Published in final edited form as:

Resuscitation. 2017 April ; 113: 56–62. doi:10.1016/j.resuscitation.2016.12.029.

Characterization of Mitochondrial Injury after Cardiac Arrest (COMICA)

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Abstract

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CONFLICT OF INTEREST:

None of the authors declare any conflict of interests.

COMPETING INTERESTS

The authors state that they have no competing interests.

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Introduction—Mitochondrial injury post-cardiac arrest has been described in pre-clinical settings but the extent to which this injury occurs in humans remains largely unknown. We hypothesized that increased levels of mitochondrial biomarkers would be associated with mortality and neurological morbidity in post-cardiac arrest subjects.

Methods—We performed a prospective multicenter study of post-cardiac arrest subjects. Inclusion criteria were comatose adults who suffered an out-of-hospital cardiac arrest. Mitochondrial biomarkers were measured at 0, 12, 24, 36 and 48 hours after return of spontaneous circulation as well as in healthy controls.

Results—Out of 111 subjects enrolled, 102 had evaluable samples at 0 hours. Cardiac arrest subjects had higher baseline cytochrome *c* levels compared to controls (2.18 ng/mL [0.74, 7.74] vs. 0.16 ng/mL [0.03, 0.91], $p<0.001$), and subjects who died had higher 0 hours cytochrome *c* levels compared to survivors (3.66 ng/mL [1.40, 14.9] vs. 1.27 ng/mL [0.16, 2.37], $p<0.001$). There were significantly higher RNAase P (3.3 [1.2, 5.7] vs. 1.2 [0.8, 1.2], $p<0.001$) and B2M (12.0 [1.0, 22.9], vs. 0.6 [0.6, 1.3], $p<0.001$) levels in cardiac arrest subjects at baseline compared to the control subjects. There were no differences between survivors and non-survivors for mitochondrial DNA, nuclear DNA, or cell free DNA.

Conclusions—Cytochrome C was increased in post-cardiac arrest subjects compared to controls, and in post-cardiac arrest non-survivors compared to survivors. Nuclear DNA and cell free DNA was increased in plasma of post-cardiac arrest subjects. There were no differences in mitochondrial DNA, nuclear DNA, or cell free DNA between survivors and non-survivors. Mitochondrial injury markers showed mixed results in post-arrest period. Future research needs to investigate these differences.

Keywords

Cardiac arrest; post-cardiac arrest; mitochondria; cytochrome c; DNA; mortality

1) INTRODUCTION

New insights into the pathophysiology of brain and other organ injury after cardiac arrest are essential to reduce the mortality and morbidity in post-cardiac arrest patients. Ischemia during arrest followed by reperfusion after ROSC, triggers systemic and organ-specific responses during the first few days following resuscitation. Preclinical studies reveal that post-ischemic cell death, particularly in the brain, occurs in concert with alterations in mitochondrial structure and function.[1, 2] We could only identify one single-center human study, evaluating how patterns of mitochondrial injury, as measured by circulating mitochondrial DNA, are related to illness severity.[3] Furthermore, the extent to which markers of mitochondrial injury can be used to track progression of post-resuscitation pathophysiology and response to therapy after cardiac arrest remains unknown. Therefore, evaluation of mitochondrial biomarkers in humans post-cardiac arrest is an important next step in understanding potential mitochondrial injury patterns.

Known mitochondrial biomarkers include cytochrome c and mitochondrial DNA (mtDNA). Cytochrome C is a small water-soluble protein that is bound to the inner mitochondrial membrane and allows for transfer of electrons between complex III and IV of the electron

transport chain (ETC) thus serving a key role in cellular respiration. [4–7] Mitochondrial DNA is located within the mitochondrial matrix and when elevated in the bloodstream, reflects mitochondrial injury. Cell free DNA and nuclear DNA (nDNA) are non-mitochondrial DNA and were used to assess levels of overall cellular damage and for correlating cellular damage to mitochondrial damage. [8–11]

We hypothesize that increased levels of mitochondrial biomarkers (specifically, cytochrome *c* and mitochondrial DNA/RNA products) are associated with mortality and neurologic morbidity in post-cardiac arrest patients. To test this hypothesis, we performed a prospective, multicenter observational study in out-of-hospital post-cardiac arrest patients.

2) METHODS

Design and Setting

The National Post-Arrest Research Consortium (NPARC) is a multicenter network for conducting research in post-cardiac arrest patients. At the time of this study, the group consisted of four urban tertiary care teaching hospitals in the United States: Beth Israel Deaconess Medical Center (Boston, MA), University of Pennsylvania (Philadelphia, PA), University of Pittsburgh (Pittsburgh, PA), and Virginia Commonwealth University (Richmond, VA). All participating centers are dedicated cardiac arrest centers and patient care protocols for these hospitals have been reported previously.[12] The current study is a prospective, observational cohort study of mitochondrial biomarkers.

Study Population

We included adults (≥ 18 years) who had suffered OHCA with sustained ROSC (defined as the presence of palpable pulses for at least 20 minutes) and who were comatose after ROSC (defined as not being able to follow commands immediately after the arrest). While not an inclusion criterion, targeted temperature management occurred in 97% of patients; when utilized the approach consistent of surface cooling devices with a target temperature between 32–34 degrees Celsius and all subject received similar post-arrest care. [12] Subjects were excluded if they had trauma as the primary cause of arrest, if they were pregnant, or if they were prisoners. Subjects were included during the period from June 2011 to March 2012. Institutional Review Boards at each participating site approved the study and in most sites granted a waiver of consent for the initial blood draw. Additional blood draws, retention of blood samples, and clinical data collection occurred only if the designated legally authorized surrogate provided written informed consent within 12 hours of ROSC.

At Beth Israel Deaconess Medical Center (Boston, MA), we enrolled healthy controls with no acute or significant chronic illness.

Data Collection and Data Management

Data were abstracted from the Emergency Medical Service (EMS) reports, emergency department charts and hospital records using standardized definitions derived by group consensus prior to the beginning of the study. We collected demographics and other baseline characteristics including initial vital signs and laboratory results. Cardiac arrest details

included initial rhythm, whether the arrest was witnessed, the presence or absence of bystander cardiopulmonary resuscitation and duration of arrest. Duration of arrest was defined as the time from estimated start of the arrest until sustained ROSC. The start of the arrest was estimated as the time of EMS call or from the EMS run sheet if the arrest was witnessed by EMS. Study data were collected and managed using the internet-based clinical research platform REDCap (Research Electronic Data Capture, Vanderbilt University, Nashville, TN) hosted at each of the four centers.[13]

Blood Sampling

Blood was drawn at 0 hours (within 6 hours of sustained ROSC), 12, 24, 36 and 48 hours after ROSC into citrated tubes and clotting tubes. We centrifuged blood within 30 minutes to separate plasma and serum. Aliquots were stored at -80°C until analysis. Due to high rate of death before the 48 hour time point, we decided *a priori* not to include this time point in our analysis.

Cytochrome c Measurement

An electrochemiluminescence immunoassay developed by Meso Scale Discovery (MSD, Rockville, Maryland, USA) was used to detect cytochrome *c* in human plasma. A matched antibody pair and standards were purchased from eBioscience (San Diego, CA, USA). 40 μL of 2 $\mu\text{g}/\text{mL}$ capture antibody diluted in phosphate-buffered saline was used to coat the plates at 4°C overnight. 5% Blocker A/TBS-T (MSD, Rockville, Maryland, USA) was used for blocking at room temperature for 1 hour. A top standard concentration of 25 ng/mL human cytochrome *c* in assay diluent was used along with a 3-fold dilution series to encompass the greater dynamic range and sensitivity of the MSD platform. Simultaneous incubation of sample plus primary detection antibody was used in this assay. Streptavidin SULFO-TAGTM (MSD, Rockville, Maryland, USA) at 1 $\mu\text{g}/\text{mL}$ in assay diluent was used as the secondary detection antibody. Finally, the plates were read in a MSD SI2400A instrument. Each plasma sample with an unknown cytochrome *c* concentration was thawed on ice and diluted 1:4 in assay diluent. All samples and standards (in a volume of 25 μL) were run in duplicates. Data analysis was performed using MSD Discovery Workbench software which developed the standard curve used to calculate the unknown concentrations in each patient sample. The lower detectable limit was 0.034 ng/mL . For undetectable values, a conservative value of 0.034 ng/mL was imputed.

Mitochondrial, Nuclear, and Cell Free DNA

Cell-free DNA was measured in plasma by centrifuging at 16,000 g for 10 minutes to remove any residual cells. The upper portion of the plasma was removed into a nuclease-free tube and stored at -80°C prior to DNA extraction. Cell-free DNA in the plasma was then isolated using plasma/serum DNA isolation kit (Abcam, Cambridge, MA) according to the manufacturer's protocol. The amount of cell free DNA were measured by NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, DE). The quality of DNA was checked by the A260/280 ratio. The amount of mitochondrial DNA (mtDNA) was measured by real-time quantitative PCR assay for both mtDNA-tRNA^{leu} and mtDNA-D loop genes which are specifically present in the mitochondrial genome. Nuclear DNA (nDNA) was measured using both the single-copy nuclear gene $\beta 2$ -Microglobulin (B2M) and TaqMan[®]

Copy Number Reference Assay for human RNase P (Applied Biosystems, Life Technologies Corporation, CA, USA) as previously described by Bai et al.[14] TaqMan real-time quantitative PCR assay was performed on a 7500 real time PCR system following the manufacture's protocol (Applied Biosystems, Life Technologies Corporation, CA, USA). DNA extracted from whole blood with a 2-fold serial dilution was used to construct the calibration curve. We measured cell free DNA, the two markers of nDNA and the two markers of mtDNA, only at the 0, 12 and 24 hour time points.

Outcome Measures

The primary outcome measure for the current study was survival to hospital discharge. For the secondary outcome of functional status at hospital discharge, we used the modified Rankin scale as recommended by recent consensus conferences.[15,16] This scale is a validated scale, ranging from 0 to 6, that was developed for measuring the performance of daily activities among patients with cerebrovascular accident, but which has been used in multiple conditions including cardiac arrest.[17, 18] Lower scores represent better performance; scores of 4 or greater represent severe disability or death. Good functional outcome was defined as a modified Rankin scale of 0–3 and poor functional outcome as a modified Rankin scale of 4–6.

Statistical Analysis

Descriptive statistics were used to summarize the study population. Data for continuous variables are presented as medians with 1st and 3rd quartiles. Categorical data are presented as counts and frequencies. Continuous variables were compared between groups using the Wilcoxon Rank Sum test and categorical data was compared using Fisher's Exact test.

We used a negative binomial regression model including age, sex and race to compare cytochrome *c* levels between cardiac arrest subjects and controls while adjusting for demographics. We used a negative binomial regression model with generalized estimating equations (with an autoregressive correlation structure to account for the correlation between repeated measures) to compare cytochrome *c* levels between non-survivors and survivors over time. We used Spearman's correlation coefficient (r_s) to determine the association between the various biomarkers.

We used logistic regression to assess the association between enrollment cytochrome *c* levels and in-hospital mortality. We first performed unadjusted analysis and then created a multivariable model considering variables reported in Table 1. We included only variables that were associated with the outcome (at a $p < 0.10$) in unadjusted analysis to limit the number of included variables and avoid overfitting. We next performed backwards selection retaining cytochrome *c* levels and variables associated with the outcome at a $p < 0.05$ to achieve a more parsimonious model. The results of the logistic regression is presented as odds ratios (OR) with 95% confidence intervals (95%CI). We furthermore report the χ^2 statistic and tested model fit for the multivariable model with the Hosmer-Lemeshow test.

We performed no adjustments for multiple comparisons.[19] All hypothesis tests were two-sided, with a significance level of $p < 0.05$. All tests of the data were performed in SAS v. 9.4 (SAS Institute Inc., Cary, NC, USA).

3) RESULTS

Patient Characteristics

Out of 111 subjects enrolled, 102 had cytochrome *c* measured at baseline and 101 had mitochondrial/nuclear/cell-free DNA measurements. The baseline characteristics of the 102 subjects are outlined in Table 1. The median age was 63 years (49, 75) and the majority (59%) were male. The median initial post-cardiac arrest lactate was 5.7 mmol/L (3.0, 9.6) and 54 (53%) received vasopressors within 3 hours after ROSC. Almost all subjects (97%) received temperature management. Overall survival was 46% and 30% had a good functional outcome. We also measured cytochrome *c* in 34 healthy controls and mitochondrial/nuclear/cell free DNA in a separate cohort of 46 healthy controls. The median age of the cytochrome controls was 37 years (25, 50), 22 (65%) were female and 22 (65%) were white. The median age of the mitochondrial/nuclear/cell free DNA controls was 31 years (24, 47), 24 (52%) were female and 39 (85%) were white.

Cytochrome *c*

Cytochrome *c* was detectable in 88 cardiac arrest subjects (86%) at the 0 hour time point and in 21 controls (62%), $p = 0.005$. Cardiac arrest subjects had higher 0 hour cytochrome *c* levels as compared to controls (2.18 ng/mL [0.74, 7.74] vs. 0.16 ng/mL [0.03, 0.91], $p < 0.001$, Figure 1). The difference remained when adjusting for demographics (age, sex and race), $p = 0.002$. 0 hour cytochrome *c* levels were weakly correlated with initial lactate levels ($r_s = 0.36$, $p < 0.001$, Figure 2). Subjects who died had higher 0 hour cytochrome *c* levels as compared to survivors (3.66 ng/mL [1.40, 14.9] vs. 1.27 ng/mL [0.16, 2.37], $p < 0.001$, Figure 1). Subjects with baseline shock had no difference in cytochrome *c* levels compared to those with no baseline shock (3.1 ng/mL [0.8, 9.9] vs. 1.9 ng/mL [0.6, 4.5], $p = 0.22$). Subjects who died of a neurologic cause had no difference in cytochrome *c* levels compared to those with other causes of death (3.3 ng/mL [1.0, 9.4] vs. 6.8 ng/mL [2.2, 18.6], $p = 0.18$). In logistic regression, 0 hour cytochrome *c* levels were not associated with mortality (OR for mortality per ng/mL increase in cytochrome *c*: 1.05 [95%CI: 1.00, 1.10], $p = 0.07$). The *c*-statistic was 0.70 (95%CI: 0.60, 0.81). Cytochrome *c* levels were higher in subjects with a poor functional outcome as compared to those with a good functional outcome (2.88 ng/mL [0.91, 9.94] vs. 1.27 ng/mL [0.29, 3.45], $p = 0.04$, eFigure 1 in the supplemental material).

There was a decrease in cytochrome *c* over time: 0 hour: 2.18 ng/mL (0.74, 7.74), 12 hours ($n = 92$): 1.58 ng/mL (0.43, 6.75), 24 hours ($n = 95$): 0.68 ng/mL (0.03, 3.55), and 36 hours ($n = 87$): 0.48 ng/mL (0.03, 2.60), $p < 0.001$ for linear decrease, Figure 3. There was no difference in the change over time between survivors and non-survivors ($p = 0.27$, Figure 3).

Mitochondrial, Nuclear DNA, and Cell-Free DNA

One hundred one subjects and 46 controls had mtDNA, nDNA, and cell-free DNA measured. Reported mtDNA and nDNA values are relative gene expression levels compared to healthy individuals, where healthy expression is set at a reference level of 1. Values for all measures are illustrated in Figure 4.

Mitochondrial DNA

There was no difference in mtDNA-tRNA^{leu} levels (1.0 [0.7, 1.3] vs. 0.8 [0.5, 1.3], $p = 0.10$) between cardiac arrest and control subjects. mtDNA-D loop levels were lower in cardiac arrest subjects as compared to controls (0.7 [0.4, 1.1] vs. 1.4 [0.6, 2.4], $p = 0.001$). There was no difference in mtDNA markers between survivors and non-survivors at the 0 hour time point (Figure 5), and there was no difference in change over time according to survival status (eFigure 2). Cytochrome *c* values were weakly correlated with mtDNA-tRNA^{leu} levels ($r_s = 0.34$, $p < 0.001$) and mtDNA-D loop levels ($r_s = 0.25$, $p = 0.01$) (eFigure 3 in the supplemental material).

Nuclear DNA

Levels of nuclear RNAase P (3.3 [1.2, 5.7] vs. 1.2 [0.8, 1.2], $p < 0.001$) and nDNA-B2M (12.0 [1.0, 22.9], vs. 0.6 [0.6, 1.3], $p < 0.001$) were higher in cardiac arrest subjects at 0 hour than in controls. Levels of n-RNAase or nDNA-B2M did not differ between survivors and non-survivors at the 0 hour time point (Figure 5). There was no difference in the change over time according to survival status for n-RNAase P, though there was a significant difference in the change over time according to survival status for nDNA-B2M with a more substantial decrease in survivors ($p = 0.03$, eFigure 2 of supplementary material). Cytochrome *c* values were not correlated with either n-RNAase ($r_s = 0.11$, $p = 0.28$) or nDNA-B2M ($r_s = 0.03$, $p = 0.77$) (eFigure 3 in the supplemental material).

Cell Free DNA

Cell free DNA was higher in cardiac arrest subjects than in controls (420ng/mL [227, 676] vs. 212ng/mL [162, 275], $p < 0.001$). There was no difference between cardiac arrest non-survivors and survivors in cell free DNA levels at baseline (454ng/mL [216, 682] vs. 409ng/mL [238, 676], $p = 0.85$). There was no difference between survivors and non-survivors in cell free DNA over time ($p = 0.49$, eFigure 4 in the supplemental material). Cell free DNA was not correlated with cytochrome *c* ($r_s = 0.05$, $p = 0.59$).

Multivariable Model

With backwards selection of variables in this multivariable model, the following variables were associated with in-hospital mortality: duration of arrest (OR per minute increase: 1.07 [95%CI: 1.03, 1.12], $p = 0.003$), initial lactate (OR per mmol/L increase: 1.26 [95%CI: 1.09, 1.46], $p = 0.002$) and chronic obstructive pulmonary disease (OR: 7.12 [95%CI: 1.26, 40.4], $p = 0.03$). Cytochrome *c* was not associated with mortality in this model (OR per nmol/mL increase: 1.03 [95%CI: 0.99, 1.07], $p = 0.21$). The c statistics for this model was 0.85 and the p-value from the Hosmer-Lemeshow test was 0.54 indicating good discrimination and calibration.

4) DISCUSSION

The principal findings in this study are that cytochrome *c* was higher at baseline and over 36 hours in post-cardiac arrest subjects as compared to controls, and in non-survivors as compared to survivors. When accounting for other variables, cytochrome *c* was not independently associated with mortality. We did not detect any differences between

mitochondrial DNA in post-cardiac arrest subjects compared to controls or between survivors and non-survivors of post-arrest subjects. We did detect differences in nDNA and cell free DNA between post-cardiac arrest subjects and controls; however, no differences were found between survivors and non-survivors.

Our results are mixed compared to the existing literature on this topic. Previous studies in animals suggest that mitochondrial injury occurs from cardiac arrest (ischemia and reperfusion injury).[1, 2] However, this has not been demonstrated clearly in humans and the exact pathway(s) remain incompletely characterized. For example, cytochrome *c* and mitochondrial DNA are increased in animal models of arrest, but we are only aware of one small single-center study after cardiac arrest in humans.[3] In that study of 85 subjects (reported as a letter to the editor), the authors measured the nuclear β -globin gene and the mitochondrial DNA encoded ATPase 8 gene. They found that both nuclear DNA (c statistic: 0.86) and mitochondrial DNA (c statistic: 0.91) were good discriminators of 3-day survival. [3]

As noted, we found that cytochrome *c* levels were higher in post-cardiac arrest patients as compared to controls, and that levels were higher in non-survivors compared to survivors. We also found that cytochrome *c* elevations were associated with elevations of various inflammatory biomarkers, which is reported elsewhere. [20] Radhakrishnan et al. previously reported that plasma cytochrome *c* levels increase in rats after cardiac arrest and that these elevations were higher in non-survivors. [21] Interventions aimed at protecting mitochondria from reperfusion injury attenuated increases in cytochrome *c* post-resuscitation in two additional studies evaluating cardiac arrest in rats. In these studies, increased levels of cytochrome *c* were associated with myocardial dysfunction.[22,23] Taken together, these preclinical studies combined with our findings support the hypothesis that mitochondrial injury occurs after arrest and cytochrome *c* may serve as an important biomarker for identifying this injury. While our results vary slightly from previous findings, our study is the first in this area conducted in humans in a large, multi-center fashion. As noted above, however, cytochrome *c* is not associated with mortality independently when accounting for other variables in a multivariable model. However, this does not necessarily detract from the importance of cytochrome *c* as a marker of injury and as an indicator of mitochondrial injury.

The fact that cytochrome *c* levels were highest at presentation and decreased thereafter suggests that the initial ischemia and reperfusion causes the bulk of mitochondrial injury. This pattern of results parallels the time-course for elevations of inflammatory biomarkers in the same dataset (i.e., inflammatory markers highest upon presentation).[20]

In contrast to elevations of cytochrome *c*, we did not find elevation of mtDNA levels in plasma in post-cardiac arrest subjects as compared to healthy controls. This finding is consistent with one previous cardiac arrest study in animals that found no brain mtDNA damage from cardiac arrest.[24] In addition, previous studies in septic shock (where mitochondrial injury is believed to occur) did not detect increases in mtDNA either. One possibility for these seemingly disparate findings (i.e., lack of mtDNA breakdown products in the face of presumed mitochondrial injury) could be faulty measurement or that we failed

to measure mtDNA fragments that are in the bloodstream. That stated, we did find a weak correlation between cytochrome *c* and mtDNA but no association between cytochrome *c* and nDNA suggesting our measurements may be valid. Assuming the data reflect a true physiologic relationship, several reasons are plausible given the physical location of mtDNA and cytochrome *c* in the mitochondria. Cytochrome *c* is located in the mitochondrial intermembrane space, so once the mitochondrial outer membrane becomes permeable from damage, diffusion of contents from the intermembrane space into the cytosol occurs, causing numerous proteins including cytochrome *c* to leak. [25] Meanwhile, mtDNA genome is located in the mitochondrial matrix, enclosed by the inner membrane. Unless the inner membrane also breaks down, the mtDNA will not be released into the cytosol or plasma.

We also found that cell free DNA and nDNA was elevated in post-arrest subjects compared to healthy controls. However, there were no differences in cell free DNA levels or nDNA levels between survivors and non-survivors. Multiple previous studies of cell free DNA in humans found that levels were higher in non-survivors as compared to survivors.[26–28] The reason for the differences in findings between these previous investigations and the current study remain unclear but could relate to measurement techniques, timing of sampling, or patient populations.

Our study has some limitations. First, all measurements were performed from plasma and/or serum and therefore we did not study mitochondrial functional assays such as oxygen consumption or other measures of mitochondrial enzymatic activity. Second, like most post-cardiac arrest cohorts, our study population was heterogeneous and mitochondrial injury either before or after cardiac arrest could differ based on pre-event co-morbid conditions or the etiology of arrest. We did not include information on the etiology of arrests into our analyses because many cases had unclear etiology. Additionally, the generalizability of our findings to other post-cardiac arrest populations remains speculative. On the other hand, our study is a multicenter prospective evaluation spanning four geographically distinct hospitals in the United States and allows for better generalizability than single center biomarker studies. Lastly, we are unsure if certain findings (particularly with regards to mtDNA and/or cell free DNA) could be related to measurement difficulties or differences in methodological technique. However, we could not identify anything suggesting that our measurements were faulty or unreliable and all measurements were performed in duplicate or triplicate.

5) CONCLUSIONS

Cytochrome *c* is increased in post-cardiac arrest subjects as compared to controls and in post-cardiac arrest non-survivors as compared to survivors. Mitochondrial DNA in the plasma of post-cardiac arrest subjects was not higher than controls and we found no difference between post-cardiac arrest survivors and non-survivors. However, there were differences in nuclear DNA and cell-free DNA between post-cardiac arrest subjects and controls. These elevations in nuclear and cell-free DNA did not differentiate survivors and non-survivors. Mitochondrial injury markers showed mixed results in post-arrest period. Future research needs to investigate these differences.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The project described was supported, in part, by the Virginia Commonwealth University CTSA award No. UL1TR000058 from the National Center for Advancing Translational Sciences. The manuscript's contents are solely the responsibility of the authors and do not necessarily represent official views of the National Center for Advancing Translational Sciences or the National Institutes of Health. Dr. Donnino is supported by NHLBI (1K02HL107447-01A1).

8) REFERENCES

1. Ayoub IM, Radhakrishnan J, Gazmuri RJ. Targeting mitochondria for resuscitation from cardiac arrest. *Critical care medicine*. 2008; 36:S440–6. [PubMed: 20449908]
2. Uchino H, Ogihara Y, Fukui H, Chijiwa M, Sekine S, Hara N, et al. Brain injury following cardiac arrest: pathophysiology for neurocritical care. *J Intensive Care*. 2016; 4:31. [PubMed: 27123307]
3. Arnalich F, Codoceo R, Lopez-Collazo E, Montiel C. Circulating cell-free mitochondrial DNA: a better early prognostic marker in patients with out-of-hospital cardiac arrest. *Resuscitation*. 2012; 83:e162–3. [PubMed: 22490673]
4. Capaldi RA. Structure and Function of Cytochrome c Oxidase. *Annual Review of Biochemistry*. 1990; 59:569–96.
5. Ayoub IM, Radhakrishnan J, Gazmuri RJ. Targeting mitochondria for resuscitation from cardiac arrest. *Crit Care Med*. 2008; 36(11 suppl):S440–S446. [PubMed: 20449908]
6. Ow YP, Green DR, Hao Z, Mak TW. Cytochrome c: functions beyond respiration. *Nat Rev Mol Cell Biol*. 2008; 9(7):532–542. [PubMed: 18568041]
7. Radhakrishnan J, Wang S, Ayoub IM, Kolarova JD, Levine RF, Gazmuri RJ. Circulating levels of cytochrome c after resuscitation from cardiac arrest: a marker of mitochondrial injury and predictor of survival. *Am J Physiol Heart Circ Physiol*. 2007; 292(2):H767–H775. [PubMed: 17040974]
8. Atamaniuk J, Vidotto C, Tschan H, Bachl N, Stuhlmeier KM, Muller MM. Increased concentrations of cell-free plasma DNA after exhaustive exercise. *Clin Chem*. 2004; 50:1668–1670. [PubMed: 15331502]
9. Stroun M, Lyautey J, Lederrey C, Olson-Sand A, Anker P. About the possible origin and mechanism of circulating DNA apoptosis and active DNA release. *Clin Chim Acta*. 2001; 313:139–142. [PubMed: 11694251]
10. Lui YY, Dennis YM. Circulating DNA in plasma and serum: biology, preanalytical issues, and diagnostic applications. *Clin Chem Lab Med*. 2002; 40:962–968. [PubMed: 12476933]
11. Yakes FM, Van Houten B. Mitochondrial DNA damage is more extensive and persists longer than nuclear DNA damage in human cells following oxidative stress. *Cell Biology. Proc Natl Acad Sci*. 1997; 94:514–519. [PubMed: 9012815]
12. Donnino MW, Rittenberger JC, Gaieski D, Cocchi MN, Giberson B, Peberdy MA, et al. The development and implementation of cardiac arrest centers. *Resuscitation*. 2011; 82:974–8. [PubMed: 21570761]
13. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)—a metadata-driven methodology and workflow process for providing translational research informatics support. *Journal of biomedical informatics*. 2009; 42:377–81. [PubMed: 18929686]
14. Bai RK, Wong LJ. Simultaneous detection and quantification of mitochondrial DNA deletion(s), depletion, and over-replication in patients with mitochondrial disease. *J Mol Diagn*. 2005; 7:613–22. [PubMed: 16258160]
15. Newcommon NJ, Green TL, Haley E, Cooke T, Hill MD. Improving the assessment of outcomes in stroke: use of a structured interview to assign grades on the modified Rankin Scale. *Stroke; a journal of cerebral circulation*. 2003; 34:377–8. author reply -8.

16. Becker LB, Aufderheide TP, Geocadin RG, Callaway CW, Lazar RM, Donnino MW, et al. Primary outcomes for resuscitation science studies: a consensus statement from the American Heart Association. *Circulation*. 2011; 124:2158–77. [PubMed: 21969010]
17. Stiell IG, Nichol G, Leroux BG, Rea TD, Ornato JP, Powell J, et al. Early versus later rhythm analysis in patients with out-of-hospital cardiac arrest. *The New England journal of medicine*. 2011; 365:787–97. [PubMed: 21879896]
18. van Alem AP, de Vos R, Schmand B, Koster RW. Cognitive impairment in survivors of out-of-hospital cardiac arrest. *American heart journal*. 2004; 148:416–21. [PubMed: 15389227]
19. Rothman KJ. No adjustments are needed for multiple comparisons. *Epidemiology*. 1990; 1:43–6. [PubMed: 2081237]
20. Peberdy MA, Andersen LW, Abbate A, Thacker LR, Gaieski D, Abella BS, et al. Inflammatory markers following resuscitation from out-of-hospital cardiac arrest-A prospective multicenter observational study. *Resuscitation*. 2016; 103:117–24. [PubMed: 26826561]
21. Radhakrishnan J, Wang S, Ayoub IM, Kolarova JD, Levine RF, Gazmuri RJ. Circulating levels of cytochrome c after resuscitation from cardiac arrest: a marker of mitochondrial injury and predictor of survival. *Am J Physiol Heart Circ Physiol*. 2007; 292:H767–75. [PubMed: 17040974]
22. Radhakrishnan J, Upadhyaya MP, Ng M, Edelheit A, Moy HM, Ayoub IM, et al. Erythropoietin facilitates resuscitation from ventricular fibrillation by signaling protection of mitochondrial bioenergetic function in rats. *Am J Transl Res*. 2013; 5:316–26. [PubMed: 23634242]
23. Radhakrishnan J, Kolarova JD, Ayoub IM, Gazmuri RJ. AVE4454B--a novel sodium-hydrogen exchanger isoform-1 inhibitor--compared less effective than cariporide for resuscitation from cardiac arrest. *Transl Res*. 2011; 157:71–80. [PubMed: 21256459]
24. White BC, Tribhuwan RC, Vander Laan DJ, DeGracia DJ, Krause GS, Grossman LI. Brain mitochondrial DNA is not damaged by prolonged cardiac arrest or reperfusion. *J Neurochem*. 1992; 58:1716–22. [PubMed: 1560228]
25. Chipuk JE, Bouchier-Hayes L, Green DR. Mitochondrial outer membrane permeabilization during apoptosis: the innocent bystander scenario. *Cell Death Differ*. 2006 Aug; 13(8):1396–402. Epub 2006 May 19. [PubMed: 16710362]
26. Huang CH, Tsai MS, Hsu CY, Chen HW, Wang TD, Chang WT, et al. Circulating cell-free DNA levels correlate with postresuscitation survival rates in out-of-hospital cardiac arrest patients. *Resuscitation*. 2012; 83:213–8. [PubMed: 21864482]
27. Gornik I, Wagner J, Gasparovic V, Milicic D, Degoricija V, Skoric B, et al. Prognostic value of cell-free DNA in plasma of out-of-hospital cardiac arrest survivors at ICU admission and 24h post-admission. *Resuscitation*. 2014; 85:233–7. [PubMed: 24145040]
28. Arnalich F, Menendez M, Lagos V, Ciria E, Quesada A, Codoceo R, et al. Prognostic value of cell-free plasma DNA in patients with cardiac arrest outside the hospital: an observational cohort study. *Critical care*. 2010; 14:R47. [PubMed: 20350299]

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Figure 1. Cytochrome *c* in survivors and non-survivors of cardiac arrest and controls

Cytochrome *c* levels in cardiac arrest subjects stratified by survival status at the 0 hour time point and in healthy controls. Each dot represents a patient and the bars represent the 1st quartile, the median and the 3rd quartile. The y-axis is logarithmic for illustrative purposes. There was a significant difference between the two groups.

Figure 2. Cytochrome *c* levels and lactate levels in cardiac arrest subjects after ROSC
The correlation between 0 hour cytochrome *c* levels and lactate levels obtained within 3 hours of ROSC. There was a weak but significant correlation (spearman's correlation coefficient = 0.36, $p < 0.001$). The x-axis is logarithmic for illustrative purposes.

Figure 3. Cytochrome *c* levels over time in non-survivors (gray) and survivors (black)
 Cytochrome *c* levels in cardiac arrest subjects comparing non-survivors and survivors. Each dot represents a patient and the bars represent the 1st quartile, the median and the 3rd quartile. The y-axis is logarithmic for illustrative purposes. There was a decrease in cytochrome *c* over time ($p < 0.001$ for linear decrease). There was no difference in the change over time between survivors and non-survivors ($p = 0.27$).

Figure 4. DNA in cardiac arrest subjects at baseline and in controls

Markers of nuclear and mitochondrial DNA in cardiac arrest subjects at the 0 hour time point and in healthy controls. The boxplots represent the 1st quartiles, median, and 3rd quartile. The whiskers represent the minimum and maximum. The y-axis is logarithmic for illustrative purposes.

Figure 5. DNA markers at baseline in cardiac arrest non-survivors and survivors

Markers of nuclear and mitochondrial DNA in cardiac arrest subjects at the 0 hour comparing non-survivors and survivors. The boxplots represent the 1st quartiles, median, and 3rd quartile. The whiskers represent the minimum and maximum. The y-axis is logarithmic for illustrative purposes.

Table 1Patient characteristics^a

	All subjects (n = 102)	Survivors (n = 47)	Non-survivors (n = 55)	p-value
Demographics				
Sex (female)	42 (41)	19 (40)	23 (42)	1.00
Age (years)	63 (49, 75)	56 (43, 73)	65 (54, 78)	0.06
Race (white)	61 (60)	29 (62)	32 (58)	0.84
Co-morbidities				
Coronary artery disease	32 (31)	13 (28)	19 (35)	0.52
Heart failure	11 (11)	4 (9)	7 (13)	0.54
Diabetes mellitus	31 (30)	10 (21)	21 (38)	0.08
COPD	19 (19)	15 (27)	4 (9)	0.02
Hypertension	57 (56)	25 (53)	32 (58)	0.69
Arrest details				
Witnessed ^b	82 (81)	41 (89)	41 (75)	0.08
Bystander CPR ^c	40 (41)	23 (50)	17 (33)	0.10
Initial rhythm shockable ^d	52 (54)	32 (73)	20 (38)	0.001
Duration of arrest (minutes) ^e	23 (13, 30)	15 (11, 25)	28 (20, 38)	0.002
Initial lab values				
pH ^f	7.2 (7.1, 7.3)	7.3 (7.2, 7.3)	7.2 (7.1, 7.3)	0.009
Lactate (mmol/L) ^f	5.7 (3.0, 9.6)	4.1 (2.2, 7.7)	7.8 (4.7, 11.1)	< 0.001
Creatinine (mg/dL) ^g	1.2 (1.0, 1.8)	1.1 (1.0, 1.5)	1.3 (1.1, 1.8)	0.05
Initial vasopressor support	54 (53)	15 (32)	39 (71)	< 0.001
Temperature management	99 (97%)	47 (100)	52 (95)	0.25

^aCategorical variables are presented as counts and frequencies and continuous variables as medians with 1st and 3rd quartiles. COPD: chronic obstructive pulmonary disease, CPR: cardiopulmonary resuscitation

^bMissing on 1 patient,

^cMissing on 4 subjects,

^dMissing on 6 subjects,

^eMissing on 12 subjects,

^fMissing on 9 subjects,

^gMissing on 3 subjects