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Clinical paper

# Inflammation, endothelial injury, and the acute respiratory distress syndrome after out-of-hospital cardiac arrest

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## Abstract

**Background**: Acute respiratory distress syndrome (ARDS) is often seen in patients resuscitated from out-of-hospital cardiac arrest (OHCA). We aim to test whether inflammatory or endothelial injury markers are associated with the development of ARDS in patients hospitalized after OHCA.

**Methods**: We conducted a prospective, cohort, pilot study at an urban academic medical center in 2019 that included a convenience sample of adults with non-traumatic OHCA. Blood and pulmonary edema fluid (PEF) were collected within 12 hours of hospital arrival. Samples were assayed for cytokines (interleukin [IL]-1, tumor necrosis factor-α [TNF-α], tumor necrosis factor receptor1 [TNFR1], IL-6), epithelial injury markers (pulmonary surfactant-associated protein D), endothelial injury markers (Angiopoietin-2 [Ang-2] and glycocalyx degradation products), and other proteins (matrix metallopeptidase-9 and myeloperoxidase). Patients were followed for 7 days for development of ARDS, as adjudicated by 3 blinded reviewers, and through hospital discharge for mortality and neurological outcome. We examined associations between biomarker concentrations and ARDS, hospital mortality, and neurological outcome using multivariable logistic regression. Latent phase analysis was used to identify distinct biological classes associated with outcomes.

**Results**: 41 patients were enrolled. Mean age was 58 years, 29% were female, and 22% had a respiratory etiology for cardiac arrest. Seven patients (17%) developed ARDS within 7 days. There were no significant associations between individual biomarkers and development of ARDS in adjusted analyses, nor survival or neurologic status after adjusting for use of targeted temperature management (TTM) and initial cardiac arrest rhythm. Elevated Ang-2 and TNFR-1 were associated with decreased survival (RR = 0.6, 95% CI = 0.3–1.0; RR = 0.5, 95% CI = 0.3–0.9; respectively), and poor neurologic status at discharge (RR = 0.4, 95% CI = 0.2–0.8; RR = 0.4, 95% CI = 0.2–0.9) in unadjusted associations.

**Conclusion**: OHCA patients have markedly elevated plasma and pulmonary edema fluid biomarker concentrations, indicating widespread inflammation, epithelial injury, and endothelial activation. Biomarker concentrations were not associated with ARDS development, though several distinct biological phenotypes warrant further exploration. Latent phase analysis demonstrated that patients with low biomarker levels aside from TNF-α and TNFR-1 (Class 2) fared worse than other patients. Future research may benefit from considering other tools to predict and prevent development of ARDS in this population.

**Keywords**: Out-of-hospital cardiac arrest, OHCA, Cardiac arrest, Acute respiratory distress syndrome, ARDS, Cardiopulmonary resuscitation, Post-arrest care

## Introduction

The acute respiratory distress syndrome (ARDS) is common after out of hospital cardiac arrest (OHCA) and associated with poor outcomes.[1,2] [1] Patients who developed ARDS after OHCA were less likely to survive with full neurologic recovery to hospital discharge than those without ARDS (35% vs 54%), had longer median

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https://doi.org/10.1016/j.resplu.2024.100590

Received 27 December 2023; Received in revised form 7 February 2024; Accepted 13 February 2024

intensive care unit stays, more days requiring mechanical ventilation, and longer hospital stays.[1]

ARDS has a characteristic biological profile that can predict both development and severity. Plasma concentrations of surfactant protein-D, angiopoietin-2 (Ang-2), interleukin (IL)-8, and tumor necrosis factor receptor-1 (TNFR-1) have been associated with both ARDS development and outcome in patients without cardiac arrest.[3,4] In addition, concentrations of pro-inflammatory mediators such as IL-1β, IL-6, IL-8, and TNF-alpha are elevated in pulmonary edema fluid in patients with ARDS.[4] Treatments aimed at reducing lung injury have been shown to alter concentrations of these pro-inflammatory mediators.[5]

The post-cardiac arrest syndrome (PCAS) is a highly inflammatory state defined by reperfusion injury, oxidative stress, and multi-organ dysfunction.[2–6] The PCAS is associated with elevated plasma concentrations of cytokines, chemokines, and markers of endothelial injury.[6,7] Additionally, the endothelial glycocalyx is damaged during cardiac arrest, and degree of injury is associated with increased mortality.[8] Thus assessing for glycocalyx damage in patients following OHCA may provide a manner for predicting unfavorable clinical outcomes, such as ARDS. While substantial pathophysiologic overlap exists between PCAS and ARDS, it is unknown whether patients who develop ARDS after OHCA have a distinct biological phenotype. Further, it is unknown whether circulating concentrations of cytokines, chemokines, and endothelial injury markers are associated with development ARDS, or whether they might be useful biomarkers for potential therapeutics.

In this pilot study, we aimed to test whether early plasma and/or alveolar fluid markers (cytokine, chemokine, and endothelial injury) in patients who experienced OHCA are associated with development of ARDS and whether distinct biological phenotypes can be identified. The goal of this study is to understand the relative contribution of epithelial injury, endothelial activation, and inflammatory pathways to ARDS onset, as well as neurologic outcomes, and mortality in the OHCA patient population. We hypothesized that patients who develop ARDS would have a biological profile that may allow for early identification of patients at-risk for ARDS, which may facilitate implementation of strategies proven to improve outcomes.

## Methods

### Ethics approval and setting

The University of Washington (UW) Human Subjects Division approved this study. Informed consent was deemed not necessary if blood volumes were limited to < 20 mL. This study site was Seattle, Washington, a city in the United States with 725,000 residents and 83.9 square miles. The Seattle Fire Department's emergency medical services system, Seattle Medic One, has been described previously.[9]

Harborview Medical Center (HMC) is a 413-bed public hospital and trauma center. HMC is a core teaching site for the UW School of Medicine and affiliated residency and fellowship programs. Combined, HMC receives approximately 65,000 emergency department (ED) visits and 110–150 patients with OHCA each year. Standard TTM guidelines dictate cooling unresponsive patients to 33 °C following OHCA.

### Study design and population

This was a prospective cohort study of adult patients who suffered non-traumatic OHCA (with any initial cardiac rhythm) between January 1, 2019 and December 31, 2019, were resuscitated, and transported to HMC. Patients were consecutively screened and included if intubated and mechanically ventilated at the time of enrollment. Patients with cardiac arrest due to trauma or hemorrhage, incarcerated persons, and pregnant patients were excluded. Patients were identified prospectively in the ED by on-call study coordinators from 8:00 am until 10:00 pm, 7 days per week.

### Outcomes

The primary outcome was development of ARDS within 7 days of hospital admission, defined according to the Berlin definition.[10] ARDS was adjudicated by three physicians trained in emergency medicine and/or critical care in a stepwise fashion (NJJ, PB, DJH). These physicians were blinded to biomarker assay results at the time of adjudication and to the reviews of the other physicians. Medical records for all patients with a qualifying partial pressure of arterial oxygen ($P_aO_2$) to fraction of inspired oxygen ($F_IO_2$) ratio (P:F ratio) of $\leq$ 300 mmHg underwent review by two physicians. The same two physicians then adjudicated chest radiographs within 24 hours of a qualifying P:F ratio as "consistent with ARDS" or "not consistent with ARDS''. For those with chest radiographs consistent with ARDS, additional medical record review was performed to ensure that hypoxemia and radiographic findings were not fully explained by heart failure or volume overload. Interobserver agreement was calculated using kappa coefficient. Where disagreement occurred, a third physician, blinded to the others' findings, performed a final review. Secondary outcomes included ICU length of stay (LOS), hospital LOS, ventilator days, hospital mortality, and favorable neurologic status at hospital discharge based on Modified Rankin Score. ICU-free days was calculated as the difference between hospital LOS and ICU LOS.

### Data and biospecimen collection

Qualifying OHCA patients were identified and enrolled within 12 hours of ED arrival. Prehospital data were obtained from the Seattle Fire Department OHCA registry.[9] ED and inpatient data were abstracted electronically from the hospital medical record by trained abstractors.

A total of 19.5 ml of blood was collected in the following tubes: 10 ml EDTA, 5 ml serum, 4.5 ml sodium citrate. Samples were centrifuged at 1,500 RPM for 10 minutes, aliquoted, and frozen to −80 °C. Pulmonary edema fluid was obtained by tracheal aspiration. A suction trap was attached to sterile in-line suction on ventilator tubing. The suction catheter was advanced blindly until it stopped, presumably wedged in a terminal airway, and edema fluid was suctioned. Edema fluid was immediately processed, centrifuged at 1,500 RPM for 10 minutes, and the supernatant was aliquoted and frozen at −80 °C. The median length of time (IQR) from hospital arrival to sample collection was 3.45 (4.42) hours.

### Assays

Plasma samples were assayed on a Meso Scale Discovery (MSD) Quickplex SQ 120 (Meso Scale Diagnostics, LLC., Rockville, MD). Assays were performed according to the manufacturer's instructions. Samples were diluted to fit the dynamic range for each analyte. Inter-assay performance was assessed with EDTA plasma control samples, and intra-assay performance was assessed with blind replicates of patient samples and the inter-assay control samples.

### Isolation and quantification of glycosaminoglycans

We quantified endothelial glycocalyx degradation by measuring shed glycosaminoglycans (GAGs) such as heparan sulfate (HS) and chondroitin sulfate (CS) from plasma specimens, as previously described.[11] Briefly, 100 μL of plasma samples were washed and desalted using a 3 kDa molecular weight cut off spin column Millipore UFC500396). GAGs were then digested with recombinant heparin lyases (I, II, III) and chondroitin lyases (ABC) in digestion buffer (50 mM ammonium acetate containing 2 mM calcium chloride adjusted to pH 7.0) at 37 °C for 4 h. Enzymes were then removed by centrifugation. Digested disaccharide products were lyophilized and labeled with 2-aminoacridone. Ultra Performance Liquid Chromatography (UPLC)-tandem mass spectrometry was performed with a Thermo UltiMate 3000 LC system and an AB Sciex QTRAP 5500 with multiple reaction monitoring (MRM) mode. A mixture containing 17 disaccharide standards prepared at 80–160 nM was similarly AMAC-labeled and used for each analysis as an external standard. Isolation and derivatization of glycosaminoglycans (GAG) and LC-MS/MS analysis was conducted according to Rizzo et al.[11].

### Statistical analysis

Descriptive statistics were performed to compare patient demographics, clinical characteristics and outcomes, by ARDS status. Biomarker concentrations were visualized via box and whisker plot, and outcomes were reported using exact binomial confidence intervals. Vital signs and biomarkers missing in over 90% of the cohort were excluded from analyses, and remaining missing data were imputed (see Supplement for additional information on multiple imputation methods applied). All biomarkers were log2-transformed to approximate normality.

#### *Bivariate and multivariate logistic regression*

Bivariate logistic regression was used to evaluate the individual association of each factor with primary and secondary outcomes. Factors associated with outcomes ($p < 0.3$), as well as clinically selected factors (based on previous literature) were considered candidate covariates to be included in backward stepwise regression and model creation (threshold for removal from model, $p = 0.2$). Using a high significance threshold to identify candidate covariates prevents premature exclusion of important variables, while the iterative process in stepwise regression of removing and refitting the model allows only the main effects to remain in the model.[12] In contrast, a relatively lower removal threshold during the stepwise process drives a more parsimonious final model to prevent overfitting. No collinearity was detected in final multivariate models.

#### *Latent phase analysis*

Latent phase analysis was performed within Stata's generalized structural equation modeling (gsem) capabilities. Additional information on latent phase analysis methods can be found in the Supplement. Two-sample tests of proportions were used to describe differences in outcomes between patients in vs. outside of each class. Exact binomial methods were used for 95% CI calculations. All statistical analyses were performed using Stata 15.

## Results

### Patient and care characteristics

A total of 41 OHCA patients were included in the cohort. Mean age was 58 years (SD ± 16), 71% were male, and 59% were white (Table 1). Of these, 16 (39% patients) survived to hospital discharge and 12 (29% patients) survived with complete neurologic recovery or mild impairment (Table 2). Descriptions of cardiac arrest care can be found in Table 1, and processes of care can be found in Table 3. Descriptions of ventilator parameters can be found in Supplemental Table 1.

### Biomarker results

Thirty nine of the 41 enrolled patients had plasma samples collected and 19 of the 41 had pulmonary fluid samples collected. Median biomarker concentrations with interquartile ranges are reported in Supplemental Table 2 Notable median plasma values include interleukin (IL)-6 (60 pg/ml), Angiopoietin (Ang)-2 (5816 pg/ml), and tumor necrosis factor receptor (TNFR)-1 (4693 pg/ml). From the pulmonary fluid, matrix metalloproteinase (MMP)-9 (69,0000 pg/ml and myeloperoxidase (MPO) (17,000,000 pg/ml) were markedly elevated. Chondroitin and heparan sulfate concentrations were also high.

### Primary outcome: ARDS incidence

A total of 7 patients (17%) met criteria for ARDS within 7 days. Of the 41 total patients enrolled, 18 never had P:F ratio $\leq 300$, 10 did not have a qualifying radiograph, and 6 had findings fully explained by heart failure or volume overload. Of the 7 patients with ARDS, median initial $P_aO_2$:$F_IO_2$ was 126 (IQR = 88–138) and 29% met hypoxemia criteria for severe ARDS ($P_aO_2$:$F_IO_2 \leq 100$; Table 2).

No clinical characteristics or biomarkers were found to be significantly associated with development of ARDS via either unadjusted or adjusted logistic regression (Tables 4–5). Fig. 1 provides a visualization of plasma biomarker concentrations by ARDS status.

### Secondary outcomes: Neurological status and mortality

The median hospital length of stay for the enrolled patients was 10 days. During hospitalization, 61% died; of the remaining, 10% were discharged to a care facility and 24% were discharged home. The distribution of neurological status at discharge is as follows: 15% made a full recovery, 15% were mildly impaired, 7% were severely impaired, and 2% were comatose (Table 2).

Clinical characteristics and biomarkers associated with neurological outcome by unadjusted logistic regression were Ang-2, TNFR1, total HS, and initial cardiac rhythm (Table 4). Multivariable modeling revealed that presence of shockable rhythm was significantly positively associated with mild to no neurological impairment.

Clinical characteristics and biomarkers associated with survival by unadjusted logistic regression were Ang-2, TNFR1, total HS, and initial cardiac rhythm. Multivariable modeling revealed that presence of shockable rhythm was significantly positively associated with survival, whereas initiation of TTM, and total HS concentration were significantly negatively associated.

### Latent phase analysis with plasma biomarkers

All nine plasma biomarkers were included for latent phase analysis (Supplementary Table 3). Fit characteristics shows the fit character-

**Table 1 – Patient and cardiac arrest characteristics.**

| Characteristic, N (%) | Cohort | No ARDS | ARDS |
|---|---|---|---|
| | (n = 41) | (n = 34) | (n = 7) |
| Age (Median, IQR) | 60 (48–69) | 59 (47–68) | 69 (53–72) |
| Weight in kg, (Median, IQR) | 80 (69–99) | 80 (70–100) | 78 (60–83) |
| Sex: Male | 29 (71) | 23 (68) | 6 (86) |
| Race: White | 24 (59) | 21 (62) | 3 (43) |
| Initial Cardiac Rhythm: Shockable | 13 (32) | 12 (35) | 1 (14) |
| First hospital temperature: < 32 °C | 37 (90) | 31 (91) | 6 (86) |
| Initial lactate (Median, IQR) | 7.5 (2.9–11) | 9.1 (3.4–12.5) | 5.4 (2.6–7.5) |
| Max troponin (Median, IQR) | 0.93 (0.16–3.57) | 0.82 (0.16–3.57) | 3.14 (0.13–4.60) |
| TTM | 29 (71) | 22 (65) | 7 (100) |
| Coronary angiography performed | 14 (34) | 11 (32) | 3 (43) |
| Percutaneous coronary intervention performed | 8 (20) | 6 (18) | 2 (29) |
| Echocardiogram performed | 33 (80) | 26 (76) | 7 (100) |
| If performed, initial LVEF (Median, IQR)* | 50 (37–59) | 48 (32–59) | 54 (60–61) |
| Cause of arrest | | | |
| Cardiac | 16 (39) | 14 (41) | 2 (28.5) |
| Respiratory | 9 (22) | 7 (21) | 2 (28.5) |
| Other** | 16 (39) | 13 (38) | 3 (43) |

ARDS = Acute Respiratory Distress Syndrome; TTM = Targeted Temperature Management; LVEF = Left Ventricular Ejection Fraction).

* Initial LVEF data was missing in 12 patients (29%).

** Other causes of arrest (n) as reported included infection (1), other medical (3), pulmonary embolism (4), trauma (2), other (unspecified further)(4), and unknown (2). Cases classified as trauma were confirmed to be eligible despite this classification.

**Table 2 – Patient outcomes.**

| Variable | Cohort | No ARDS | ARDS |
|---|---|---|---|
| | N = 41 | N = 34 | N = 7 |
| **Vent-Free Days** median (IQR) | 0 (0–5) | 0 (0–5) | 0 (0–0) |
| **ICU-Free Days** median (IQR) | 0 (0–0) | 0 (0–0) | 0 (0–0) |
| **Hospital length of stay** | 6 (4–11) | 5 (3–8) | 11 (7–52) |
| **DNAR during hospitalization** | 26 (63) | 20 (59) | 6 (86) |
| **Hospital mortality** | 25 (61) | 20 (59) | 5 (72) |
| **Discharge location** | | | |
| Home | 10 (26) | 9 (29) | 1 (14) |
| Care facility | 4 (10) | 3 (10) | 1 (14) |
| Expired | 24 (63) | 19 (61) | 5 (72) |
| **Neurologic status at discharge** | | | |
| Full Recovery | 6 (15) | 6 (18) | 0 (0) |
| Mildly Impaired | 3 (7) | 5 (15) | 1 (14) |
| Severely Impaired | 3 (7) | 3 (9) | 0 (0) |
| Comatose | 1 (2) | 0 (0) | 1 (14) |
| Expired | 25 (61) | 20 (59) | 5 (71) |
| **Independence at discharge** | | | |
| Yes | 5 (12) | 5 (15) | 0 (0) |
| No | 11 (27) | 9 (26) | 2 (29) |
| Expired | 25 (61) | 20 (59) | 5 (72) |

(DNAR = Do Not Attempt Resuscitation; ARDS = Acute Respiratory Distress Syndrome; ICU = Intensive Care Unit).

istics of LPA models, beginning with a two-class model. Model fit characteristics improved in three- and four-class models, as measured by AIC (Aikake's Information Criteria) and BIC (Bayesian Information Criteria). Ultimately, the three-class solution was identified as optimal for both model fit characteristics and having substantial class memberships ($N \geq 12$ in each class) allowing further inter-class comparisons, while the four-class solution included a class with only one member and was excluded from further analysis.

Based on the three-class model, all nine plasma biomarkers were evaluated for interclass differences. To generate the most parsimonious final model, the biomarkers with the least significant differences between classes were sequentially removed from the model, beginning with PSPD ($p = 0.32$), then MPO ($p = 0.18$), and finally Ang-2 ($p = 0.04$). Although fit characteristics would have continued to improve with removal of any of the remaining biomarkers from the model, all showed practically indistinguishable, highly significant

**Table 3 – Processes of Care.**

| Variable | Cohort | No ARDS | ARDS |
|---|---|---|---|
| | *N = 41* | *N = 34* | *N = 7* |
| $P_aO_2$:$F_iO_2$[**] ≤300[**], N (%) | 23 (56) | 16 (47) | 7 (100) |
| Qualifying chest radiograph, N (%) | 11 (27) | 4 (12) | 7 (100) |
| $P_aO_2$:$F_iO_2$[**], Median (IQR) | 207 (124–306) | 228 (128–312) | 148 (122–195) |
| ARDS severity (worst $P_aO_2$:$F_iO_2$) | | | |
| Severe ARDS (≤100) | 6 (19) | 5 (21) | 1 (14) |
| Moderate ARDS (101–200) | 9 (29) | 4 (17) | 5 (71) |
| Mild ARDS (201–300) | 8 (26) | 7 (29) | 1 (14) |
| Not ARDS (301 + ) | 8 (26) | 8 (33) | 0 (0) |
| NMB, N (%) | 18 (44) | 14 (41) | 4 (57) |
| Proning, N (%) | 0 (0) | 0 (0) | 0 (0) |
| Inhaled pulmonary vasodilators, N (%) | 1 (2) | 1 (3) | 0 (0) |
| Diuretics, N (%) | 14 (34) | 9 (26) | 5 (71) |
| ECMO, N (%) | 1 (2) | 1 (3) | 0 (0) |

[**] Worst (lowest) P:F ratio during encounter (10 measurements per day over initial 7 days in hospital) ($P_aO_2$ = Partial Pressure of $O_2$ in arterial blood; $F_iO_2$ = Fraction of inspired Oxygen; ARDS = Acute Respiratory Distress Syndrome; NMB = Neuromuscular Blockade; ECMO = Extracorporeal Membrane Oxygenation).

interclass differences ($p \leq 0.001$) and were therefore retained as important discriminatory factors. As such, the final model included IL-1β, IL-6, IL-8, TNF-α and TNFR-1, resulting in the following three classes: Class 1. Low concentrations of all biomarkers; Class 2. Low concentrations of all biomarkers, except TNF-α and TNFR-1; and Class 3. Elevated concentrations of all biomarkers. Supplemental Fig. 1 shows raw (untransformed) biomarker concentrations across these classes via box and whisker plots. Medians and IQRs can be found in Supplemental Table 4. Post-hoc analyses were then performed to identify differences in outcomes between classes (Supplemental Figure 2). With respect to development of ARDS and mortality, no statistically significant differences between classes were detected. With respect to neurological status, Class 2 fared significantly worse than average with 0/13 patients surviving with mild to no neurological impairment.

## Discussion

In a prospective cohort of 41 patients with out-of-hospital cardiac arrest, we measured markedly elevated plasma and pulmonary edema fluid concentrations of pro-inflammatory cytokines, chemokines, and markers of endothelial injury, but did not demonstrate differences in comparing patients who developed ARDS and those who did not (Fig. 1).

ARDS is common after both in-hospital cardiac arrest (IHCA) and OHCA.[13] The incidence of ARDS following in-hospital cardiac arrest is approximately 20% higher than OHCA, likely due to the differences in baseline comorbidities and a greater proportion of patients with respiratory etiology. We recently described that 48% of OHCA patients who survived 48 hours met the Berlin Definition of ARDS.[1] Previous studies described an incidence ranging from 5-65% depending on definitions and timing.[14,15] In our cohort, ARDS was associated with higher mortality, poor neurological outcome, and more hospital resource utilization. Subsequent studies have demonstrated an association between ventilation with low tidal volumes and improved outcome after OHCA, implying that mitigating lung injury or ARDS is a potentially modifiable therapeutic target after OHCA.[16].

Following cardiac arrest, a sepsis-like inflammatory state known as the PCAS may develop.[17] Plasma concentrations of IL-6 and IL-10 have previously been described to correlate with severity of PCAS and severity of cardiovascular dysfunction.[14,18] IL-6 levels have also been associated with neurological outcome after OHCA.[19] Little is known about the association between circulating pro-inflammatory mediators and respiratory function or ARDS after OHCA, or whether these pathways represent a therapeutic target for improving outcome after OHCA. One possible explanation of our findings is that the pathophysiology of ARDS is different than other more inflammatory causes of ARDS such as pneumonia or sepsis. For example, in a cohort of patients from two other ARDS clinical trials where sepsis and pneumonia were the commonest etiologies, average concentrations of IL-6 were 4 times higher than our cohort, though IL-8 concentrations were similar.[19] A number of factors may contribute to lung injury in the cardiac arrest patient, including aspiration, pulmonary contusion (from chest compressions), and reperfusion injury. Additionally, brain-lung crosstalk has been proposed as a mechanism lung and brain injury.[20] A variety of mechanisms have been proposed, including neurohormonal activation release of damage-associated molecular patterns (DAMPs). Further supporting brain-lung interactions, several studies have noted that ventilation parameters are associated with neurological recovery.[16,21]

Concordantly, distinct biological profiles have also been associated with development of ARDS. Plasma concentrations of surfactant protein-D, Ang-2, IL-8, and TNFR-1 have been associated with both ARDS development and outcome.[3,4] Concentrations of pro-inflammatory cytokines such as IL-1β, IL-6, IL-8, and TNF-α are elevated in alveolar or pulmonary edema fluid in patients with ARDS.[4] ARDS, however, is a heterogenous syndrome and numerous sub-phenotypes have been characterized, most notably hypoinflammatory and hyperinflammatory phenotypes, each with unique biological profiles, illness trajectories, outcomes. [9,22] Patients with different sub-phenotypes may respond to treatments distinctly.[9,22] Based on this pilot study, it appears that post-arrest ARDS is less inflammatory compared with etiologies such as pneumonia and sepsis.[19]

**Table 4a – Unadjusted Analysis: Full Cohort.**

| Characteristic | Association with ARDS | Association with Mortality | Association with Neuro Outcome |
|---|---|---|---|
| **(A) Full Cohort (N = 41)** | | | |
| Age | 0.02 (-0.03, 0.08) | 0 (-0.04, 0.04) | 0.02 (-0.03, 0.06) |
| Weight | −0.03 (-0.07, 0.02) | 0.01 (-0.02, 0.04) | 0.01 (-0.02, 0.04) |
| Sex | −1.05 (-3.29, 1.18) | −0.16 (-1.53, 1.22) | −0.27 (-1.72, 1.18) |
| Temperature (first 32 + ) | 0.54 (-1.88, 2.97) | 0.72 (-1.64, 3.07) | 0.24 (-2.13, 2.61) |
| Lactate (first) | −0.17 (-0.38, 0.04) | 0.11 (-0.03, 0.24) | 0.08 (-0.06, 0.23) |
| Troponin (maximum) | −0.05 (-0.19, 0.1) | 0.01 (-0.04, 0.07) | 0 (-0.05, 0.06) |
| Shockable Initial Cardiac Rhythm | −1.19 (-3.42, 1.05) | −1.91 (-3.37, −0.45) | −2.26 (-3.8, −0.72) |
| TTM | N/A (All ARDS pts received TTM) | 1.13 (-0.26, 2.53) | 0.81 (-0.62, 2.24) |
| ARDS | N/A (Outcome = ARDS) | 0.56 (-1.22, 2.34) | 1.05 (-1.18, 3.29) |
| *Plasma Markers (log2-transformed, pg/ml)* | | | |
| IL1-β | 0.11 (-0.27, 0.49) | 0.12 (-0.19, 0.42) | −0.02 (-0.34, 0.29) |
| IL-6 | −0.01 (-0.28, 0.27) | 0.05 (-0.17, 0.26) | 0.04 (-0.19, 0.28) |
| IL-8 | −0.06 (-0.35, 0.24) | 0.17 (-0.07, 0.42) | 0.13 (-0.12, 0.39) |
| TNF-α | 0.39 (-0.44, 1.21) | 0.36 (-0.26, 0.99) | 0.7 (-0.02, 1.43) |
| MMP-9 | −0.1 (-0.43, 0.23) | 0.16 (-0.14, 0.46) | 0.06 (-0.23, 0.35) |
| MPO | 0.11 (-0.27, 0.49) | 0.12 (-0.12, 0.36) | 0.04 (-0.18, 0.25) |
| PSPD | 0.08 (-0.42, 0.58) | −0.19 (-0.59, 0.21) | −0.08 (-0.49, 0.34) |
| Ang-2 | 0.05 (-0.26, 0.35) | 0.58 (0.04, 1.11) | 0.95 (0.21, 1.7) |
| TNFR-1 | 0.29 (-0.29, 0.87) | 0.62 (0.06, 1.18) | 0.84 (0.14, 1.55) |
| Total HS | −0.01 (-0.34, 0.32) | 0.39 (0.02, 0.77) | 0.28 (-0.03, 0.6) |
| Total CS | −0.95 (-2.52, 0.62) | 0.04 (-1.11, 1.2) | −0.6 (-1.89, 0.68) |

**Table 4b – Unadajusted analysis: Pulmonary Markers Subset.**

| **(B) Subset: Pulmonary Markers (N = 19) log2-transformed, pg/ml** | | | |
|---|---|---|---|
| IL1-β | −0.1 (-0.46, 0.27) | −0.15 (-0.49, 0.19) | −0.14 (-0.5, 0.23) |
| IL-6 | 0.25 (-0.38, 0.89) | 0.01 (-0.27, 0.3) | 0.03 (-0.28, 0.33) |
| IL-8 | 0.05 (-0.44, 0.53) | −0.52 (-1.68, 0.64) | −0.65 (-2.03, 0.72) |
| TNF-α | 0.11 (-0.39, 0.62) | −0.01 (-0.31, 0.3) | 0.03 (-0.3, 0.35) |
| MMP-9 | −0.48 (-1.27, 0.31) | −0.43 (-1.07, 0.2) | −0.68 (-1.62, 0.25) |
| MPO | −0.44 (-1.2, 0.32) | −0.27 (-0.71, 0.17) | −0.37 (-0.87, 0.13) |
| PSPD | 0.76 (-0.25, 1.77) | 0.23 (-0.13, 0.58) | 0.34 (-0.1, 0.78) |
| Ang-2 | 0.2 (-0.32, 0.72) | 0.32 (-0.05, 0.7) | 0.69 (0.02, 1.35) |
| TNFR-1 | 0.08 (-0.69, 0.86) | 0.23 (-0.35, 0.8) | 1.05 (-0.39, 0.85) |

**Table $4_{A,B}$. Unadjusted Analysis.** Full cohort (A) and subset of those patients with pulmonary samples available (B).

(TTM = Targeted Temperature Management; ARDS = Acute Respiratory Distress Syndrome; IL1-β = Interleukin 1β; IL-6 = Interleukin 6; IL-8 = Interleukin 8; TNF-α = Tumor Necrosis Factor α; TNFR-1 = Tumor Necrosis Factor Receptor 1; HS = Heparan Sulfate; CS = Chondroitin Sulfate; PSPD = Pulmonary Surfactant-Associated Protein D; MPO = Myeloperoxidase; Ang-2 = Angiopoietin 2; MMP-9 = Matrix Metalloproteinase-9).

We demonstrated elevated concentrations of some pro-inflammatory cytokines and chemokines. Modulating the inflammatory response after cardiac arrest has been of great interest with mixed clinical results.[23] Three clinical trials have demonstrated potential benefit with administration of corticosteroids (along with vasopressin and epinephrine) during in-hospital and out-of-hospital cardiac arrest, but the exact mechanism is unknown.[23–25] In post-arrest patients with refractory shock, administration of hydrocortisone in patients with shock reduced IL-6 levels, but did not improve hemodynamics or clinical outcome.[26] A recent trial which randomized patients after OHCA found that a one-time infusion of tocilizumab, an IL-6 receptor antagonist, reduced C-reactive protein and leukocyte levels, as well as markers of myocardial injury, but was not powered for clinical endpoints.[27] Additionally, a study found that a high-dose administration of methylprednisolone in the prehospital setting was associated with a reduced level of IL-6 compared with placebo.[28]

The endothelial glycocalyx is known to degrade in sepsis, a comparable disease state to PCAS. Blood levels of glycocalyx components correlate with organ dysfunction, illness severity, and mortality.[27] Glycocalyx components have been associated with cognitive dysfunction in sepsis,[29] and may mediate alveolar microvascular dysfunction in sepsis-induced ARDS.[30] Endothelial injury and glycocalyx shedding have been demonstrated after OHCA, with sustained elevation in syndecan-1, sE-selectin, and thrombomodulin correlating with PCAS severity at 24, 48, and 72 hours.[6–8] Our study similarly demonstrated significant endothelial injury and glycocalyx disruption occurs, perhaps reflecting the effects of reperfusion injury.

**Table 5 – Multivariable Analysis.**

| *Outcome = ARDS* | | *Outcome = No to Mild Neurological Impairment* | | *Outcome = Survival* | |
|---|---|---|---|---|---|
| **Covariate** | **RR (LCI, UCI)** | **Covariate** | **RR (LCI, UCI)** | **Covariate** | **RR (LCI, UCI)** |
| First Lactate | 0.8 (0.7, 1) | Shockable Rhythm | 33.9 (1.7, 677) | Shockable Rhythm | 90.7 (1.6, 5304.5) |
| Weight | 1 (0.9, 1) | ARDS | 2.4 (0.1, 63.3) | ARDS | 4.5 (0.2, 121.7) |
| | | Ang-2 (Plasma) | 0.5 (0.2, 1.1) | Ang-2 (Plasma) | 0.4 (0.1, 1.2) |
| | | TTM Initiated | 0.1 (0, 1.4) | TTM Initiated | 0 (0, 0.8) |
| | | Total HS (Plasma) | 0.6 (0.3, 1.2) | Total HS | 0.3 (0.1, 0.9) |
| | | | | TNF-α (Plasma) | 2.4 (0.3, 21.2) |
| | | | | IL-6 (Plasma) | 1 (0.5, 2) |

TTM = Targeted Temperature Management; ARDS = Acute Respiratory Distress Syndrome; IL6 = Interleukin 6; TNF-α = Tumor Necrosis Factor α; HS = Heparan Sulfate; Ang-2 = Angiopoietin 2.

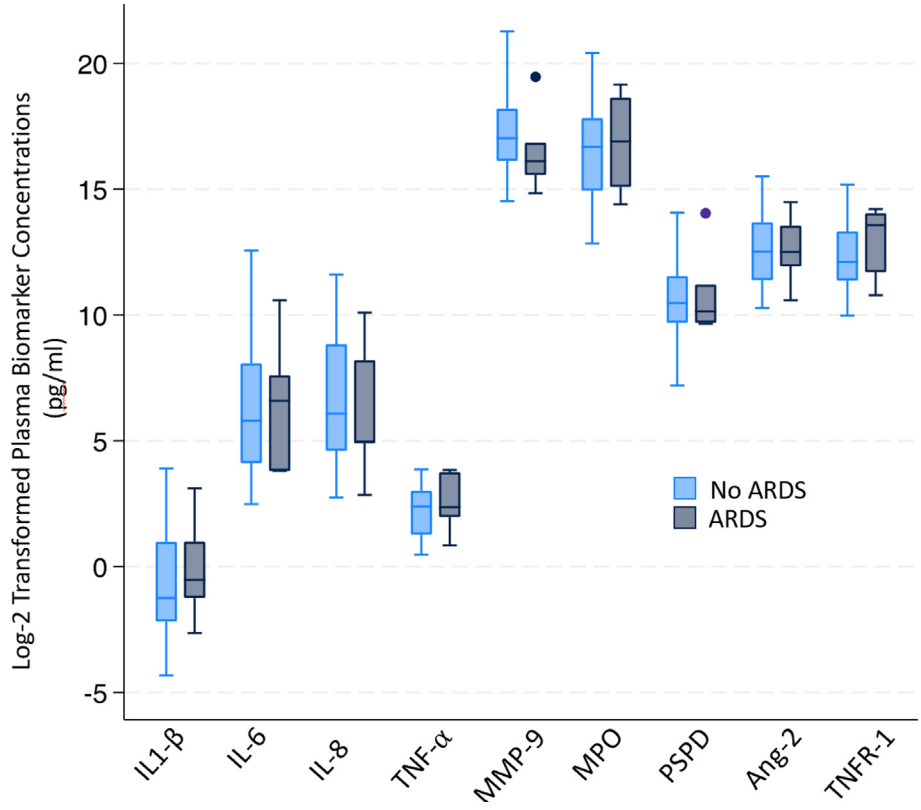


**Fig. 1 – Plasma Biomarker Concentrations by ARDS Status. (IL1-b = Interleukin 1b; IL-6 = Interleukin 6; IL-8 = Interleukin 8; TNF-a = Tumor Necrosis Factor a; MMP-9 = Matrix Metalloproteinase-9; MPO = Myeloperoxidase; PSPD = PSPD = Pulmonary Surfactant-Associated Protein D; Ang-2 = Angiopoietin 2; TNFR-1 = Tumor Necrosis Factor Receptor 1).**

While endothelial activation and injury is a clear feature of PCAS, their associations with pulmonary and neurologic dysfunction remains unknown.

## Limitations

This was a pilot, single-center study, and therefore results may not generalize to all settings. Patients were enrolled in 2019 and analysis was delayed due to the COVID-19 pandemic, however this cohort represents cases unaffected by COVID-19. We estimated an incidence of ARDS of 50% based on prior work, but only 18% of patients in this cohort developed ARDS. As a result of this and of low enrollment numbers, the study may have lacked sufficient power to detect differences among groups. It is unclear if timing of sample collection following arrest has an impact on the association between biomarker levels and development of ARDS. All patients were enrolled and samples collected within 12 hours but an even shorter time window, as well as serial sample collections may inform whether timing has an impact, and to what extent.

## Conclusions

The findings from this pilot study suggest potential relationships between early biomarker levels and markers of severity of injury following OHCA, including neurological status and mortality. OHCA patients have markedly elevated biomarker concentrations, indicating inflammation, epithelial injury, and endothelial activation. Biomarker concentrations were not associated with ARDS development in this patient population, though several distinct biological phenotypes warrant further exploration. Future research may benefit from considering other tools to predict and prevent development of ARDS in this population.

Although there were no demonstrated associations between the level of any specific biomarker and ARDS status in this population, it will be important to examine relationships in future studies. Because this was a small study prone to type 2 error, it may have been underpowered to detect an association between specific biomarkers and ARDS status, and thus further study of the association is warranted.

## Funding sources/disclosures

This study was funded by a research grant from the Medic One Foundation. NJJ also receives funding from the National Institutes of Health, Center for Disease Control and Prevention, and the University of Washington Royalty Research Fund. EPS received funding from the National Institutes of Health. The authors have no other disclosures.

## CRediT authorship contribution statement

**Sarah C. Katsandres:** Writing – original draft, Project administration, Methodology, Data curation. **Jane Hall:** Writing – review & editing, Visualization, Methodology, Formal analysis. **Kyle Danielson:** Methodology, Conceptualization. **Sana Sakr:** Formal analysis. **Sarah G. Dean:** Data curation. **David J. Carlbom:** Writing – review & editing, Conceptualization. **Mark M. Wurfel:** Writing – review & editing, Validation, Methodology, Formal analysis. **Pavan K. Bhatraju:** Writing – original draft, Methodology, Formal analysis. **Joseph A. Hippensteel:** Writing – review & editing, Validation, Formal analysis. **Eric P. Schmidt:** Writing – review & editing, Supervision, Formal analysis. **Kaori Oshima:** Supervision, Investigation, Formal analysis. **Catherine R. Counts:** Writing – review & editing, Resources, Methodology, Data curation, Conceptualization. **Michael R. Sayre:** Writing – review & editing, Visualization, Supervision. **Daniel J. Henning:** Resources, Investigation, Conceptualization. **Nicholas J. Johnson:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.resplu.2024.100590.

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