STUDY PROTOCOL

REOXYGENATION AFTER CARDIAC ARREST II (REOX II) STUDY

SPONSOR: NATIONAL INSTITUTES OF HEALTH
NATIONAL HEART, LUNG, AND BLOOD INSTITUTE

GRANT # R01 HL112815: NEUROLOGICAL AND COGNITIVE EFFECTS OF HYPEROXIA AFTER CARDIAC ARREST

VERSION 1, 12/3/2015

Principal Investigator:
Stephen Trzeciak, MD, MPH
Professor and Head of Critical Care Medicine
Cooper University Health Care
Cooper Medical School of Rowan University
Camden, New Jersey 08103
Phone: 856-342-2633
Email: trzeciak-stephen@cooperhealth.edu

A. SIGNIFICANCE

Out-of-hospital cardiac arrest (OHCA) is common, lethal, and neurologically devastating for many survivors, but tremendous potential exists to improve outcomes with post-resuscitation interventions.

A.1. Scope of the problem

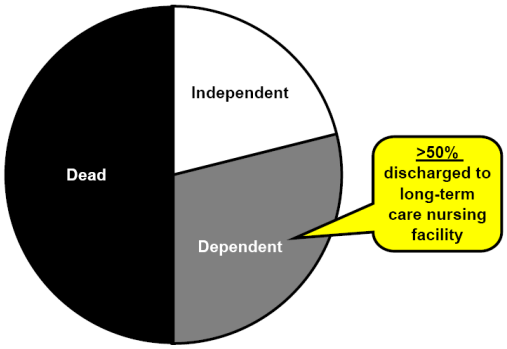
OHCA is common and lethal. Approximately 295,000 Americans are treated by Emergency Medical Services for OHCA annually[1], with an overall survival to hospital discharge of <10%, making OHCA a leading cause of death in the U.S.[2] Victims of OHCA are relatively young (median age 67 years), and women are affected as often as men. Often, OHCA is the first manifestation of cardiovascular disease, with two-thirds of arrests occurring without prior recognition of an underlying condition.[3] Relative to other common emergency conditions, the number of deaths from OHCA is extremely high (**Table 1**).[2,4,5]

Table 1: Annual U.S. deaths from out-of-hospital cardiac arrest (OHCA) compared to other emergency conditions.

Disease	Mortality (est.)
OHCA	***265,000***
Severe sepsis	215,000
Acute myocardial infarction	141,000
Stroke	137,000
Trauma	124.000

Even if initial resuscitation from OHCA is successful, the rate of subsequent death and disability is staggering, primarily due to *anoxic brain injury*. Although cardiopulmonary resuscitation (CPR) and defibrillation may be successful in achieving return of spontaneous circulation (ROSC), the subsequent ischemia/reperfusion insult may have crippling neurological effects. Anoxic brain injury is the most common (~70%) cause of death among patients resuscitated from OHCA.[6] In a large (n=8736) U.S. multi-center cohort study of patients who survived to intensive care unit (ICU) admission following successful resuscitation from cardiac arrest, our research team found that ***50%*** of patients did not survive to hospital discharge.[7] Of those who did survive, the majority of patients were functionally dependent, and more than half of these patients were discharged to a long-term care facility (**Figure 1**). Furthermore, survivors discharged with functional independence are considered to have "good outcomes"; however, substantial cognitive and psychomotor deficits are common among these survivors. Recently, our research team published the results of an NIH-funded study of neuropsychological deficits among OHCA survivors (see *Preliminary Studies* in section C.3.7).[8] Among patients who recovered alertness but remained confused at 7 days, we performed neuropsychological testing at 90 days post-arrest. We found that while two-thirds of patients manifested mild deficits in memory and psychomotor function with other cognitive domains preserved, one-third of patients manifested severe memory and psychomotor deficits and impairment in all cognitive domains. Overall, only ~25% of these patients were able to return to work. ***In summary, patients resuscitated from OHCA commonly suffer severe neurological injury leading to death or long-term disability. Research on new methods to improve outcomes from this devastating condition is a crucial and urgent priority. Given the magnitude of death and disability that OHCA imparts upon Americans, even modest improvements would provide a major public health benefit.***

Figure 1: Outcomes for 8736 patients admitted to an intensive care unit after successful resuscitation from cardiac arrest (*Trzeciak et al Crit Care Med 2009).*



A.2. Importance of *post-resuscitation* interventions.

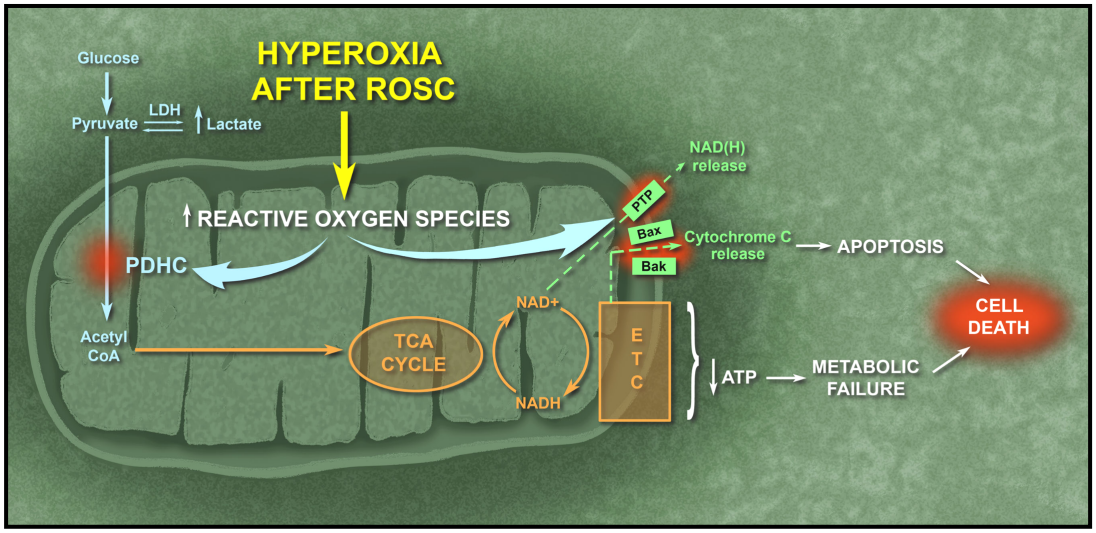
Until recently, neurological injury after resuscitation from cardiac arrest was largely thought to be *untreatable*. Now it is recognized that the trajectory of this disease can in fact be modulated.[9] Although the initial ischemic insult has already occurred, therapeutic interventions applied *after* ROSC may mitigate the severity of ***reperfusion injury***. Neuronal death after reperfusion is a *dynamic* process that does not occur immediately after ROSC. Laboratory investigations demonstrate that histopathological changes in the brain commonly take several hours or days after ROSC to manifest.[9] These data indicate that a *window of opportunity* exists after ROSC in which brain injury may be attenuated. Target temperature management (TTM), a strategy to control body temperature for 12-24 hours after ROSC, is the first proven therapy to reduce brain injury after resuscitation from cardiac arrest. Two landmark clinical trials found significantly improved neurological outcomes for patients treated with TTM after OHCA due to ventricular fibrillation (VF).[10,11] These pivotal

findings were the first evidence that brain injury after ROSC is in fact *treatable*. The American Heart Association (AHA) now recommends TTM for adults with OHCA due to VF who remain comatose after ROSC (Class I recommendation).[12] Although TTM represents a truly groundbreaking discovery, ***the mortality and disability among OHCA victims treated with TTM remain unacceptably high***.[9] Finding ***additional post-ROSC interventions*** to further attenuate reperfusion injury and improve outcomes is a crucial research priority for resuscitation science with potential impact for thousands of Americans annually.[13]

A.3. Significance of the proposed research

Another potentially modifiable factor in post-resuscitation care is the amount of oxygen delivered to the brain after reperfusion. Current resuscitation guidelines recommend a fraction of inspired oxygen (FiO_2) of 100% during CPR, as this may help achieve ROSC; however, clinicians frequently maintain high FiO_2 for prolonged and highly variable periods of time *after* circulation is restored to ensure adequate oxygenation.[9] This strategy may not be innocuous, as there appears to be a paradox regarding oxygen delivery to the injured brain. Insufficient cerebral oxygen delivery can exacerbate cerebral anoxia, but hyperoxia (i.e. excessively high arterial oxygen partial pressure [PaO_2] and saturation [SaO_2] due to an excessively high FiO_2) potentially accelerates reperfusion injury. Preclinical studies have found that hyperoxia after ROSC worsens brain damage, evidenced on functional neurological testing[14-16] and histopathology,[17,18] and worsens survival.[19] The postulated mechanism is that ***hyperoxia drives an increase in formation of reactive oxygen species***. This increase may cause ***oxidative impairment of mitochondrial respiration and cerebral energy metabolism*** **(Figure 2)**. Oxidative modification to mitochondrial proteins may exacerbate the reperfusion-dependent inactivation of brain pyruvate dehydrogenase complex (PDHC), the only bridge between anaerobic and aerobic metabolism.[20] Increased reactive oxygen species may also activate the mitochondrial permeability transition pore (PTP) to release NAD(H) into the cytosol, depleting a vital metabolic cofactor. These changes may impair oxidative phosphorylation and cerebral adenosine triphosphate (ATP) generation.[20] Metabolic failure may ensue, resulting in decreased cerebral consumption of glucose and oxygen, increased production of lactate, and delayed neuronal cell death.[18,21] In addition, increased reactive oxygen species may impair electron transport chain activity by forming mitochondrial membrane pores that release cytochrome *c* into the cytosol.[20] Release of cytochrome *c* may trigger caspase-dependent apoptosis. Apoptosis may also be triggered as mitochondrial dysfunction disrupts cellular calcium ion homeostasis, resulting in an influx of intracellular calcium. Increased reactive oxygen species may also cause oxidation of brain lipids (i.e. lipid peroxidation), which may have physiologic effects (e.g. alteration of blood flow, neutrophil chemoattraction) as well as cellular toxic effects (e.g. excitotoxicity, neurodegeneration).[15,19] Increased reactive oxygen species may also promote cellular inflammatory reactions, specifically the activation of microglia and astrocytes in the neuronal microenvironment leading to increased neuronal cell death.[14] Lastly, hyperoxia may also have a direct vasoconstrictor effect, which may reduce cerebral blood flow after ROSC, exacerbating ischemic injury.[22,23]

Figure 2: Mitochondrial effects of hyperoxia after ROSC. Hyperoxia increases reactive oxygen species, which may inhibit pyruvate dehydrogenase complex (PDHC), and open mitochondrial membrane permeability transition pore (PTP), depleting NAD(H). These effects inhibit adenosine triphosphate (ATP) generation, leading to metabolic failure. Increased reactive oxygen species may also, via Bcl-2-associated proteins (Bax/Bak), induce mitochondrial release of cytochrome C, triggering caspase-dependent apoptosis. [TCA = tricarboxylic acid; NAD = nicotinamide adenine dinucleotide; ETC = electron transport chain]

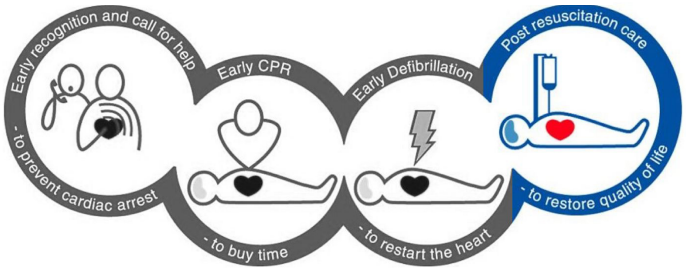


These data from animal models support the hypothesis that hyperoxia after ROSC may worsen outcomes in post-resuscitation patients. However, clinical data are *few*. Three preliminary retrospective multi-center analyses have been published. Two of these were published by our research team.[24,25] In a large (n=6326) cohort of patients admitted to one of 120 U.S. ICUs after cardiac arrest, we found that post-ROSC hyperoxia was ***common, an independent predictor of in-hospital death, and associated with lower functional independence among survivors.***[24] In a secondary analysis using the same ICU database, we found that a linear, ***"dose-dependent" relationship exists between supranormal PaO_2 after ROSC and mortality***[25] (see *Preliminary Studies* in sections *C.3.1* and *C.3.2*). However, a recent study from the Australian and New Zealand Intensive Care Society (ANZICS) investigators reported somewhat different results.[26] Using a retrospective methodology that replicated our initial study, they found that among 12,000 patients admitted to an ICU after cardiac arrest, patients with hyperoxia (defined by our previous methodology as a PaO_2 ≥300 mmHg) had significantly higher mortality (59% [95%CI 56-61%]) than patients with normoxia (47% [95% CI 45-50%]), but not patients with hypoxia (60% [95% CI 59-61%]). In a multivariable model adjusting for illness severity, hyperoxia had an odds ratio for mortality of 1.2 (95% CI 1.1-1.6). However, when statistical models with a number of additional adjustments were applied, the association with increased mortality was lost. They concluded that hyperoxia has no consistently reproducible independent association with mortality.[26] Therefore, **whether hyperoxia is associated with poor outcome in post-resuscitation patients remains unclear**. Given that post-ROSC hyperoxia exposure is extremely common[25], this unanswered question represents a ***critical knowledge gap*** for resuscitation science. Accordingly, this project will generate ***new, critically important knowledge about a fundamental element of post-cardiac arrest care.***

B. INNOVATION

Supplemental oxygen administration is considered a cornerstone of effective CPR, and the belief that increased oxygen delivery is beneficial is historically one of the most basic assumptions of resuscitation. In this application, we ***challenge the present paradigm*** regarding the role of supplemental oxygen in the treatment of cardiac arrest. Supplemental oxygen may be a *drug with important toxicity* after ROSC. Specifically, we propose an *alternative hypothesis* that hyperoxia after ROSC due to excessive supplemental oxygen is associated with harm. Hypotheses that challenge the prevailing thinking are the hypotheses with the greatest capacity to produce results that challenge conventional clinical practice. This project is further innovative in that we aim to ***identify a new potential therapeutic target*** in post-resuscitation care. **Figure 3** below displays the classical *Chain of Survival* paradigm for the treatment of cardiac arrest victims, with a new final link emphasizing the importance of post-resuscitation care.[27] Rather than studying a completely new treatment strategy (e.g. hypothermia), the proposed study will generate new knowledge about a standard component of *supportive care* (i.e. supplemental oxygen administration) that may be contributing to poor outcomes. This project is further innovative in that we will measure ***neuropsychological function*** among survivors at 180 days. Historically, cardiac arrest studies have used crude outcome measures.[28] For example, the most often used outcome, Cerebral Performance Category (CPC), requires no cognitive testing. The CPC-1 is compatible with cognitive impairment, so the precise nature of residual deficits is largely unknown. *To date, we are only aware of 5 high quality prospective studies of neuropsychological deficits after cardiac arrest published in the literature*[8,29-32] *(including work from our research team*[8]*), and these studies only included 245 patients combined.* Recently, a consensus conference on outcome measures in cardiac arrest research (led by the AHA and with participation of the NIH and FDA)[33] stressed the importance of cognitive and functional outcomes, thus we are responsive to a recognized need for innovation in the field. This project is further innovative in that we will use the ***Emergency Department*** (ED) as our main clinical laboratory. In contrast to other clinical research networks in the U.S. that focus on *pre-hospital* elements of resuscitation or other neurological emergencies, the ***Emergency Medicine Shock Research Network (EMShockNet)*** (described in section *C.2*) is focused on the ED phase of therapy and continuum of care to the ICU. Because reduction of supplemental oxygen in OHCA patients typically begins in the ED and is continued

Figure 3: The *new Chain of Survival* paradigm including postresuscitation care.



in the ICU, our network is an ideal environment to test this hypothesis. We are not aware of any U.S. hospital-based cardiac arrest clinical research consortium, and thus EMShockNet is a unique resource. Lastly, this project is innovative in that it uses gas chromatography negative ion chemical ionization mass spectrometry (GC/NICI/MS) to identify ***novel biomarkers of post-resuscitation oxidative injury*** (i.e. plasma/urine isoprostanes and isofurans). Due to their GC/NICI/MS characteristics, isoprostane and isofuran assays are innovative methodologies that apply advanced technologies.

C. APPROACH

C.1. The investigators

Table 2: The team of investigators. [ARDS = acute respiratory distress syndrome; EMShockNet = Emergency Medicine Shock Research Network; CUH = Cooper University Hospital; UMC = University of Mississippi Medical Center; BIDMC = Beth Israel Deaconess Medical Center; MH = Methodist Hospital, Indianapolis, IN; HUP = Hospital of the University of Pennsylvania; PRES = Penn-Presbyterian Medical Center]

Investigator	Role	Qualifications	Responsibilities
Stephen Trzeciak, MD, MPH	PI	Dr. Trzeciak is co-founder of EMShockNet. His research has been funded by NIH, Shock Society, Emergency Medicine Foundation, and American Heart Association. He has prior experience as PI directing a prospective multi-center (EMShockNet) study using high frequency oxygenation measurements and analyses of cumulative oxygenation exposure over time in the resuscitation phase of therapy.[34]	Overall PI and study director overseeing all aspects of the project.
Alan E. Jones, MD	Co-I	Dr. Jones is co-founder of EMShockNet. He is Vice Chair for Research, Department of Emergency Medicine, UMC. He has >60 papers related to the resuscitation of critically ill patients, and was PI on a pivotal NIH-funded (GM076652) EMShockNet trial recently published in JAMA[35] (see D.2. below).	Site PI (UMC)
Nathan I. Shapiro, MD, MPH	Co-I	Dr. Shapiro is co-founder of EMShockNet. He is Vice Chair for Research, Department of Emergency Medicine, BIDMC. He has extensive experience as PI on multi-center observational studies, including work funded by NIH (HL091757), and has >80 papers related to the resuscitation of critically ill patients.	Site PI (BIDMC)
Jeffrey A. Kline, MD	Co-I	Dr. Kline is Vice Chair of Emergency Medicine at MH. He is a renowned leader in Emergency Medicine research. He was awarded the 2009 Excellence in Research Award from the Society for Academic Emergency Medicine. He has a long track record of NIH funding and over 150 papers related to critically ill patients.	Site PI (MH)
Benjamin S. Abella, MD, MPhil	Co-I	Dr. Abella is Director of Clinical Research, Center for Resuscitation Science, HUP. He has >60 papers in the field of cardiac arrest including work funded by NIH (HL083082). He was a co-author on the 2010 CPR guidelines. He established and directs a highly successful training course for post-cardiac arrest care.	Site PI (HUP and PRES)
J. Hope Kilgannon, MD	Co-I	Dr. Kilgannon has extensive and unique experience related to the proposed research. She was first author (with Dr. Trzeciak as senior author) on both of our recent papers on hyperoxia after cardiac arrest[24, 25] (see *Preliminary Studies*).	Site PI (CUH)
Ramona Hopkins, PhD	Co-I	Dr. Hopkins is a Professor of Psychology and Neuroscience, Brigham Young University. She has conducted extensive prior research assessing long-term cognitive, psychiatric, physical, and quality of life outcomes in survivors of critical illness and anoxia/hypoxia, including NIH/NHLBI ARDS Network-2 studies.	Oversee all aspects of neuropsychological testing.

The **Principal Investigator, Stephen Trzeciak, MD, MPH**, has extensive experience related to the proposed topic, established long-standing collaborative relationships with the research team proposed here, and is highly committed and uniquely suited to lead the proposed multi-center study. Our investigators have proven track records of NIH funding and successful publication in the domain of cardiac arrest and resuscitation. The proposed investigators have collaborated on and coauthored (i.e. two or more authors from our research team) ***30*** peer-reviewed original science papers published in the biomedical literature in the 36 months preceding this application.

C.2. Our research network

The ***Emergency Medicine Shock Research Network*** was founded in 2007. Its mission is to conduct innovative clinical investigation for the advancement of generalizeable knowledge about circulatory shock states in humans. Although the first EMShockNet studies were focused on septic shock, we have expanded our research portfolio to focus on the most severe manifestation of circulatory shock, namely cardiac arrest. ***Our network has already demonstrated the ability to successfully complete multicenter studies of the size and complexity proposed in this application.*** To date we have enrolled ***more than 800 patients*** in prospective research protocols in the Emergency Department (ED) including real-time data collection in the ED and through the ICU course. We performed the largest randomized trial published to date comparing different goal-directed resuscitation strategies in ED patients with septic shock (*JAMA 2010; 303(8):739-46*). Therefore ***we are experienced at enrolling patients in ED-based time-sensitive research protocols in the resuscitation phase of patient care***. In addition to the original EMShockNet sites (CUH, BIDMC, CMC), four additional centers will participate in the proposed study. The University of Mississippi became an EMShockNet site in 2010 when Dr. Jones was recruited to UMC from CMC. MH became an EMShockNet site at the onset of REOX I. Both HUP (which has previously enrolled patients in EMShockNet research[36]) and Penn-Presbyterian will participate, with Dr. Abella as PI for both sites. Dr. Abella's team has been enrolling OHCA patients in prospective research protocols at both sites since 2004.

Our sites also have extensive experience implementing aggressive clinical treatment protocols for critically ill patients in the ED setting, and analyzing the effects using a before-and-after study design. Specifically, we have effectively implemented protocols for post-cardiac arrest care and hemodynamic optimization for sepsis.[37-42] Importantly, we also have experience continuing these aggressive treatment protocols into the ICU. Compared to our previous treatment protocols, which required the introduction of invasive hemodynamic monitoring or other complex procedures in the ED setting, our protocol for rapid FiO_2 optimization after resuscitation from cardiac arrest is relatively ***straight-forward and quite feasible***. Our sites' clinical rationale for implementing the protocol in this application is that rapid FiO_2 optimization is now recommended in the American Heart Association (AHA) Guidelines for Cardiopulmonary Resuscitation and Emergency Cardiovascular Care[43] (and is now included in Advanced Cardiac Life Support [ACLS]) and a consensus statement on post-resuscitation care from the International Liaison Committee on Resuscitation (ILCOR).[9] Therefore, the implementation of a rapid FiO_2 optimization protocol after resuscitation from cardiac arrest will be consistent with current consensus treatment guidelines, but at the same time will generate the most valuable clinical data to date regarding this fundamental element of post-cardiac arrest care.

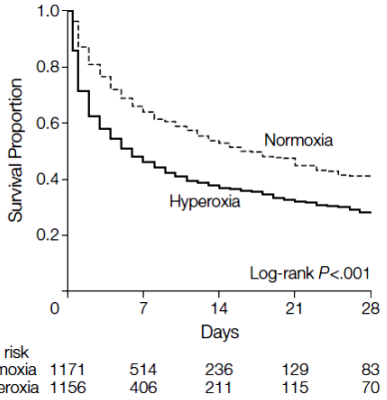
C.3. Preliminary studies from the research team proposed in this application

C.3.1. Arterial hyperoxia after resuscitation from cardiac arrest is associated with poor outcome.

Kilgannon JH, Jones AE, Shapiro NI, Angelos MG, Milcarek B, Hunter K, Parrillo JE, Trzeciak S. Association between arterial hyperoxia following resuscitation from cardiac arrest and in-hospital mortality. JAMA 2010; 303(21):2165-71.

Using Project IMPACT, a database of ICU admissions from 120 U.S. hospitals, we tested the hypothesis that exposure to hyperoxia (defined as a $PaO_2 \geq 300$ mmHg on the first arterial blood gas recorded in the ICU) was associated with increased in-hospital death among 6326 patients admitted to an ICU after cardiac arrest. We found that the patients with hyperoxia had significantly higher in-hospital mortality (63% [95% CI 60%-66%]) compared to patients with normoxia (45% [95% CI 43%-48%]; proportion difference 18% [95% CI 14%-22%]), and also compared to patients with hypoxia (57% [95% CI 56%-59%]; proportion difference, 6% [95% CI, 3%-9%]). Survival over time appears in **Figure 4**. In a model controlling for potential confounders (e.g., age, preadmission functional status, comorbid conditions, vital signs, and other physiological indices) including sensitivity analyses that adjusted for hospital factors and propensity of hyperoxia exposure, hyperoxia had an odds ratio for death of 1.8 (95% CI, 1.5-2.2). Among survivors, hyperoxia was associated with a lower proportion of functional independence at discharge compared to patients with normoxia (29% vs. 38%, respectively; proportion difference, 9% [95% CI, 3%-15%].

Figure 4: In-hospital death between hyperoxia and normoxia groups.

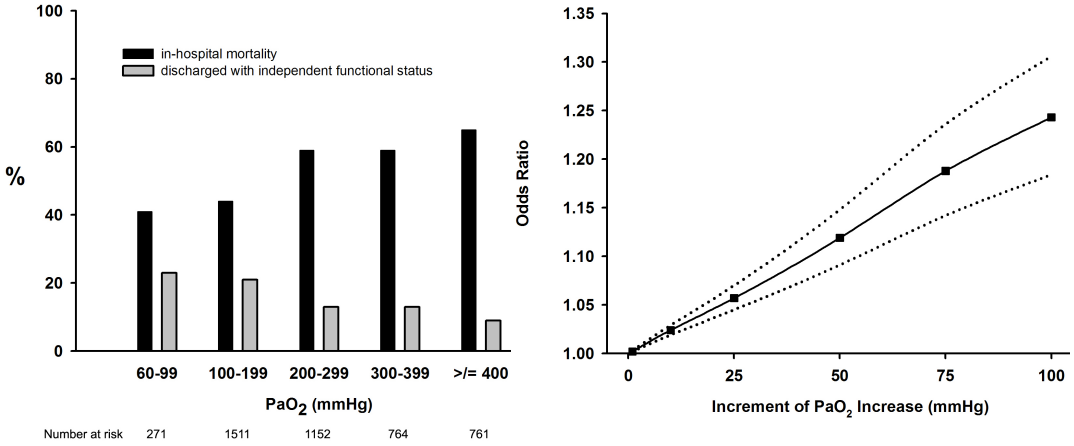


C.3.2. Dose-dependent relationship between supranormal PaO_2 and risk of death after cardiac arrest.
Kilgannon JH, Jones AE, Parrillo JE, Dellinger RP, Milcarek B, Hunter K, Shapiro NI, Trzeciak S. Relationship between supranormal oxygen tension and outcome after resuscitation from cardiac arrest. Circulation 2011; 123(23):2717-22.

Using the same ICU database, we tested the hypothesis that a linear, dose-dependent relationship exists between post-resuscitation supranormal oxygen tension (highest PaO_2 over the first 24 hours in the ICU) and in-hospital mortality. Among 4459 patients, the median PaO_2 was 231 (IQR 149-349) mmHg. Over ascending ranges of oxygen tension, we found significant linear trends of increasing in-hospital mortality and decreasing survival as functionally independent, p<0.0001 (**Figure 5**). On multivariable analysis, a 100 mmHg increase in PaO_2 was associated with a 24% increase in mortality risk (odds ratio 1.24 [95% CI 1.18-1.31] (**Figure 6**).

Figure 5 (left): Proportions of in-hospital mortality and independent functional status among survivors over increasing oxygen tensions. We found linear trends for increasing mortality and decreasing functional independence with increasing oxygen tension, p<0.0001.

Figure 6 (right): Odds ratios (solid line) and 95% confidence intervals (dotted lines) for in-hospital death with increasing increments of oxygen tension.



C.3.3. Development of a clinical protocol for rapid FiO_2 optimization.
The method of rapid FiO_2 optimization in REOX II must be comprised of a detailed protocol (see *Methods* section below) rather than a simple directive to titrate FiO_2 to keep SaO_2 or PaO_2 in a predetermined range, because rigorous uniformity is needed in the approach across all study sites, including uniformity in the increments and frequency of FiO_2 reductions. The therapeutic goal of our protocol is a ***PaO_2 of 60-99 mmHg***, because this is the PaO_2 range that was associated with the lowest risk of poor outcome in our previously published work.[25] We also use PaO_2 (measured by arterial blood gas [ABG] analysis) as the ultimate goal rather than SaO_2 measured by pulse oximetry because an SaO_2 value <100% on pulse oximetry monitoring does not always exclude supranormal PaO_2. The protocol in this application begins with very rapid reduction of FiO_2 as much as possible according to SaO_2 values, and when FiO_2 is maximally reduced by SaO_2 an ABG is measured, followed by finer adjustment of FiO_2 to achieve a PaO_2 60-99 mmHg. The protocol must not only prescribe each downward titration of FiO_2 but it must also include detailed limbs for *upward* titration of FiO_2 to account for potential "overshoot" in FiO_2 reduction. We acknowledge that the flowchart for FiO_2 titration in the *Methods* section (**Figure 9**) may appear complex, but our experience is that this level of detail is needed in order to ensure that FiO_2 optimization will not only be rapid and effective, but also safe. We believe using this protocol will allow for rapid achievement of the target PaO_2 after ROSC and no significant hypoxic events related to overshoot.

C.3.4. Optimal assessment of oxidative stress *in vivo*.

Roberts LJ, and colleagues. Biomarkers of Oxidative Stress Study II: Are oxidation products of lipids, proteins, and DNA markers of CCL_4 poisoning? Free Radic Biol Med 2005;38(6):698-710.

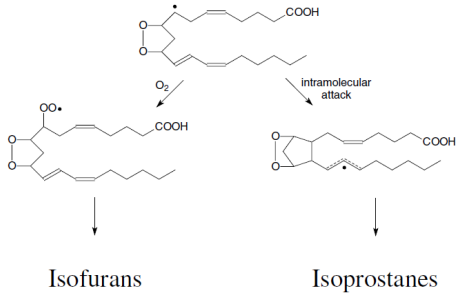
Oxidative stress refers to the overproduction of oxygen free radicals and/or an insufficiency of antioxidant/radical-detoxifying systems resulting in deleterious modification of proteins, nucleic acids, and lipids (i.e. lipid peroxidation). The proposed study will assess the degree of oxidative stress in post-resuscitation patients, and therefore will require the most sensitive and specific biomarkers of oxidant injury *in vivo*. The optimal biomarkers to measure remained unclear until the Biomarkers of Oxidative Stress Study (BOSS) that was recently conducted by the National Institute of Environmental Health Sciences (NIEHS). **Dr. Jack Roberts** (Vanderbilt University), who is among the **Key Personnel on this application**, was one of the NIEHS-BOSS Principal Investigators. It was a comprehensive, multi-laboratory validation study directly comparing multiple different methods of assessing oxidative stress using identical samples from a rat model of massive oxidative injury (acute carbon tetrachloride poisoning). The purpose of the study was to identify the most reliable, sensitive and specific noninvasive biomarkers of oxidative damage. The study compared isoprostanes (prostaglandin F2-like compounds that are formed nonenzymatically by free radical-induced peroxidation of arachidonic acid), lipid hydroperoxides, thiobarbituric acid-reactive substances (TBARS), malondialdehyde (MDA), protein carbonyls, methionine sulfoxidation, tyrosine products, 8-hydroxy-2V-deoxyguanosine, leukocyte DNA–MDA adducts, and DNA-strand breaks. ***Isoprostanes (IsoPs) measured in plasma or urine by gas chromatography negative ion chemical ionization mass spectrometry (GC/NICI/MS) were found to be the most reproducible, sensitive, and specific biomarkers of in vivo oxidative stress***. None of the other biomarkers tested were found to be sufficiently reliable for oxidative stress assessment *in vivo*. Dr. Roberts and his laboratory have two decades of experience with these techniques for measuring IsoPs.

C.3.5. Assessment of oxidative stress with exposure to hyperoxia.

Roberts LJ, et al. Discovery of lipid peroxidation products formed in vivo with a substituted tetrahydrofuran ring (isofurans) that are favored by increased oxygen tension. Proc Natl Acad Sci 2002;99(26):16713-18.

Dr. Roberts and his laboratory were the investigators who *discovered* ***isofurans*** (IsoFs), which are isomeric products of lipid peroxidation characterized by a substituted tetrahydrofuran ring. Isofuran assays are essential for the proposed study. Under conditions of increasing ambient oxygen concentrations (e.g. high FiO_2 producing supranormal PaO_2) an oxygen molecule is added to the carbon-centered radical to yield a peroxyl radical, and this favors the production of IsoFs instead of IsoPs (**Figure 7**). These reactions are competing, mutually exclusive, and ***differentially regulated by the ambient oxygen concentration***. Although IsoPs are an excellent assessment of oxidative stress in general, under hyperoxic conditions measurement of IsoPs alone would underestimate the extent of lipid peroxidation. A complete picture across all FiO_2 concentrations is only possible with measurement of ***both IsoPs and IsoFs***. Given that IsoFs are a remarkably sensitive indicator of hyperoxia-induced oxidative injury, and the proposed study is focused on hyperoxia exposure, measuring IsoFs in addition to IsoPs in this study is vital. Importantly, and pertinent to the feasibility of the proposed study, IsoF and IsoP assays are remarkably stable and therefore are applicable to stored specimens. In addition, IsoFs and IsoPs are quantified in the same GC/NICI/MS assay using a single purification protocol, allowing for maximum analytical simplicity. We will measure plasma IsoPs and IsoFs in this study.

Figure 7: Differential effects on isofuran and isoprostane formation by ambient oxygen. Excessive ambient oxygen favors isofurans.



C.3.6. Association between isofuran levels after resuscitation and clinical outcome.

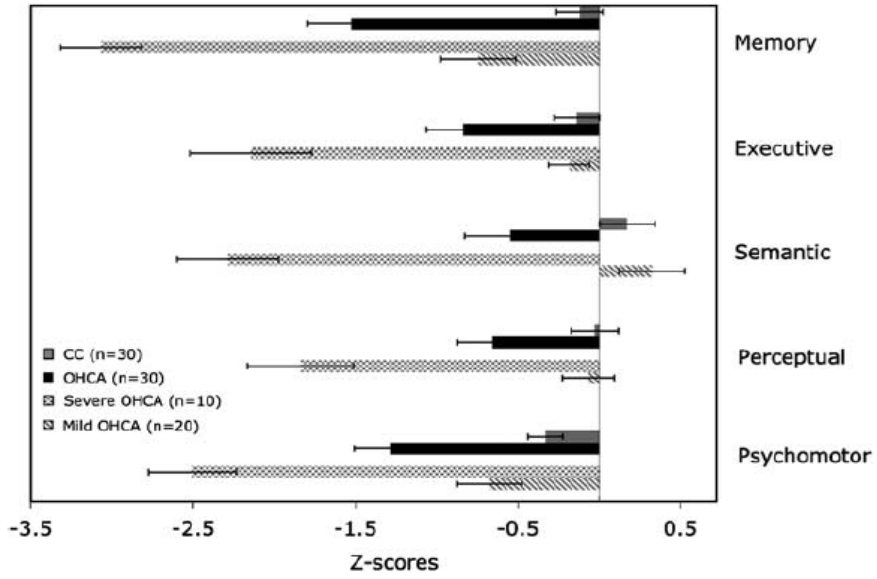
Roberts LJ, et al. Preterm resuscitation with low oxygen causes less oxidative stress, inflammation, and chronic lung disease. Pediatrics 2009;124(3):e439-49.

In this clinical trial comparing resuscitation with 90% FiO_2 versus 30% FiO_2 at the time of delivery among extremely low gestational age neonates, Dr. Roberts and colleagues assessed *in vivo* oxidative stress by measuring urine isofurans by GC/NICI/MS. They found that neonates resuscitated with 30% FiO_2 had significantly lower isofuran levels over the first day after resuscitation compared to those resuscitated with 90% FiO_2, and with this reduction in isofurans, neonates in the 30% FiO_2 arm also had improved clinical outcomes (reduction in acute lung injury and bronchopulmonary dysplasia).

C.3.7. Neuropsychological deficits among survivors of out-of-hospital cardiac arrest.
Alexander MP, Lafleche G, Schnyer D, Lim C, Verfaellie M. Cognitive and functional outcome after out of hospital cardiac arrest. J Int Neuropsychol Soc 2011; 17(2):364-8.

This NIH-funded (R01HD046442) prospective study determined the nature and severity of residual cognitive deficits for survivors of OHCA. Thirty OHCA patients who recovered alertness within 1-3 days of ROSC but remained confused at one week, and 30 non-OHCA coronary care admissions (matched controls) were administered neuropsychological testing at 90 days in five cognitive domains: memory, executive function, lexical-semantic, visuoperceptual, and psychomotor (**Figure 8**). Subjects fell into two deficit profiles. Two-thirds had only mild memory deficits and borderline psychomotor deficits compared to controls; 40% in this mild group had returned to work. The other one-third of patients had severe impairments in all domains. In the severe group, only as impairment in memory or psychomotor domains increased did impairments in other domains appear. Follow-up evaluation at one year revealed discouraging little further cognitive improvement over the levels reached at 90 days. Only one additional subject returned to work. In addition, we assessed health-related quality of life (QOL) at one year using the Sickness Impact Profile-68 (SIP-68)[44], a measure of health-related functional status in rehabilitation medicine, and found that the OHCA patients reported more adverse effects on health than the cardiac control patients due to poorer psychological health and social participation, not due to effects on physical health.

Figure 8: Composite z-scores – mean and standard deviation – for neuropsychological function in five cognitive domains among cardiac controls (CC), all out-of-hospital cardiac arrests (OHCA), and mild and severely impaired OHCA patients separately. Deficits after OHCA were most common and most severe in memory and psychomotor domains.



The proposed study will use a similar methodology for neuropsychological testing and functional assessment at 180 days after resuscitation among survivors.

D.1. RESEARCH DESIGN AND METHODS

D.1.1 Specific Aims

We will test if a protocol for rapid FiO_2 optimization following ROSC from cardiac arrest is associated with:

- *Reduced in vivo oxidative stress during the post-resuscitation phase of therapy (Aim 1)*
- *Reduced neurological disability at hospital discharge (Aim 2)*
- *Reduced neuropsychological and functional deficits among survivors at 180 days (Aim 3)*

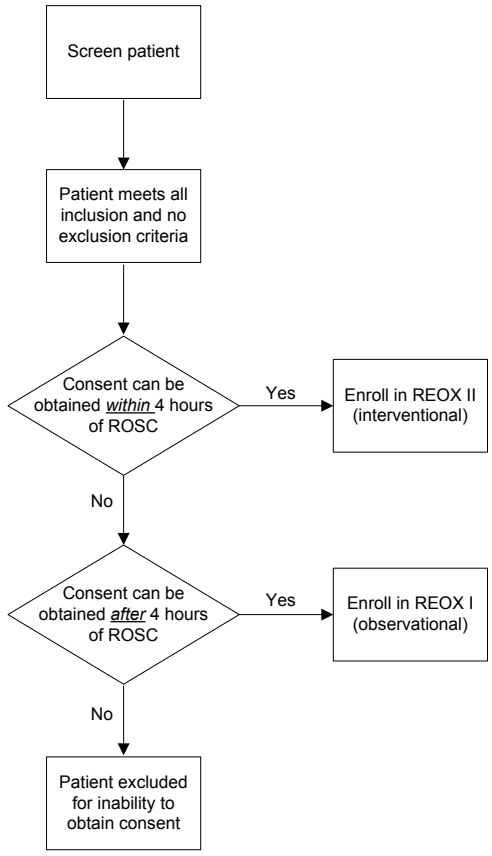
D.1.2. Overview

Design: Prospective protocol implementation study with an observational comparison group [REOX II (intervention component) versus REOX I (observational component)]
Participants: Adult patients resuscitated from cardiac arrest
Enrollment location: Emergency Departments of 6 urban academic medical centers
Intervention: Implementation of a clinical protocol for rapid FiO_2 optimization after ROSC from cardiac arrest.
Primary outcome: Good neurological outcome (defined as Modified Rankin Scale ≤ 3)[45] at hospital discharge.
Secondary outcomes: Neuropsychological tests and ability to return to work among survivors at 180 days.
Mechanistic outcomes: Oxidative stress markers (IsoPs/IsoFs) in plasma 0-6 hours after ROSC.
Estimated sample size and study duration: 280 patients (205 from REOX I and 75 from REOX II).

D.2. REOX II Study Protocol

***Study setting and population*:** This study is the second phase in a two-phase study. The initial phase, REOX I, was an observational-only study. As in REOX I, REOX II will identify subjects with return of spontaneous circulation (ROSC) after cardiac arrest in 6 hospitals (Cooper University Hospital, Camden, NJ; Hospital of the University of Pennsylvania and Penn-Presbyterian Medical Center, both in Philadelphia, PA; University of Mississippi Medical Center, Jackson, MS; Methodist Hospital, Indianapolis, IN; and Beth Israel Deaconess Medical Center, Boston, MA). The inclusion criteria are: age > 17 years; cardiac arrest; ROSC achieved; comatose (Glasgow Coma Scale motor score <6) after ROSC; and clinician intent to treat with target temperature management. The exclusion criteria are: presumed etiology of arrest is trauma, hemorrhage or sepsis; resident of a nursing home or other long-term care facility; known or clinically apparent pregnancy; known lack of commitment to aggressive support by next of kin; and any other condition, that, in the opinion of the investigator, would preclude the subject from being a suitable candidate (e.g. end stage chronic illness with no reasonable expectation of survival to hospital discharge).

Figure 9 Flow diagram for enrollment



***Patient identification and recruitment*:** Each of the participating centers currently has a mechanism in place for real-time notification of study personnel when an OHCA subject arrives in the emergency department (ED) or when a cardiac arrest occurs in-hospital. Each center has previously validated its notification mechanism to ensure that subjects will not be missed and the population of potentially eligible subjects will be *consecutive* cardiac arrest subjects. Study personnel will assess patient eligibility and will immediately enroll subjects meeting all inclusion and no exclusion criteria.

***Informed consent*:** In line with REOX I (observational-only component), in REOX II we will begin initial collection of cardiac arrest and post-resuscitation data immediately after ROSC. As per the inclusion criteria all subjects will be comatose during the initial post-ROSC period and therefore will not be able to consent themselves for the study intervention (i.e. protocol for rapid FiO_2 optimization after cardiac arrest). Once a subject is identified to have all inclusion and no exclusion criteria, we will approach surrogates (i.e. next of kin or legally authorized representative)

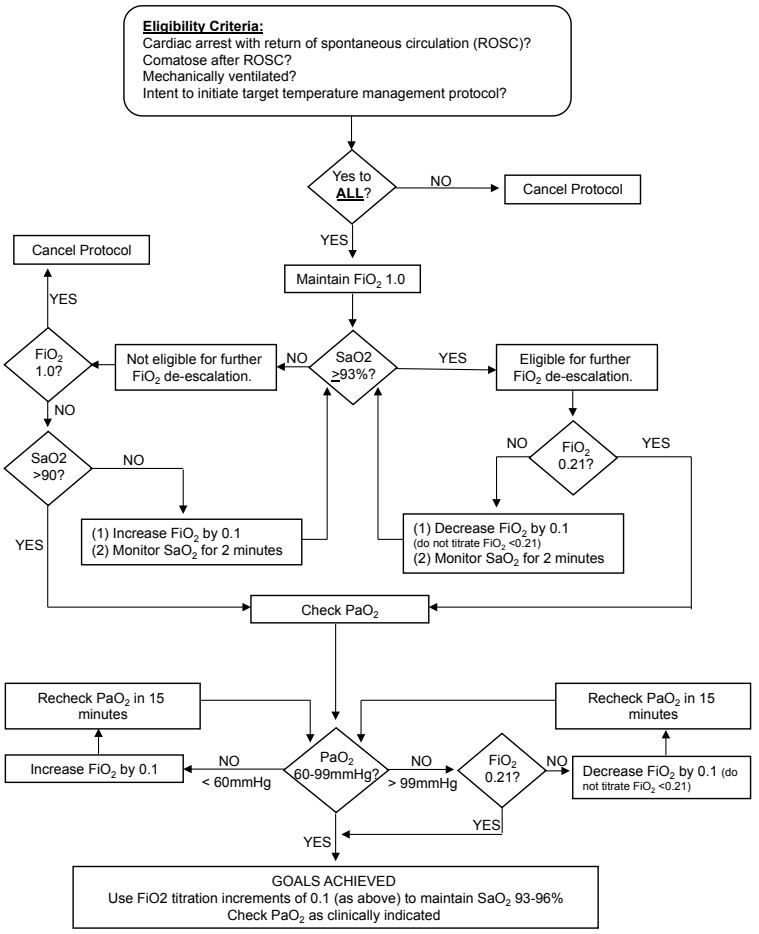
within the first four hours after ROSC for written informed consent and HIPAA authorization prior to implementation of the study intervention. If communication with a surrogate within the first four hours after ROSC is not achieved the subject will not be eligible for the study intervention. However, for subjects who are excluded from the study intervention due to lack of informed consent within the first four hours after ROSC, we will approach subjects or surrogates at a later time for written informed consent and HIPAA authorization for participation in the observational only (REOX I) component of the study including (a) use of plasma samples for research purposes (both stored samples obtained at the time of initial resuscitation and new samples); (b) determination of Modified Rankin Scale at hospital discharge; and (c) follow-up visits for neuropsychological testing at 180 days (**Figure 9**).

Table 3: Standard elements of post-cardiac arrest care across all centers. (TH = therapeutic hypothermia; GCS = Glasgow Coma Scale; ROSC = return of spontaneous circulation)

Standard care elements
24/7 capability for interventional cardiac catheterization (if needed)
Standardized patient selection for TH (i.e. GCS motor <6 after ROSC)
Target temperature goal for TH of 32-36ºC
Maintain TH target temperature 24 hours
Controlled rewarming to avoid hyperpyrexia after TH completion
24/7 capability for goal-directed hemodynamic support interventions
24/7 capability for continuous electroencephalographic monitoring
Evidence-based approach to neurological prognostication (e.g. wait ≥72 hours after ROSC before support limitations for poor neurological prognosis)

***Therapeutic interventions*: Table 3** displays the standard elements of post-resuscitation care across all centers. All sites are experienced in target temperature management after ROSC and target 32-36ºC for 24 hours using external cooling devices (Arctic Sun, Medivance, USA, or Gaymar III, Gaymar Industries, USA). Using a standardized order set for target temperature management induction, we demonstrated successful achievement of target temperature in 96% of patients and a median time from ROSC to target temperature of four hours (i.e. faster than the landmark randomized trial) in our routine clinical care.[40] All standard elements in **Table 3** reflect current AHA recommendations.[12] All subjects in this study will receive standard post-resuscitation care. Since none of the sites previously had a protocol in place for FiO_2 titration after ROSC and our standardized order sets for post-resuscitation care do not have orders for FiO_2 titration, subjects in REOX I (observational only component) had clinician-directed FiO_2 titration after ROSC.

For the interventional component of this study we will implement the protocol for rapid FiO_2 optimization after cardiac arrest (**Figure 10**) at all sites. Once a subject is identified and written informed consent is obtained from a surrogate, the study intervention (i.e. rapid FiO_2 optimization protocol) will be activated by the investigator who receives notification in real-time. (see *"Patient identification and recruitment"* above). The on call investigator will communicate directly with the clinical care team at the bedside and will ensure activation and adherence to the clinical protocol. Subjects enrolled in the study intervention will receive standard post-resuscitation care in **Table 3** *plus* rapid FiO_2 optimization according to **Figure 10**. Our sites have an outstanding track record of sustained clinical adherence to new resuscitation protocols for multi-year time periods.[37-42] Subjects who are excluded from the study intervention due to lack of informed consent within the first four hours after ROSC, will still be eligible for the observational only aspect of this study (REOX I). These subjects will receive standard post-resuscitation care outlined in **Table 3**, but will not have activation of the rapid FiO_2 optimization.



Instead these patients will have clinician-directed FiO_2 titration after ROSC. Data will be prospectively collected on these patients (see *"Data Collection"* below) and we will approach subjects or surrogates at a later time for written informed consent and HIPAA authorization for participation in the observational component of the study.

***Data collection*:** We will collect baseline data (e.g. demographics, comorbidities, etc.) and intra-arrest data (e.g. location, initial rhythm, length of downtime, etc.) using the Utstein template for cardiac arrest research[46] including the recommended variables for post-resuscitation research.[47] We will capture multiple indicators of post-resuscitation care quality in accordance with the 2010 AHA guidelines.[12] As mandated by the protocol (**Figure 10**) above, our standardized order set for post-resuscitation care will include multiple arterial blood gas (ABG) analyses for partial pressure of arterial oxygen (PaO_2) measurement after ROSC. As part of standard care each institution already obtains ABG analyses at 0- and 6-hours after ROSC. Any additional ABG analyses required to achieve the PaO_2 goal described in **Figure 10** will be considered for research purposes and will be funded by the study. We will record all ABG results over the initial 36 hours after ROSC. We will capture SaO_2 continuously for the initial 36 hours after ROSC. We will also capture every adjustment in FiO_2 from respiratory therapy records and the precise time of the adjustment. We will obtain plasma samples after ROSC at 0- and 6-hours after ROSC and ship the samples to the laboratory of our collaborator Jack Roberts (Vanderbilt) for measurement of isoprostanes (IsoPs) and isofurans (IsoFs) by gas chromatography negative ion chemical ionization mass spectrometry (GC/NICI/MS). IsoP and IsoF assays are remarkably stable and therefore are applicable to stored specimens. We can measure IsoPs and IsoFs on clinical plasma samples obtained immediately after ROSC because (after informed consent is secured) we can use samples left over in the clinical laboratory that are routinely discarded.

Table 4: Modified Rankin Scale

Scale	Description
0	No symptoms.
1	No significant disability. Able to carry out all usual activities, despite some symptoms.
2	Slight disability. Able to look after own affairs unassisted, but unable to carry out all previous activities.
3	Moderate disability. Requires some help, but able to walk unassisted.
4	Moderately severe disability. Unable to walk and attend to own bodily needs unassisted.
5	Severe disability. Requires constant nursing care and attention, bedridden, incontinent.
6	Dead

***Outcome measures*:** The primary outcome will be neurological disability at hospital discharge assessed with the Modified Rankin Scale (mRS),[48,49] a well-validated and widely used outcome measures in stroke clinical trials. The scale runs from 0-6, from perfect health without symptoms to death (**Table 4**). All raters will be trained and certified in mRS assessment[50] and will use a structured questionnaire and interview, which have been shown to produce very good interobserver reliability.[51,52] We will measure the Full Outline of UnResponsiveness (FOUR) Score[53,54] at 72 hours post-ROSC (**Table 5**). The FOUR Score consists of four components with a maximum score of four for each component and allows for assessing intubated patients and brainstem reflexes. We will also perform neuropsychological testing at 180 days after resuscitation for all patients who survived to hospital discharge. We will use an NHLBI-sponsored methodology for performing centralized, telephone-based cognitive testing, which has been previously validated for use in multi-center clinical studies.[55-60] Neuropsychological testing will use validated instruments across five cognitive domains: (1) attention, Wechsler Adult Intelligence Scale-IV-digit span; (2) reasoning, Wechsler Adult Intelligence Scale-IV-similarities; (3) immediate and delayed memory, Wechsler Memory Scale-III-logical memory I and II; (4) verbal fluency, Controlled Oral Word Association Test; and (5) executive functioning, Hayling Sentence Completion Test. We will also assess

Table 5: Full Outline of UnResponsiveness Score

Component	Scoring
Eye Response	4 = eyelids open or opened, tracking, or blinking to command 3 = eyelids open but not tracking 2 = eyelids closed but open to loud voice 1 = eyelids closed but open to pain 0 = eyelids remain closed with pain
Motor Response	4 = thumbs-up, fist, or peace sign 3 = localizing to pain 2 = flexion response to pain 1 = extension response to pain 0 = no response to pain or generalized myoclonus status
Brainstem Reflexes	4 = pupil and corneal reflexes present 3 = one pupil wide and fixed 2 = pupil or corneal reflexes absent 1 = pupil and corneal reflexes absent 0 = absent pupil, corneal, and cough reflex
Respiration	4 = not intubated, regular breathing pattern 3 = not intubated, Cheyne-Stokes breathing pattern 2 = not intubated, irregular breathing 1 = breathes above ventilator rate 0 = breathes at ventilator rate or apnea

orientation and judgment using the COGNISTAT- Neurobehavioral Cognitive Status Exam (NCSE). Functional outcome assessment will use validated measures of ability to return to work. The telephone-based cognitive battery allows for uniformity in cognitive assessment for patients enrolled across all study sites. Subjects who complete the telephone-based cognitive battery will be compensated $100 for their time. *Other measures*: Post-ROSC hyperoxia may also have extracerebral effects. We will calculate the extracerebral Sequential Organ Failure Assessment (SOFA) score,[61] which quantifies respiratory, cardiovascular, renal, hepatic, and coagulation system failure for 0-6 hours and 6-36 hours. We have experience using SOFA as an outcome measure to quantify organ failure.[61-64] These data will permit secondary analyses of the association between hyperoxia exposure and non-neurologic organ failure (i.e. total SOFA minus the neurological component[61]).

Analysis

We will begin the analysis with descriptive statistics for the entire cohort. We will report categorical data as proportions with 95% confidence intervals and continuous data as means with standard deviations or medians with interquartile ranges as appropriate. Using the data collected for PaO_2 and SaO_2 over time as described above, we will compute the time-weighted average (TWA) PaO_2 and SaO_2 over the first 24 hours after ROSC. Computing TWA is a common method for quantifying exposures over time in epidemiological research,[65-67] including studies of exposures and subsequent cognitive function.[68] The equations are:

TWA-PaO_2 = [(PaO_2*a* * Time *a*) + (PaO_2*b* * Time *b*) + … (PaO_2*z* * Time *z*)] / (Time *a* + Time *b* + … Time *z*)

TWA-SaO_2 = [(SaO_2*a* * Time *a*) + (SaO_2*b* * Time *b*) + … (SaO_2*z* * Time *z*)] / (Time *a* + Time *b* + … Time *z*)

We will build a multivariable regression model to test the association between TWA-PaO_2 and mRS at hospital discharge, adjusting for covariates previously demonstrated to be associated with poor neurological outcome after cardiac arrest. Covariates to be considered include: (1) pre-arrest comorbidities (Charlson index[69]), (2) prolonged downtime (downtime > 20 minutes) (yes/no), (3) initial arrest rhythm (pulseless electrical activity/asystole vs. ventricular tachycardia/ventricular fibrillation), and (4) arrest location (in-hospital vs. out-of-hospital).[70-75] In addition, in order to account for patients with lung injury (i.e. inability to maintain adequate oxygenation) we will add PaO_2/FiO_2 < 100mmHg (yes/no) to the regression model. We will build separate models to test the association between TWA-PaO_2 (adjusting for the five covariates above) and each of the five cognitive domains in our neuropsychological testing. We will then repeat these analyses using TWA-SaO_2 as the exposure variable of interest. This approach will not only allow us to test for an independent association between the exposure and neurological disability and neuropsychological deficits, but will also allow us to pinpoint one or more types of cognitive deficit (e.g. attention, executive function) most closely associated with the exposure. Given outcome measures that are quantitative (i.e. z-score for each cognitive domain) or semi-quantitative (i.e. mRS at hospital discharge), we will treat these outcomes as *continuous* dependent variables for modeling. Using continuous measures of the exposure and outcomes (rather than collapsing the data into categorical variables) will permit detection of subtle effects that could be missed if only categorical analyses were employed.

Secondary analyses: We will compare proportions of good neurological outcome [defined as a mRS ≤ 3 (based on the preliminary data of our collaborator, Dr. Clifton Callaway, University of Pittsburgh)],[45] *between subjects who receive the study intervention (i.e. rapid FiO_2 optimization) vs. those who do not receive the study intervention*, using binomial test. Among survivors, we will compare the 180-day neuropsychological measures (composite z-scores for each cognitive domain) between the same two groups using t-test or Mann-Whitney U as appropriate with corrections for multiple comparisons. We will compare the proportions of patients able to return to work between the two groups using binomial test. We will compare plasma IsoPs/IsoFs at each time point between the two groups using t-test or Mann-Whitney U as appropriate with corrections for multiple comparisons.

Sample Size: The sample size calculation in the initial study proposal was based on the primary outcome, mRS at hospital discharge, and we planned to enroll a total of 266 subjects. To ensure adequate power to test the relationships between the above independent variables and cognitive domains, we assumed a medium relationship strength between the six independent variables and each of the five cognitive domains ($R^2 = 0.15$). In order to achieve 80% power and an $\alpha = 0.05$ we would require a minimal of 85 subjects to undergo neuropsychological testing at 180 days. This sample size will be among the largest prospective studies of

neuropsychological outcomes that we are aware of in the literature.[8,29-32] Assuming a 15% drop out rate for lost to follow up for neuropsychological testing at 180 days we would require 98 subjects to be discharged with a mRS ≤ 4. Based on preliminary data from REOX I we expect 35% of patients will be discharged with a mRS ≤ 4 and undergo cognitive testing at 180 days. We would therefore require a minimal total sample size of 280 subjects between REOX I and II combined for the multivariable testing. We have enrolled 200 subjects in REOX I to date. We anticipate additional subjects to be enrolled in REOX I and therefore plan for 75 subjects to be enrolled in REOX II to ensure a total sample size of at least 280 subjects.

***Overview of the safety monitoring plan and serious adverse event tracking*:** In order to help ensure adequate protection of human subjects enrolled in this clinical study, we established an independent three-member **Data Safety Monitoring Board (DSMB)** (see **DSMB Charter**). The Chair of the DSMB will be Dr. Christopher W. Jones. Dr. Jones is an established investigator and is currently the Director of Clinical Research, Department of Emergency Medicine, Cooper University Hospital, Camden, NJ. The DSMB will be charged with reviewing all serious adverse event reports and will also perform interim analysis of the data after one-third (n=25) and two-thirds (n=50) of the patients have been enrolled in REOX II. Given neither REOX I nor REOX II are blinded, all data reviewed by the DSMB will be unmasked. At both interim analyses the committee will be asked to render a decision as to whether the trial should be continued, modified, or stopped based on the safety profile of the interim data. We have defined the following a priori as a reportable serious adverse event (SAE): (1) any events resulting in death or repeat cardiac arrest; (2) sustained hypoxia (defined as SaO_2 < 88% for more than three consecutive minutes); (3) heart rate < 50 for greater than one minute; (4) documented cyanosis at any time; or (5) withdrawal of a subject from the trial for any reason. We will define excessive rates of any single SAE *a priori* as a >15% difference in the prevalence (% of total number in each group) between the two groups [i.e. subjects who receive the study intervention (i.e. rapid FiO_2 optimization) vs. those who do not receive the study intervention] at either interim analysis. A clinician can withdraw a subject from the study for worsening clinical status including worsening respiratory status (based on clinical judgment, arterial blood gas analysis, or increasing FiO_2 requirement). The site PIs at each collaborating institution will be responsible for reporting all predefined SAEs, as well as any other adverse events felt to be related to the study protocol, to the coordinating site, Cooper University Hospital. In addition to reporting all adverse events to the DSMB, all adverse events will be submitted to each institution's IRB for review.

***Registration*:** This study will be registered on clinicaltrials.gov

References

1. Nichol G, Thomas E, Callaway CW, et al. Regional variation in out-of-hospital cardiac arrest incidence and outcome. *Jama.* Sep 24 2008;300(12):1423-1431.
2. Association AH. Heart disease and stroke statistics: 2010 update. Dallas, TX2010.
3. Zheng ZJ, Croft JB, Giles WH, Mensah GA. Sudden cardiac death in the United States, 1989 to 1998. *Circulation.* Oct 30 2001;104(18):2158-2163.
4. Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Carcillo J, Pinsky MR. Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. *Critical care medicine.* Jul 2001;29(7):1303-1310.
5. Surgeons ACo. *National Trauma Data Bank Report 2006.* Chicago, IL2006.
6. Laver S, Farrow C, Turner D, Nolan J. Mode of death after admission to an intensive care unit following cardiac arrest. *Intensive care medicine.* Nov 2004;30(11):2126-2128.
7. Trzeciak S, Jones AE, Kilgannon JH, et al. Significance of arterial hypotension after resuscitation from cardiac arrest. *Critical care medicine.* Nov 2009;37(11):2895-2903; quiz 2904.
8. Alexander MP, Lafleche G, Schnyer D, Lim C, Verfaellie M. Cognitive and functional outcome after out of hospital cardiac arrest. *J Int Neuropsychol Soc.* Mar 2011;17(2):364-368.
9. Neumar RW, Nolan JP, Adrie C, et al. Post-cardiac arrest syndrome: epidemiology, pathophysiology, treatment, and prognostication. A consensus statement from the International Liaison Committee on Resuscitation (American Heart Association, Australian and New Zealand Council on Resuscitation, European Resuscitation Council, Heart and Stroke Foundation of Canada, InterAmerican Heart Foundation, Resuscitation Council of Asia, and the Resuscitation Council of Southern Africa); the American Heart Association Emergency Cardiovascular Care Committee; the Council on Cardiovascular Surgery and Anesthesia; the Council on Cardiopulmonary, Perioperative, and Critical Care; the Council on Clinical Cardiology; and the Stroke Council. *Circulation.* Dec 2 2008;118(23):2452-2483.
10. Mild therapeutic hypothermia to improve the neurologic outcome after cardiac arrest. *The New England journal of medicine.* Feb 21 2002;346(8):549-556.
11. Bernard SA, Gray TW, Buist MD, et al. Treatment of comatose survivors of out-of-hospital cardiac arrest with induced hypothermia. *The New England journal of medicine.* Feb 21 2002;346(8):557-563.
12. Peberdy MA, Callaway CW, Neumar RW, et al. Part 9: post-cardiac arrest care: 2010 American Heart Association Guidelines for Cardiopulmonary Resuscitation and Emergency Cardiovascular Care. *Circulation.* Nov 2;122(18 Suppl 3):S768-786.
13. Gazmuri RJ, Nolan JP, Nadkarni VM, et al. Scientific knowledge gaps and clinical research priorities for cardiopulmonary resuscitation and emergency cardiovascular care identified during the 2005 International Consensus Conference on ECC and CPR Science with Treatment Recommendations. A consensus statement from the International Liaison Committee on Resuscitation; the American Heart Association Emergency Cardiovascular Care Committee; the Stroke Council; and the Cardiovascular Nursing Council. *Resuscitation.* Dec 2007;75(3):400-411.
14. Hazelton JL, Balan I, Elmer GI, et al. Hyperoxic reperfusion after global cerebral ischemia promotes inflammation and long-term hippocampal neuronal death. *Journal of neurotrauma.* Apr 2010;27(4):753-762.
15. Liu Y, Rosenthal RE, Haywood Y, Miljkovic-Lolic M, Vanderhoek JY, Fiskum G. Normoxic ventilation after cardiac arrest reduces oxidation of brain lipids and improves neurological outcome. *Stroke; a journal of cerebral circulation.* Aug 1998;29(8):1679-1686.
16. Zwemer CF, Whitesall SE, D'Alecy LG. Cardiopulmonary-cerebral resuscitation with 100% oxygen exacerbates neurological dysfunction following nine minutes of normothermic cardiac arrest in dogs. *Resuscitation.* Mar 1994;27(2):159-170.
17. Danilov CA, Fiskum G. Hyperoxia promotes astrocyte cell death after oxygen and glucose deprivation. *Glia.* May 2008;56(7):801-808.
18. Vereczki V, Martin E, Rosenthal RE, Hof PR, Hoffman GE, Fiskum G. Normoxic resuscitation after cardiac arrest protects against hippocampal oxidative stress, metabolic dysfunction, and neuronal death. *J Cereb Blood Flow Metab.* Jun 2006;26(6):821-835.
19. Mickel HS, Vaishnav YN, Kempski O, von Lubitz D, Weiss JF, Feuerstein G. Breathing 100% oxygen

after global brain ischemia in Mongolian Gerbils results in increased lipid peroxidation and increased mortality. *Stroke; a journal of cerebral circulation.* Mar-Apr 1987;18(2):426-430.
20. Fiskum G, Danilov CA, Mehrabian Z, et al. Postischemic oxidative stress promotes mitochondrial metabolic failure in neurons and astrocytes. *Annals of the New York Academy of Sciences.* Dec 2008;1147:129-138.
21. Richards EM, Fiskum G, Rosenthal RE, Hopkins I, McKenna MC. Hyperoxic reperfusion after global ischemia decreases hippocampal energy metabolism. *Stroke; a journal of cerebral circulation.* May 2007;38(5):1578-1584.
22. Dyson A, Stidwill R, Taylor V, Singer M. The impact of inspired oxygen concentration on tissue oxygenation during progressive haemorrhage. *Intensive care medicine.* Oct 2009;35(10):1783-1791.
23. Lu J, Dai G, Egi Y, et al. Characterization of cerebrovascular responses to hyperoxia and hypercapnia using MRI in rat. *Neuroimage.* May 1 2009;45(4):1126-1134.
24. Kilgannon JH, Jones AE, Shapiro NI, et al. Association between arterial hyperoxia following resuscitation from cardiac arrest and in-hospital mortality. *Jama.* Jun 2 2010;303(21):2165-2171.
25. Kilgannon JH, Jones AE, Parrillo JE, et al. Relationship between supranormal oxygen tension and outcome after resuscitation from cardiac arrest. *Circulation.* 2011;(in press).
26. Bellomo R, Bailey M, Eastwood GM, et al. Arterial hyperoxia and in-hospital mortality after resuscitation from cardiac arrest. *Crit Care.* Mar 8 2011;15(2):R90.
27. Nolan JP, Soar J, Zideman DA, et al. European Resuscitation Council Guidelines for Resuscitation 2010 Section 1. Executive summary. *Resuscitation.* Oct 2010;81(10):1219-1276.
28. Trzeciak S, Jones AE, Kilgannon JH, et al. Outcome measures utilized in clinical trials of interventions for post-cardiac arrest syndrome: a systematic review. *Resuscitation.* Jun 2009;80(6):617-623.
29. Roine RO, Kajaste S, Kaste M. Neuropsychological sequelae of cardiac arrest. *Jama.* Jan 13 1993;269(2):237-242.
30. Sauve MJ, Doolittle N, Walker JA, Paul SM, Scheinman MM. Factors associated with cognitive recovery after cardiopulmonary resuscitation. *Am J Crit Care.* Mar 1996;5(2):127-139.
31. Tiainen M, Poutiainen E, Kovala T, Takkunen O, Happola O, Roine RO. Cognitive and neurophysiological outcome of cardiac arrest survivors treated with therapeutic hypothermia. *Stroke; a journal of cerebral circulation.* Aug 2007;38(8):2303-2308.
32. van Alem AP, de Vos R, Schmand B, Koster RW. Cognitive impairment in survivors of out-of-hospital cardiac arrest. *American heart journal.* Sep 2004;148(3):416-421.
33. Becker LB, Aufderheide TP, Geocadin RG, et al. Primary outcomes for resuscitation science studies: a consensus statement from the American Heart Association. *Circulation.* Nov 8 2011;124(19):2158-2177.
34. Trzeciak S, Jones AE, Shapiro NI, et al. A prospective multicenter cohort study of the association between global tissue hypoxia and coagulation abnormalities during early sepsis resuscitation. *Critical care medicine.* Apr 2010;38(4):1092-1100.
35. Jones AE, Shapiro NI, Trzeciak S, Arnold RC, Claremont HA, Kline JA. Lactate clearance vs central venous oxygen saturation as goals of early sepsis therapy: a randomized clinical trial. *Jama.* Feb 24 2010;303(8):739-746.
36. Pope JV, Jones AE, Gaieski DF, Arnold RC, Trzeciak S, Shapiro NI. Multicenter study of central venous oxygen saturation (ScvO(2)) as a predictor of mortality in patients with sepsis. *Ann Emerg Med.* Jan 2010;55(1):40-46 e41.
37. Gaieski DF, Band RA, Abella BS, et al. Early goal-directed hemodynamic optimization combined with therapeutic hypothermia in comatose survivors of out-of-hospital cardiac arrest. *Resuscitation.* Apr 2009;80(4):418-424.
38. Gaieski DF, Fuchs B, Carr BG, et al. Practical implementation of therapeutic hypothermia after cardiac arrest. *Hosp Pract (Minneap).* Dec 2009;37(1):71-83.
39. Jones AE, Focht A, Horton JM, Kline JA. Prospective external validation of the clinical effectiveness of an emergency department-based early goal-directed therapy protocol for severe sepsis and septic shock. *Chest.* Aug 2007;132(2):425-432.
40. Kilgannon JH, Roberts BW, Stauss M, et al. Use of a standardized order set for achieving target temperature in the implementation of therapeutic hypothermia after cardiac arrest: a feasibility study. *Acad Emerg Med.* Jun 2008;15(6):499-505.

41. Shapiro NI, Howell MD, Talmor D, et al. Implementation and outcomes of the Multiple Urgent Sepsis Therapies (MUST) protocol. *Critical care medicine.* Apr 2006;34(4):1025-1032.
42. Trzeciak S, Dellinger RP, Abate NL, et al. Translating research to clinical practice: a 1-year experience with implementing early goal-directed therapy for septic shock in the emergency department. *Chest.* Feb 2006;129(2):225-232.
43. Peberdy MA, Callaway CW, Neumar RW, et al. Part 9: post-cardiac arrest care: 2010 American Heart Association Guidelines for Cardiopulmonary Resuscitation and Emergency Cardiovascular Care. *Circulation.* Nov 2 2010;122(18 Suppl 3):S768-786.
44. Post MW, de Bruin A, de Witte L, Schrijvers A. The SIP68: a measure of health-related functional status in rehabilitation medicine. *Arch Phys Med Rehabil.* May 1996;77(5):440-445.
45. Rittenberger JC, Raina K, Holm MB, Kim YJ, Callaway CW. Association between Cerebral Performance Category, Modified Rankin Scale, and discharge disposition after cardiac arrest. *Resuscitation.* Aug 2011;82(8):1036-1040.
46. Jacobs I, Nadkarni V, Bahr J, et al. Cardiac arrest and cardiopulmonary resuscitation outcome reports: update and simplification of the Utstein templates for resuscitation registries: a statement for healthcare professionals from a task force of the International Liaison Committee on Resuscitation (American Heart Association, European Resuscitation Council, Australian Resuscitation Council, New Zealand Resuscitation Council, Heart and Stroke Foundation of Canada, InterAmerican Heart Foundation, Resuscitation Councils of Southern Africa). *Circulation.* Nov 23 2004;110(21):3385-3397.
47. Langhelle A, Nolan J, Herlitz J, et al. Recommended guidelines for reviewing, reporting, and conducting research on post-resuscitation care: the Utstein style. *Resuscitation.* Sep 2005;66(3):271-283.
48. Farrell B, Godwin J, Richards S, Warlow C. The United Kingdom transient ischaemic attack (UK-TIA) aspirin trial: final results. *J Neurol Neurosurg Psychiatry.* Dec 1991;54(12):1044-1054.
49. van Swieten JC, Koudstaal PJ, Visser MC, Schouten HJ, van Gijn J. Interobserver agreement for the assessment of handicap in stroke patients. *Stroke; a journal of cerebral circulation.* May 1988;19(5):604-607.
50. Quinn TJ, Lees KR, Hardemark HG, Dawson J, Walters MR. Initial experience of a digital training resource for modified Rankin scale assessment in clinical trials. *Stroke; a journal of cerebral circulation.* Aug 2007;38(8):2257-2261.
51. Wilson JT, Hareendran A, Grant M, et al. 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.* Sep 2002;33(9):2243-2246.
52. Wilson JT, Hareendran A, Hendry A, Potter J, Bone I, Muir KW. Reliability of the modified Rankin Scale across multiple raters: benefits of a structured interview. *Stroke; a journal of cerebral circulation.* Apr 2005;36(4):777-781.
53. Stead LG, Wijdicks EF, Bhagra A, et al. Validation of a new coma scale, the FOUR score, in the emergency department. *Neurocritical care.* 2009;10(1):50-54.
54. Wijdicks EF, Bamlet WR, Maramattom BV, Manno EM, McClelland RL. Validation of a new coma scale: The FOUR score. *Annals of neurology.* Oct 2005;58(4):585-593.
55. Christie JD, Biester RC, Taichman DB, et al. Formation and validation of a telephone battery to assess cognitive function in acute respiratory distress syndrome survivors. *Journal of critical care.* Jun 2006;21(2):125-132.
56. Mikkelsen ME, Christie JD, Lanken PN, et al. The adult respiratory distress syndrome cognitive outcomes study: long-term neuropsychological function in survivors of acute lung injury. *Am J Respir Crit Care Med.* Jun 15 2012;185(12):1307-1315.
57. Mikkelsen ME, Shull WH, Biester RC, et al. Cognitive, mood and quality of life impairments in a select population of ARDS survivors. *Respirology.* Jan 2009;14(1):76-82.
58. Needham DM, Dinglas VD, Bienvenu OJ, et al. One year outcomes in patients with acute lung injury randomised to initial trophic or full enteral feeding: prospective follow-up of EDEN randomised trial. *Bmj.* 2013;346:f1532.
59. Needham DM, Dinglas VD, Morris PE, et al. Physical and cognitive performance of patients with acute lung injury 1 year after initial trophic versus full enteral feeding. EDEN trial follow-up. *Am J Respir Crit Care Med.* Sep 1 2013;188(5):567-576.
60. Taichman DB, Christie J, Biester R, et al. Validation of a brief telephone battery for neurocognitive

assessment of patients with pulmonary arterial hypertension. *Respiratory research.* 2005;6:39.
61. Roberts BW, Kilgannon JH, Chansky ME, et al. Multiple organ dysfunction after return of spontaneous circulation in postcardiac arrest syndrome. *Crit Care Med.* Jun 2013;41(6):1492-1501.
62. Jones AE, Trzeciak S, Kline JA. The Sequential Organ Failure Assessment score for predicting outcome in patients with severe sepsis and evidence of hypoperfusion at the time of emergency department presentation. *Critical care medicine.* May 2009;37(5):1649-1654.
63. Trzeciak S, McCoy JV, Phillip Dellinger R, et al. Early increases in microcirculatory perfusion during protocol-directed resuscitation are associated with reduced multi-organ failure at 24 h in patients with sepsis. *Intensive care medicine.* Dec 2008;34(12):2210-2217.
64. Sciences NIoGM. Randomized trial of inhaled nitric oxide to augment tissue perfusion in sepsis (NCT00608322). clinicaltrials.gov. Accessed May 1, 2011.
65. Doria A, Wojcik J, Xu R, et al. Interaction between poor glycemic control and 9p21 locus on risk of coronary artery disease in type 2 diabetes. *JAMA : the journal of the American Medical Association.* Nov 26 2008;300(20):2389-2397.
66. Egi M, Bellomo R, Stachowski E, et al. The interaction of chronic and acute glycemia with mortality in critically ill patients with diabetes. *Critical care medicine.* Jan 2011;39(1):105-111.
67. Finney SJ, Zekveld C, Elia A, Evans TW. Glucose control and mortality in critically ill patients. *JAMA : the journal of the American Medical Association.* Oct 15 2003;290(15):2041-2047.
68. Jacobson AM, Musen G, Ryan CM, et al. Long-term effect of diabetes and its treatment on cognitive function. *The New England journal of medicine.* May 3 2007;356(18):1842-1852.
69. Murray SB, Bates DW, Ngo L, Ufberg JW, Shapiro NI. Charlson Index is associated with one-year mortality in emergency department patients with suspected infection. *Acad Emerg Med.* May 2006;13(5):530-536.
70. Gaul GB, Gruska M, Titscher G, et al. Prediction of survival after out-of-hospital cardiac arrest: results of a community-based study in Vienna. *Resuscitation.* Oct 1996;32(3):169-176.
71. Hajbaghery MA, Mousavi G, Akbari H. Factors influencing survival after in-hospital cardiopulmonary resuscitation. *Resuscitation.* Sep 2005;66(3):317-321.
72. Langhelle A, Tyvold SS, Lexow K, Hapnes SA, Sunde K, Steen PA. In-hospital factors associated with improved outcome after out-of-hospital cardiac arrest. A comparison between four regions in Norway. *Resuscitation.* Mar 2003;56(3):247-263.
73. Nadkarni VM, Larkin GL, Peberdy MA, et al. First documented rhythm and clinical outcome from in-hospital cardiac arrest among children and adults. *Jama.* Jan 4 2006;295(1):50-57.
74. Peberdy MA, Kaye W, Ornato JP, et al. Cardiopulmonary resuscitation of adults in the hospital: a report of 14720 cardiac arrests from the National Registry of Cardiopulmonary Resuscitation. *Resuscitation.* Sep 2003;58(3):297-308.
75. Tok D, Keles GT, Toprak V, Topcu I. Assessment of in-hospital cardiopulmonary resuscitation using Utstein template in a university hospital. *Tohoku J Exp Med.* Apr 2004;202(4):265-273.
76. Davis DP, Idris AH, Sise MJ, et al. Early ventilation and outcome in patients with moderate to severe traumatic brain injury. *Crit Care Med.* Apr 2006;34(4):1202-1208.
77. Del Castillo J, Lopez-Herce J, Matamoros M, et al. Hyperoxia, hypocapnia and hypercapnia as outcome factors after cardiac arrest in children. *Resuscitation.* Dec 2012;83(12):1456-1461.
78. Roberts BW, Kilgannon JH, Chansky ME, Mittal N, Wooden J, Trzeciak S. Association between postresuscitation partial pressure of arterial carbon dioxide and neurological outcome in patients with post-cardiac arrest syndrome. *Circulation.* May 28 2013;127(21):2107-2113.
79. Roberts BW, Kilgannon JH, Chansky ME, Mittal N, Wooden J, Trzeciak S. Response to letter regarding article, "association between postresuscitation partial pressure of arterial carbon dioxide and neurological outcome in patients with post-cardiac arrest syndrome". *Circulation.* Jan 7 2014;129(1):e10.