for MICU patients, but not for non-MICU patients	- *28-Mortality: 40/243* - mtDNA was significantly ↑ in NS *v.* S - median: 7,457 copies/ μL *v.* 2,846 copies/ μL; p = 5×10^{-6}
Arnalich 2013 (8)	Pulmonary Embolism	Within 7h of ED Admission	NA	- mtDNA correlated with H-FABP (r = 0.476; p < 0.01), lactate (r = 0.451; p < 0.01), and sFas (r = 0.379; p < 0.01)	- *15-Day PE Related Mortality: 18/74* - In massive pulmonary embolism, mtDNA was significantly ↑ in NS *v.* S - median: 4,220 GE/mL *v.* 1,830 GE/mL; p < 0.01 - AUC for mtDNA and mortality was significantly ↑ than troponin and mortality - mtDNA: 0.89 [95% CI 0.78 – 0.99] - trop: 0.59 [95% CI 0.41 – 0.79]; p = 0.015

Author and Year of Publication	Time Points	Peak	Observations	Mortality
SURGERY - TRAUMA				
Simmons 2017 (27)	Within 8h of Transfusion → 24h to 48h Post-transfusion	Non-ARDS: Initial ARDS: 24 to 48h	- mtDNA was significantly ↑ in FFP and platelets than PRBCs - FFP: 195.8 ng/mL ± 58.8 *v.* Plt: 93.9 ng/mL ± 24.3 v. PRBC: 3.0 ng/mL ± 0.4; p < 0.01 - mtDNA differed by shelf-life in PRBCs and FFP - PRBC: young - 2.2 ng/mL ± 0.6 FFP: young - 265.2 ng/mL ± 83.5 moderate - 3.5 ng/mL ± 0.8 moderate - 87.1 ng/mL ± 39.1 old - 2.9 ng/mL ± 0.6 old - 19.3 ng/mL ±7.6 p = 0.009 p < 0.001 - mtDNA differed significantly by ABO blood type - A: 189.4 ng/mL ±81.1 B: 33.7 ng/mL ± 21.1 - AB 15.0 ng/mL ± 5.4 O: 49.3 ng/mL ±16.2 p < 0.001 - mtDNA did not differ in PRBCs by sex, but trended toward being ↑ in ♀ platelets relative to ♂ platelets (198.5 ng/mL ±172.5 *v.* 33.86 ng/mL ± 5.412; p = 0.29) - mtDNA was significantly ↑ in ♀ FFP relative to ♂ FFP - 690.5 ng/mL ± 128.1 *v.* 178.6 ng/mL ± 67.3; p = 0.046 - Patients who developed ARDS received significantly more mtDNA DAMPs during transfusions than patients who did not develop ARDS - 3.8 x 10^4 ng/mL ± 9.6 x 10^3 *v.* 1.9 x 10^4 ng/mL ± 6.6 x 10^3; p < 0.05) - Serum mtDNA concentration after transfusion correlated with cumulative transfused mtDNA (r^2 = 0.74, p < 0.01)	- *Mortality, Unspecified: 1/14* - Investigators did not comment upon the relationship between mtDNA and mortality
Mohamed 2016 (31)	ED Admission	NA	- mtDNA was significantly ↑ in patients relative to controls - mtDNA was significantly ↑ in patients who developed post-trauma complications relative to those who did not - Patients who developed ARDS had significantly ↑ levels of mtDNA than those who developed sepsis, which in turn were significantly ↑ than those who developed acute myocardial infarction - ROC curve analysis suggested admission mtDNA had high sensitivity as predictor of ICU mortality	- *ICU Mortality: 11/61* - mtDNA was significantly ↑ in NS v. S - 11,040.9 copies/µl ± 9116 *v.* 4,011.6 copies/µl ± 3885; p = 0.045
Timmermans 2016 (37)	Pre-hospital → ED Admit → Day 1, 3, 5, 7, 10 Post-trauma	Pre-Hospital	- Pre-hospital and Day 1 mtDNA was significantly ↑ in patients relative to controls - mtDNA was non-significantly ↑ in patients relative to controls at all other time points - mtDNA did not correlate with HLA-DRA mRNA expression (r = -0.09, p = 0.33) - mtDNA, at ED Admit, was significantly ↑ in patients who developed infection within 28-days *v.* those who did not (2.5-fold inc. [1.4 - 6.6] *v.* 1.4-fold inc. [0.5 – 4.0]; p = 0.046)	- *28-Day Mortality: 39/166* - Investigators did not comment upon the relationship between mtDNA and mortality
McIlroy 2015 (41)	Pre-op → Post-op →	Day 5	- mtDNA was significantly ↑ at all time points relative to controls	- *Hospital Mortality: 0/35*

	7h post-op → 24h post-op → Day 3, 5		- Pre-operative mtDNA levels correlated with post-operative levels (p = 0.0138) - Immediate post-operative mtDNA levels were negatively correlated with intraoperative fluid administration (p = 0.0017) - mtDNA ↑ was independent of tissue necrosis markers (CK, LDH, AST)	
Simmons 2013 (45)	Within 8h of ICU Admit (Day 0) → Day 1, 2, 6	Day 6	- mtDNA was significantly ↑ in patients who developed SIRS within 48h of presentation	- *Mortality, Unspecified:* 4/13 - mtDNA was significantly ↑ in NS *v.* S - COX1: RR 20.4 [95% CI 1.3 – 318] - D-Loop: RR 8.0 [95% CI 1.16 – 55.2] - ND1: RR 8.0 [95% CI 1.15 – 55.8] - ND6: RR 20.4 [95% CI 1.3 – 318] - $p < 0.05$
Gu 2013 (46)	ICU Admit	NA	- mtDNA was significantly ↑ in trauma patients relative to healthy controls ($p < 0.001$) - mtDNA was significantly ↑ in patients who developed post-traumatic SIRS *v.* those that did not - 1,774.03 pg/mL [IQR 564.87 - 10,901.3] *v.* 500.496 [145.415 - 1,285.6]; $p < 0.001$ - mtDNA correlated with APACHE II ($r = 0.230$, $p = 0.034$) & ISS ($r = 0.454$, $p < 0.001$) - Subgroup analysis of post-traumatic patients with SIRS, found mtDNA to be significantly associated with the degree of inflammatory response, including organ dysfunction	- *Hospital Mortality: 0/86*
Lam 2004 (57)	ED Admit	NA	- mtDNA was significantly ↑ in patients relative to controls - median 8,586,300 copies/mL vs. 1,607,000 copies/mL; p = 0.003	- *Mortality, Unspecified: 2/38* - mtDNA was significantly ↑ in NS *v.* S - median: 340,000,000 copies/mL *v.* 8,325,000 copies/mL; p = 0.02

Author and Year of Publication	Time Points	Peak	Observations	Mortality
SURGERY – CARDIOPULMONARY BYPASS				
Paunel-Görgülü 2017 (25)	Admission → Immediately Post-op → Post-op Day 1, 3, 5, 8	Day 8	- mtDNA was significantly ↑ in patients with long-CPB relative to those with short-CPB - mtDNA ↑ during the post-operative period in patients with long-CPB, with most significant elevations noted on Day 3 and 8	- *Hospital Mortality: 4/48* - Investigators did not comment upon the relationship between mtDNA and mortality
Qin 2016 (34)	Admission → End of Cardiopulmonary Bypass → 6h, 12h, 24h Post-op	12h	- mtDNA was significantly ↑ at the end of bypass and all subsequent time points relative to admission ($p < 0.01$) - mtDNA at 12h displayed significant correlation with peak CRP ($r = 0.72$, $p < 0.01$), peak BNP ($r = 0.639$, $p < 0.01$), and peak PCT levels ($r = 0.588$, $p < 0.01$)	- *Hospital Mortality: 0/46*

Author and Year of Publication	Population	Time Points	Peak	Observations	Mortality
SURGERY - MISCELLANEOUS					
Leijte 2018 (23)	Post CRS-HIPEC	Induction → Post CRS → Post HIPEC → Post ICU Admit → 24hr Post-op	Day 1	- mtDNA was significantly ↓ in patients at baseline relative to controls - mtDNA was not significantly ↑ in patients relative to controls - mtDNA did not correlate with cytokines (TNF-α, IL-6, IL-8, IL-10, MCP-1, MIP-1α, and MIP-1β)	- *28-Day Mortality: 0/20*
Hampson 2017 (26)	Burn	Admission (Day 1) → Day 3, 7, 14, 21, 28 → Month 2, 3, 6, 12	Day 14	- mtDNA was not significantly ↑ - Relative to controls, neutrophils from burn patients released significantly less DNA on Day 3 and Day 7 and were partially to resistant induction of NETosis by PMA	- *Mortality, Unspecified: 20/63* - mtDNA was not associated with mortality
Simmons 2017 (2)	Ventilator Associated Pneumonia	At time of BAL → 24h to 48h Post-BAL	24h	- mtDNA did not differ at time of BAL between patients - mtDNA was significantly ↑ at 24h in patients who would be diagnosed with VAP relative to those who were not - 159.60 ng/mL ± 77.37 *v.* 10.43 ng/mL ± 4.36; $p < 0.05$ - mtDNA in BAL was significantly ↑ in patients with VAP *v.* without - 248.70 ng/mL ± 109.7 *v.* 43.91 ng/mL ± 16.61; $p < 0.05$	- *Hospital Mortality: 0/31*
Chou 2008 (56)	Corrosive Ingestion	ED Admit → 12h later	12h	- In the survival group, there was no significant difference in mtDNA at presentation and 12 hours later - In the mortality group, mtDNA were significantly ↑ in both the emergency room and 12 h later	- *Hospital Mortality: 10/48* - mtDNA was significantly ↑ at admission in NS *v.* S - 235 kiloGE/L *v.* 76 kiloGE/L; $p = 0.000$

Author and Year of Publication	Population	Time Points	Peak	Observations	Mortality
MEDICINE & SURGERY					
Krychtiuk 2015 (38)	Mixed	Within 24h of ICU Admission	NA	- mtDNA was significantly ↑ in medical patients relative to surgical patients and controls - 24.1 ng/mL [IQR 10.7 – 42.6] *v.* 13.4 ng/mL [IQR 6.6 – 29.9] *v.* 13.8 ng/mL [IQR 6.5 – 28.5]; p < 0.05 - mtDNA was not ↑ in surgical patients relative to controls - For medical patients, mtDNA was highest in patients with sepsis (33.2 ng/mL [IQR 17.3 – 62.0]) and heart failure or cardiogenic shock (29.8 ng/mL [IQR 17.2 - 53.6]) *v.* other medical conditions (18.4 ng/mL [IQR 9.3 – 34.1 ng/mL] p < 0.05) - mtDNA was not associated with APACHE II (r = 0.06; p = 0.35), SAPS II (r = 0.06; p = 0.37), or SOFA (r = 0.02; p = 0.82) - mtDNA did not correlate with CRP, procalcitonin, or leukocyte count	- *30-Day Mortality: 59/228* - mtDNA was significantly ↑ in NS *v.* S - 26.9 ng/mL [IQR 11.2 – 60.6] *v.* 19.7 ng/mL [IQR 9.5 – 34.8]; p < 0.05 - When analyzed separately, mtDNA was significantly associated with mortality in medical (27.2ng/mL [IQR 12.5 – 60.6] *v.* 21.1 ng/mL [IQR 9.6 – 37.2]; p < 0.05), but not surgical patients (4.4 ng/mL [IQR 3.3 – 74.5] *v.* 13.6 ng/mL [IQR 8.8 – 29.9]; p – 0.20) - Patients with mtDNA in the highest quartile had a 2.4-fold higher risk of dying than patients in the first quartile (p = 0.008)
Yamanouchi 2013 (44)	Trauma	Admission (Day 1) → Day 2, 3, 5	Day 1	- mtDNA separated by centrifugation was more than 100 times higher than that in plasma after filtration through a 0.22µm filter - mtDNA, on Day 1, was significantly ↑ relative to controls - 0.23 µg/mL [IQR 0.04 – 0.58] *v.* 0.02 µg/mL [IQR 0.2 – 0.03]; p < 0.01 - mtDNA was not significantly ↑ on Day 2, 3, 5 - mtDNA correlated with CPK (r^2 = 0.463; p < 0.05) and ISS (r^2 = 0.362; p < 0.05)	- *28-Day Mortality: 2/37* - mtDNA, on Day 1, was significantly ↑ in NS *v.* S (p < 0.05)
	Sepsis			- mtDNA was significantly ↑ relative to controls on - Day 1: 0.20 µg/mL [IQR 0.06 – 0.80]; p < 0.01 - Day 2: 0.15 µg/mL [IQR 0.05 – 1.04]; p < 0.05 - Day 3: 0.18 µg/mL [IQR 0.06 – 0.16]; p < 0.05 - mtDNA was not significantly ↑ on Day 5 - mtDNA did not correlate with CPK (r^2 = 0.28; p = 0.44) - mtDNA did not correlate with initial lactate, SOFA, or APACHE II	- *28-Day Mortality: 3/23* - mtDNA, on Day 1, was not significantly associated with mortality
Jansen 2018 (24)	Non-Infectious SIRS	Within 24hr of ICU Admit	NA	- mtDNA was significantly ↑ in patients with SIRS vs without - mtDNA level was not further increased by concomitant presence of AKI - mtDNA did not correlate with IL-8, IL-6, PF4, and creatinine:albumin - Urine mtDNA was significantly ↑ in SIRS with AKI vs. SIRS without AKI and ICU controls	- *28-Day Mortality:* 11/37 - *90-Day Mortality:* 15/37 - Investigators did not comment upon the relationship between mtDNA and mortality

				- Urine mtDNA correlated with urinary inflammatory markers (IL-8, IL-6, prothrombin fragment F1+2, vWF, PF4)	
Aslami 2018 (22)	Post-Cardiac Arrest	Admission → 24hr post admit → Return of Core Temp to 36°C (for the 33°C Cohort)	Admission	- mtDNA significantly ↑ in patients relative to controls ($p < 0.05$) - Cooling to 33°C was associated with a relative risk reduction in levels of mtDNA (NADH2, NADH2, COX3, but not cytochrome b) at 24h compared to baseline - mtDNA levels in patients kept at 36°C remained unchanged from baseline - mtDNA (cytochrome B, but not NADH1, NAD2H, and COX3) remained significantly lower after rewarming than patients kept at 36°C	- *ICU Mortality:* 6/20 - Investigators did not comment upon the relationship between mtDNA and mortality

Author and Year of Publication	Population	Time Points	Peak	Observations	Mortality
NEUROLOGY					
Wang 2014 (42)	Traumatic Brain Injury	Within 24h of TBI → Day 4, Day 7 Post-TBI	Day 7	- mtDNA, at presentation, was significantly ↑ in patients relative to controls - 30.2 ng/mL *v.* 12.7 ng/mL; p < 0.001 - mtDNA was not significantly ↑ in the poor outcome group *v.* the good outcome group	- *Hospital Mortality: 0/88*
Wang 2013 (47)	Subarachnoid Hemorrhage	Within 24h of SAH onset (Day 1) → Day 4, 8, 11, 14	Day 8	- mtDNA in plasma and CSF was not significantly ↑ in patients with thick SAH vs. minimal SAH - mtDNA in plasma was significantly ↑ in the poor outcome group vs. the good outcome group (median 51.2 ng/ml vs. 5.9 ng/ml, p = 0.011) on Day 8, only - mtDNA in CSF was significantly ↑ in the poor outcome *v.* the good outcome group on: - Day 1: median – 72.3 ng/mL *v.* 18.0; p = 0.011 - Day 4: median – 49.2 ng/ml *v.* 19.0 ng/ml; p = 0.020	- *6-Month Mortality: 1/21*
Wang 2012 (48)	Intracerebral Hemorrhage	Within 24h of ICH → Day 4, 7, 10, 14 after ICH onset	Good Outcome: Day 1 Bad Outcome: Day 7	- mtDNA was not significantly ↑ in patients with poor outcome vs. good outcome from Day 1 to Day 14	- *Mortality, Unspecified:* 0/60
Tsai 2011 (54)	Acute Ischemic Stroke	Within 48h of stroke onset → Day 7, 30	Day 1	- mtDNA was significantly ↑ on Day 1, 7, and 30 relative to controls (p < 0.001) - mtDNA was not significantly ↑ in the poor outcome group relative to the good outcome group - 3120.9 kiloGE/L ± 970.0 *v.* 2333.1 kiloGE/L ± 272.7; p = 0.30	- *3-Month Mortality: 0/50*
Lu 2010 (55)	Bacterial Meningitis	Admission (Day 1) → Day 7, 14	Day 1	- mtDNA was significantly ↑ in bacterial meningitis relative to controls (p = 0.002) - mtDNA was not significantly ↑ in aspectic meningitis relative to controls - In bacterial meningitis, mtDNA was significantly ↑ relative to controls from Day 1 to Day 14 (p = 0.0001 and p = 0.016, respectively)	- *3-Month Mortality:* < 7 - mtDNA was significantly ↑ on Day 1 in the poor outcome group relative to the good outcome group (median 86ng/ml vs 20ng/ml, p = 0.015) - mtDNA was not significantly ↑ on Day 7 and Day 14 in the poor outcome group vs the good outcome group

Author and Year of Publication	Population	Time Points	Peak	Observations	Mortality
PEDIATRICS					
Di Caro 2016 (35)	Sepsis	Within 24h of ICU Admission	NA	- mtDNA was significantly ↑ in septic patients vs. critically ill non-septic patients and controls - 1.75 x 10^5 copies/µL [IQR 6.64 x 10^4 – 3.67 x 10^5] *v.* 5.73 x 10^3 copies/µL [IQR 3.90 x 10^3 – 1.28 x 10^4] *v.* 6.64 x 10^3 copies/µL [IQR 5.22 x 10^3 – 1.63 x 10^4]; p = 0.001 - mtDNA was not significantly ↑ in critically ill non-septic patients vs controls (p = 1.0) - mtDNA was significantly ↑ in patients admitted with shock vs. those without shock - 1.77 x 10^5 copies/µL [IQR 9.50 x 10^4 – 4.27 x 10^5] *v.* 6.89 x 10^4 copies/µL [IQR 4.96 x 10^4 – 8.56 x 10^4; p = 0.23 - mtDNA was significantly ↑ in patients with MOF vs. patients without MOF - 3.2 x 10^5 copies/µL [IQR 1.41 x 10^5 – 1.08 x 10^6] *v.* 2.9 x 10^4 [IQR 2.47 x 10^4 – 5.43 x 10^4]; p < 0.05	- *ICU Mortality: 2/28*

e-Table 3: QUIPS

Study Author and Date of Publication	Study Participation	Study Attrition	Prognostic Factor Measurement	Outcome Measurement	Study Confounding	Statistical Analysis and Reporting
Aslami 2018 (22)						
Leijte 2018 (23)						
Jansen 2018 (24)						
Paunel-Görgülü 2017 (25)						
Hampson 2017 (26)						
Simmons 2017* (27)						
Donnino 2017 (28)						
Qin 2017 (29)						
Simmons 2017† (2)						
Marenzi 2016 (30)						
Mohamed 2016 (31)						
Timmermans 2016‡ (32)						
Omura 2016 (33)						
Qin 2016 (34)						
Di Caro 2016 (35)						
Schäfer 2016 (36)						
Timmermans 2016§ (37)						
Krychtiuk 2015 (38)						
Timmermans 2015 (39)						
Bhagirath 2015 (40)						
McIlroy 2015 (41)						
McGill 2014 (7)						

Wang 2014 (42)						
Fernández-Ruiz 2014 (43)						
Nakahira 2013 (6)						
Yamanouchi 2013 (44)						
Simmons 2013 (45)						
Gu 2013 (46)						
Arnalich 2013 (8)						
Wang 2013 (47)						
Wang 2012 (48)						
Arnalich 2012 (49)						
Puskarich 2012 (50)						
Kung 2012 (51)						
McGill 2012 (52)						
Garrabou 2011 (53)						
Tsai 2011 (54)						
Lu 2010 (55)						
Chou 2008 (56)						
Lam 2004 (57)						

Footnotes:

The green, yellow, and red circles denote low, moderate, and high risk of bias, respectively.

*, †: There are two separate Simmons 2017 studies. Data represented by * corresponds to to their study on trauma patients receiving blood transfusions while data represented by † corresponds to their study on ventilator associated pneumonia.

‡, §: There are two separate Timmermans 2016 studies. Data represented by ‡ corresponds to their study on sepsis while data represented by § corresponds to their study in patients with trauma.