

Title: **Linking Endotypes and Outcomes in Pediatric Acute Respiratory Distress Syndrome (LEOPARDS)**

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**ABBREVIATIONS AND DEFINITIONS OF TERMS**

AE	Adverse event
ARDS	Acute respiratory distress syndrome
CART	Classification and regression tree
CHOP	Children's Hospital of Philadelphia
CRF	Case report form
ELISA	Enzyme Linked Immunosorbent Assay
HIPAA	Health Insurance Portability and Accountability Act
IRB	Institutional review board
LCA	Latent class analysis
PICU	Pediatric intensive care unit
PELOD	Pediatric logistic organ dysfunction score
PRISM-III	Pediatric risk of mortality-III
VPS	Virtual PICU System

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## ABSTRACT

Context: Acute respiratory distress syndrome (ARDS) is a heterogeneous syndrome of acute respiratory failure which affects 45,000 children in the United States annually. Heterogeneity has contributed to negative trials, as interventions useful in one sub-type may not be useful in another. Biomarkers have the potential to identify sub-phenotypes of pediatric ARDS which may benefit from targeted therapy.

Objectives: Our overall goal is to risk stratify pediatric ARDS patients and to identify sub-phenotypes with shared biology in order to appropriately target therapies in future trials. Specifically, we aim to:

Aim 1: Validate and refine PARDSEVERE, a published protein biomarker-based pediatric ARDS risk stratification tool

Aim 2: Stratify pediatric ARDS into sub-phenotypes using a known 100-gene expression-based classifier to group subjects according to shared underlying biology

Aim 3: Identify *de novo* sub-phenotypes in pediatric ARDS using biomarkers and whole genome transcriptomics of peripheral blood

### Study Design:

This is a prospective, multicenter study of 500 intubated children with ARDS, with planned blood collection within 24 hours of ARDS onset and subsequent measurement of plasma protein biomarkers and peripheral blood gene expression.

### Setting/Participants:

We will enroll 500 intubated children with ARDS from 17 academic pediatric intensive care units in the United States.

### Study Interventions and Measures:

We will measure pre-determined biomarkers with known or suspected association with ARDS severity or outcome. Simultaneously, we will measure gene expression of peripheral blood. Both plasma biomarkers and gene expression profiles will be analyzed using various machine learning techniques, including classification and regression tree, latent class analysis, and hierarchical clustering with the goal of identifying sub-phenotypes of ARDS. These sub-phenotypes will be examined for association with outcome (primary is 28-day mortality), and explicitly tested for variation in response to exogenous treatments (e.g., corticosteroids).

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## 1 BACKGROUND INFORMATION AND RATIONALE

### 1.1 Introduction

**Acute respiratory distress syndrome (ARDS) is common and deadly in children:** ARDS is characterized by acute onset of diffuse bilateral pulmonary edema and severe hypoxemia not fully explained by cardiac dysfunction [1, 2]. Primarily defined for adults, ARDS affects 45,000 children in the United States annually [3], representing 10% of mechanically ventilated children in pediatric intensive care units (PICUs)[4], with a mortality rate of 20% in the United States and 30% worldwide [5-7]. There are no specific pharmacological therapies for adult [8-19] or pediatric [20-23] ARDS despite several trials, and supportive care with lung-protective ventilation [24] and fluid restriction [25] remains the mainstay of treatment. ARDS is a heterogeneous syndrome, with patients having distinct co-morbidities and inciting etiologies (pneumonia, non-pulmonary sepsis). This heterogeneity has contributed to negative trial results, as therapies effective in some patients are ineffective in others [26]. Methods to reduce heterogeneity, including sub-phenotyping using protein and mRNA biomarkers, have been proposed for improving patient selection for future clinical trials [27].

**Studies are needed specifically in pediatric ARDS:** In children, a lack of therapies is further compounded by uncertainty in management, as guidelines are typically extrapolated from adult ARDS, with uncertain applicability [28]. However, pediatric ARDS possesses a distinct epidemiology [29], outcomes [30], and pathobiology [31], necessitating studies specific to this population. We have published how the lower mortality rate in children necessitates alternative patient-centered outcomes for observational and interventional studies [32]. Additionally, as risk factors and co-morbidities differ from adult ARDS [29, 33, 34], studies specifically investigating the pathophysiologic changes leading to increased alveolar permeability in children are sorely needed, and are currently lacking. For example, pediatric ARDS spans birth to 18 years of age, encompassing different stages of lung development. Over 13% of children with ARDS have a history of prematurity [35] and are at high risk for impaired pulmonary vascular and parenchymal growth, which contributes to hypoxemia, cardiac dysfunction, organ failure, and outcomes in pediatric ARDS in a manner distinct from adult ARDS [36].

### 1.2 Relevant Literature and Data

**Adult ARDS possesses distinct subtypes representing different pathophysiologic mechanisms:** In adult ARDS, studies of biomarkers collected during clinical trials [37-39] have led to the discovery of endotypes, or sub-phenotypes with shared pathophysiologic, biomarker, or transcriptomic profiles [27]. Two distinct endotypes, dubbed “hypo-” and “hyperinflammatory” have consistently been discovered in 4 trials using latent class analysis (LCA), a form of cluster analysis. The hyper-inflammatory endotype was characterized by higher proportions of patients in shock, with sepsis, and higher levels of inflammatory biomarkers. Three biomarkers (interleukin-8 [IL-8], bicarbonate, and soluble tumor necrosis factor-1 [sTNFR1]) adequately discriminated the two endotypes. These

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endotypes have clinical relevance, as they showed differential association with higher positive end-expiratory pressure (PEEP) [37], conservative fluid management [38], and to simvastatin [39], with benefits of these interventions only seen in the hyperinflammatory endotype. Additional work in adult ARDS has demonstrated different biomarker profiles between direct (primarily pulmonary) and indirect (primarily non-pulmonary) inciting etiologies, with elevated lung epithelial damage biomarkers seen in direct ARDS, and elevated endothelial biomarkers in indirect [40], suggesting that these may also represent endotypes. However, to date, there has not been demonstration of differential response to therapies based on stratification by direct or indirect ARDS biomarker profiles.

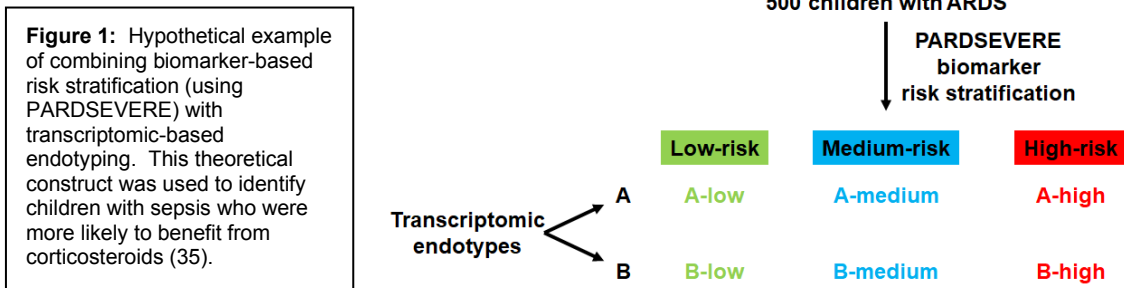
**Pediatric ARDS possesses distinct subtypes representing different pathophysiological mechanisms:** Investigations of whether clinically relevant endotypes exist in pediatric ARDS are in their infancy. Our group has demonstrated that infectious and non-infectious ARDS, defined using clinical variables, have different predictors of mortality [34], suggesting the possibility of distinct endotypes. Very recently, endotypes defined by differential circulating levels of matrix metalloproteinases (MMP) were reported in a separate, smaller cohort of pediatric ARDS, with higher mortality in the “hyperinflammatory” endotype [41]. However, this is the sole example of biomarker-based endotyping in pediatric ARDS, and requires replication in a larger, prospective cohort. Furthermore, these endotypes were defined solely using MMP pathway proteins, and it is unclear whether endotypes defined using a broader array of biomarkers would prove more informative.

**Identification of endotypes allows for predictive enrichment in clinical trials:** Identifying endotypes with common underlying biology is requisite for predictive enrichment – selection of subjects more likely to respond to trial interventions based on a biological mechanism [26, 27]. In adult ARDS, the association between mortality and higher PEEP, conservative fluid management, or simvastatin was only observed in the hyperinflammatory endotype [37-39], suggesting that future trials of these interventions, which had no mortality benefit in the parent trials enrolling heterogeneous ARDS, should be restricted to this responsive endotype. Pediatric ARDS, with its lower mortality rate and distinct etiologic and co-morbidity profile, requires its own endotyping strategy, and cannot *a priori* rely on adult evidence. In pediatric sepsis, Dr. Hector Wong (a co-Investigator on this proposal) has designed a gene expression-based endotyping strategy which identified two distinct endotypes with differential expression of glucocorticoid receptor signaling genes and with divergent response to corticosteroids [42-44]. Whether pediatric ARDS possesses clinically relevant endotypes with differential response to therapies remains unknown, and is the key goal of Aims 2 and 3.

**Protein biomarkers can stratify mortality risk and allow for prognostic enrichment in pediatric ARDS:** In addition to predictive enrichment, biomarkers have also been proposed for prognostic enrichment – the selection of subjects more likely to have the outcome of interest to improve power to detect an effect of an intervention [26, 27]. We developed and published a biomarker-based risk prediction tool (PARDSEVERE) which stratifies pediatric ARDS into low-, medium-,

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and high-risk for mortality in 152 subjects from a single center [45]. PARDSEVERE used age and three plasma biomarkers (IL-8, macrophage inflammatory protein 1- $\alpha$  [MIP1 $\alpha$ /CCL3], and heat shock 70kDa protein 1B [HSPA1B]), and discriminated non-survivors with an area under the receiver operating characteristic curve (AUROC) of 0.85, outperforming other severity of illness scores. The low-risk strata had mortality < 5%, while the high-risk strata had mortality > 30%. If validated in Aim 1, this strategy could be used to identify subjects who should be either excluded (low-risk subjects with low probability of improved mortality from intervention) or included (subjects with higher predicted mortality) in an interventional trial. Furthermore, risk stratification and predictive enrichment can be combined to further identify endotypes which may have differential responses to therapies (Figure 1).



**Biomarker-based endotyping and risk stratification offers advantages over clinical variables:** Clinical variables offer little detail about underlying pathophysiology. This is relevant when considering targeted therapies beyond supportive care. For example, we have shown that angiotensin-2 (ANG2) and soluble receptor for advanced glycation end-products (sRAGE), markers of endothelial and lung epithelial dysfunction, respectively, are elevated in pediatric ARDS non-survivors [46], suggesting utility for prognostic enrichment as well as mechanistic implications. Therapies targeting the ANG2 [47] and RAGE pathways [48] are already in pre-clinical trials. Biomarker characterization of pediatric ARDS would allow restriction of trial subjects to those with evidence of dysregulated ANG2 or RAGE pathways, combining prognostic and predictive enrichment.

Our group has demonstrated advantages of biomarker-based prognostic and predictive enrichment over clinical variables alone. Recently, we showed how a positive response to inhaled nitric oxide (iNO), defined by an improvement in oxygenation of  $\geq 20\%$ , was associated with shorter ventilator duration in pediatric ARDS [49]. No clinical variables, including age, history of prematurity, vasopressor support, echocardiographic evidence of pulmonary hypertension, etiology of ARDS, or baseline oxygenation predicted oxygenation response to iNO. However, levels of ANG2, a biomarker of endothelial dysfunction, predicted a positive oxygenation response. Separately, we developed PARDSEVERE using classification and regression tree (CART) methodology [45]. We tested several clinical variables, including known predictors of mortality (oxygenation, organ failure, immunocompromised status, vasopressor support) for inclusion in the model. However, CART selected age plus three biomarkers (IL-8, MIP1 $\alpha$ , HSPA1B) as the final model. A possible explanation for this finding is that the mortality risk from



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being immunocompromised were better explained using biomarkers, rather than a clinical designation of immune status. In both cases, we demonstrated how biomarker-based enrichment and risk stratification can add value above clinical variables alone.

### **1.3 Compliance Statement**

This study will be conducted in full accordance all applicable Children's Hospital of Philadelphia Research Policies and Procedures and all applicable Federal and state laws and regulations including 45 CFR 46. All episodes of noncompliance will be documented.

The investigators will perform the study in accordance with this protocol, will obtain consent and assent, and will report unanticipated problems involving risks to subjects or others in accordance with The Children's Hospital of Philadelphia IRB Policies and Procedures and all federal requirements. Collection, recording, and reporting of data will be accurate and will ensure the privacy, health, and welfare of research subjects during and after the study.

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## 2 STUDY OBJECTIVES

The proposed studies will establish the presence and clinical relevance of protein and mRNA biomarker-defined endotypes in pediatric ARDS. Additionally, the studies will validate a mortality risk stratification tool which can be developed to provide real-time prognostic enrichment in future trials. Successful completion of the Aims will significantly advance our understanding of pediatric ARDS, provide insight into underlying pathophysiology, and provide testable hypotheses on whether the association between specific treatments used in pediatric ARDS and outcomes differ by endotypes.

### 2.1 Primary Objective (or Aim)

This protocol has 3 specific Aims:

Aim 1: Validate and refine PARDSEVERE, a published protein biomarker-based pediatric ARDS risk stratification tool

Aim 2: Stratify pediatric ARDS into endotypes using a known 100-gene expression-based classifier to group subjects according to shared underlying biology

Aim 3: Identify *de novo* endotypes in pediatric ARDS using biomarkers and whole genome transcriptomics of peripheral blood

### 2.2 Secondary Objectives (or Aim)

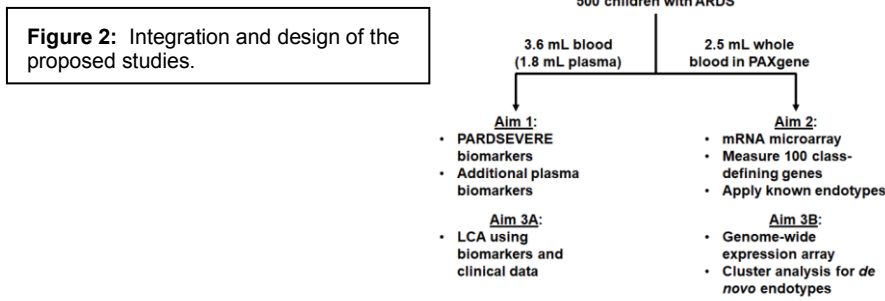
The secondary objectives are to:

- Associate endotypes with 28-day mortality
  - Associate endotypes with duration of mechanical ventilation
  - Associate differential response of therapies (e.g., corticosteroids) dependent upon endotypes.
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### 3 INVESTIGATIONAL PLAN

#### 3.1 General Schema of Study Design

The proposal is a prospective observational cohort study of pediatric ARDS taking place at 17 centers in the United States. The study involves simultaneous collection of plasma (for measurement of circulating biomarkers) and RNA-stabilized whole blood (for microarrays) within 24 hours of ARDS onset in 500 children (Figure 2).



#### 3.2 Study Duration, Enrollment and Number of Sites

The study will be funded for 5 years (anticipated start date of July 1, 2019 until June 30, 2024). We anticipate enrollment beginning January 1, 2020 and lasting until December 31, 2022 (3 years). We have budgeted ample time for start-up (6 months) and extension of enrollment (up to 2 years) in case of slower than expected enrollment. Seventeen US sites have expressed interest:

- Children's Hospital of Philadelphia (DCC and main site)
- Cincinnati Children's Hospital Medical Center (Cincinnati, OH)
- Nationwide Children's Hospital (Columbus, OH)
- Texas Children's Hospital (Houston, TX)
- Children's National Medical Center (Washington, DC)
- Children's Hospital of Wisconsin (Milwaukee, WI)
- Akron Children's Hospital (Akron, OH)
- Nicklaus Children's Hospital (Miami, FL)
- Children's Mercy Hospital (Kansas City, MO)
- Penn State Hershey Children's Hospital (Hershey, PA)
- Saint Barnabas Medical Center (Livingston, NJ)
- Riley Children's at Indiana University Health (Indianapolis, IN)
- Columbia University Medical Center (New York, NY)
- Arkansas Children's Hospital (Little Rock, AR)
- Children's Healthcare of Atlanta (Atlanta, GA)

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- Children's Hospital Colorado (Aurora, CO)
  - Washington University (St. Louis, MO)

### **3.3 Total Number of Study Sites/Total Number of Subjects Projected**

#### **3.3.1 Duration of Study Participation**

The study duration per subject will be up to PICU discharge. Subject involvement will be limited to the single approach for consent, and a single blood draw, both within 24 hours of eligibility (i.e., ARDS onset). The subject will be followed until PICU discharge for collection of clinical data (mortality status, duration of ventilation, therapies used).

#### **3.3.2 Total Number of Study Sites/Total Number of Subjects Projected**

The study will be conducted at 17 investigative sites in the United States.

Recruitment will stop when 500 subjects are recruited. It is expected that all 500 subjects will be enrolled to produce 500 evaluable subjects for the plasma biomarker aims, and 475 evaluable subjects for the mRNA aims.

### **3.4 Study Population**

#### **3.4.1 Inclusion Criteria**

- 1) acute ( $\leq 7$  days of risk factor) respiratory failure requiring invasive mechanical ventilation
- 2) age  $> 44$  weeks corrected gestational age and  $< 17.5$  years
- 3) invasive mechanical ventilation via endotracheal tube
- 4) bilateral infiltrates on chest radiograph
- 5) oxygenation index (OI)  $\geq 4$ ; or oxygen saturation index (OSI)  $\geq 5$  on 2 consecutive measurements at least 4 hours apart but  $< 24$  hours apart
- 6) invasively ventilated  $\leq 7$  days before meeting above radiographic and oxygenation criteria
- 7) invasive blood drawing access (central venous catheter, arterial catheter, or blood-drawing IV)

#### **3.4.2 Exclusion Criteria**

- 1) weight  $< 3$  kilograms
  - 2) cyanotic congenital heart disease (other than Patent Foramen Ovale (PFO) or Patent Ductus Arteriosus (PDA))
  - 3) tracheostomy at time of screening
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- 4) invasively ventilated for > 7 days when meet ARDS criteria above
  - 5) cardiac failure as predominant cause of respiratory failure
  - 6) primary obstructive airway disease (asthma, bronchiolitis) by judgement of clinician as the primary cause of respiratory failure
  - 7) alternative known chronic lung disease as cause of respiratory failure (cystic fibrosis, eosinophilic pneumonia, interstitial pneumonitis, pulmonary hemosiderosis, cryptogenic organizing pneumonia)
  - 8) severe neurologic morbidity not expected to survive > 72 hours
  - 9) any limitations of care at time of screening
  - 10) previous enrollment in this study

Subjects that do not meet all of the enrollment criteria may not be enrolled. Any violations of these criteria must be reported in accordance with IRB Policies and Procedures. We limited enrollment to subjects < 17.5 years to preclude any subjects from turning 18 during their PICU stay (median length of ventilation for ARDS in 9 days).

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## **4 STUDY PROCEDURES**

### **4.1 Screening Visit**

Subjects will be screened daily at the respective PICUs by trained research coordinators. Intubated subjects will be assessed for study eligibility by chart review, and if necessary, discussion with the treating team. No subjects or parents/guardians will be approached for consent until deemed eligible. Potential subjects will not be asked screening questions prior to obtaining informed consent.

All individuals meeting all inclusion criteria and none of the exclusion criteria will be approached for study enrollment within 24 hours of meeting eligibility criteria. After confirming eligibility, patients will be approached for informed consent

### **4.2 Study Period**

#### **4.2.1 Enrollment window**

Study procedures will be performed at enrollment for all subjects. Subjects will be eligible  $\leq 24$  hours after eligibility, defined as the time-point of the second qualifying OI or OSI (see Inclusion criteria). Study procedures are:

- Start of paper CRF and uploading into web-based Redcap database
- Blood collection for plasma and mRNA analysis

#### **4.2.2 Discharge**

Subjects will be followed for duration of PICU stay until PICU discharge, death, or 90 days after enrollment. Data for the stay will be collected in the paper CRF and uploaded on-site to a web-based Redcap database.

### **4.3 Subject Completion/Withdrawal**

Subjects may withdraw from the study at any time without prejudice to their care. It will be documented whether or not each subject completes the clinical study.

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## 5 STUDY EVALUATIONS AND MEASUREMENTS

### 5.1 Screening and Monitoring Evaluations and Measurements

#### 5.1.1 Medical Record Review

All clinical data will be generated at the discretion of the care team and attending physician. The following data will be abstracted from the medical chart (paper or electronic):

Category	Data to be Collected
Demographic	Date of birth, date of PICU admission/transfer, date of discharge, gender, race/ethnicity, weight, height
Medical history	Comorbid illnesses, lung injury diagnosis, organ failure score
Blood gas data	PaO <sub>2</sub> , PaCO <sub>2</sub> , pH
Ventilator data	Mode of ventilation, peak inflating pressure, mean airway pressure, positive end-expiratory pressure, compliance, dead space fraction
Laboratory data	White blood cell count, chemistries, lactate
Outcomes	Ventilator days, PICU survival
Vital Status	Alive or deceased at PICU discharge

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#### 5.1.2 Laboratory Evaluations

Example: Blood sampling will be performed for the following laboratory evaluations

- Plasma biomarker measurements
- Genome-wide mRNA expression from whole blood

##### 5.1.2.1 Blood Sampling

A total of 6.1 mL whole blood will be taken from each patient within 24 hours of meeting study eligibility via existing blood-drawing access.

##### 5.1.2.2 Blood Processing

Whole blood will be collected in 3 tubes. Two sodium citrate (“light blue top”) tubes will receive 1.8 mL each (total = 3.6 mL). These two tubes will be centrifuged within 30 minutes (2000 g for 20 minutes at 20°C). Plasma samples (supernatant) will be divided into aliquots and stored at -20°C at sites other than CHOP, and then sent to CHOP every 2 weeks on dry ice, where they will be stored long-term at -80°C.

An additional 2.5 mL will be stored in a PAXgene tube for RNA, stored upright at room temperature for 4 to 24 hours, and then stored at -20°C at sites other than CHOP, and then sent to CHOP every 2 weeks on dry ice, where they will be stored

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long-term at -30°C. Analyses will be conducted on both plasma and mRNA at CHOP, using enzyme-linked immunosorbent assays (ELISAs) or the Molecular Biology Core for microarray, as appropriate.

## **5.2 Safety Evaluation**

Patient safety will be monitored by noting any adverse events that occur during or as a consequence of study blood collection. Study blood collection should pose no increased risk for complications greater than that of standard clinical blood collection, and total blood volume collected does not exceed age/weight-appropriate maximums (which is why < 3 kg weight is an exclusion criteria).



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## 6 STATISTICAL CONSIDERATIONS

### 6.1 Primary Endpoint

The primary endpoint of Aim 1 is successful performance of the PARDSEVERE risk stratification tool to discriminate 28-day mortality in pediatric ARDS. The primary endpoint of Aim 2 is application of a known 100-gene classifier to test whether transcriptomic endotypes exist in pediatric ARDS. The endpoint of Aim 3 is generation of novel proteomic and transcriptomic endotypes.

### 6.2 Secondary Endpoints

Secondary endpoints will include association of endotypes with mortality and ventilator-free days at 28 days.

### 6.3 Control of Bias and Confounding

While not completely possible to control for bias in an observational study, we have taken certain steps to minimize the risk of confounding. The primary exposure is biomarker (protein or mRNA)-defined endotypes. Clinical data will be collected on paper CRFs on-site and uploaded to a central, web-based Redcap server. Biomarker measurements will be made in bulk post-clinical data collection. Thus, clinical data is being collected blinded to the biomarker levels or endotype assignments. Finally, the statisticians involved in this data analysis will be blinded to outcomes (mortality); endotype assignments will be performed on presenting clinical variables and biomarker measurements.

### 6.4 Statistical Methods

#### 6.4.1 Baseline Data

Baseline and demographic characteristics will be summarized by standard descriptive summaries (e.g., means and standard deviations or medians with interquartile ranges for continuous variables and proportions for categorical variables). Comparisons between survivors and non-survivors, and later between different endotypes, will be tested using unpaired t-test or Wilcoxon rank sum test for continuous variables. The analogues of these tests for > 2 groups (ANOVA and Kruskal-Wallis) will be used if > 2 endotypes are identified. Fisher's exact or chi-squared test will be used for categorical variables.

#### 6.4.2 Analysis of Primary Outcome of Interest

For Aim 1, PARDSEVERE biomarker levels will be used to classify subjects according to the previously published PARDSEVERE decision rules without modifications [45]. This allows for assignment of 28-day mortality risk based on allocation of study subjects to one of 5 terminal nodes. Performance of PARDSEVERE will be assessed by AUROC for discriminating 28-day mortality and other diagnostic accuracy measures, including sensitivity, specificity, and positive and negative predictive values at different probability cutoffs. PARDSEVERE will

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be considered validated if sensitivity is  $\geq 0.85$  and specificity is  $\geq 0.65$ , both with 95% CI width  $\leq 0.16$ .

For Aim 2, we will perform mRNA gene expression, and identify endotypes using a previously published 100-gene classifier [42, 43].

For Aim 3, we will identify *de novo* endotypes using LCA including biomarker and clinical variables, or novel endotypes using the entire gene expression pattern.

For the transcriptomic part of Aims 2 and 3, the cohort will be split 60:40 into a derivation and test set. In Aim 2, we will determine genome-wide differential gene expression. After filtering and normalizing, gene expression values will be ranked by median absolute deviation across all patient samples. We will then perform unsupervised agglomerative hierarchical clustering on the top 5000 probesets, with clusters allowed  $k = 2-12$ , as has been done to discover mRNA-based endotypes in adult sepsis [50]. To estimate  $k$  (number of endotypes), we will combine cumulative distribution functions and cophenetic distance correlation analysis to assess clustering stability. Using a random forest classifier (supervised classification with high dimensional data methods)[50, 51], we will assess ARDS endotype classification with ten-fold cross-validation of stepwise increments in gene numbers. We will decide on the final number of endotype-defining genes when the process yields a cross-validation misclassification error rate of  $< 10\%$ .

For the protein biomarker part of Aim 3, the cohort will be split 60:40 into a derivation and test set. We will use all measured plasma biomarkers and select clinical variables recorded within 24 hours of ARDS onset, including age, vasopressor-inotrope score [52], specific laboratory values including PELOD 2, bicarbonate, and OI at ARDS onset. These variables were chosen based on their ability to discriminate mortality in previous studies of adult [37] or pediatric ARDS [35], including by our group [34], and their ready availability within the first 24 hours of ARDS. All analyses will be performed blinded to outcome. As scales of variables vary considerably, continuous variables will be standardized to mean = 0 and standard deviation = 1 prior to analysis. We will consider models between 2 and 5 subclasses, as has been done for adult ARDS [37-39]. For LCA, criteria for model selection will be based on the Bayesian Information Criteria, the Vuong-Lo-Mendell-Rubin likelihood ratio test, and size of the smallest class. Model estimation will be based on full-information maximum likelihood methods. To avoid local maximum likelihood solutions, we will use 100 random starting values, of which the best 20 will be optimized.

For secondary aims, assuming a mortality rate of 16%, a 50:50 split between endotypes, and  $\alpha = 0.05$ , with 475 subjects we have 80% power to detect an association with an odds ratio  $\geq 2.0$  between endotype and 28-day mortality. Importantly, as the goal of endotype discovery is not mortality prediction [53], we are not powering for this outcome. Rather, we provide this calculation here for reference of what effect size endotype has on mortality that we will be able to observe.

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## 6.5 Sample Size and Power

For Aim 1, assuming a mortality rate of 16% and a PARDSEVERE sensitivity of 0.85 and specificity of 0.65, the sample size needed for two-sided 95% CI width of  $\leq 0.16$  (i.e., sensitivity of 0.85 with 95% CI 0.77 to 0.93) is 454 subjects. In our single-center study of 152 subjects and 16% mortality [45], our sensitivity was 0.96 and specificity was 0.66, suggesting that these assumptions are realistic. We have increased this to 500 total subjects to account for ~10% of subjects being unevaluable due to sample loss or degradation during transport and storage.

For the transcriptomic Aims 2 and 3, we anticipate 475 evaluable subjects with sufficient RNA from the total cohort of 500, assuming a conservative estimate of 5% drop-out due to neutropenia. As we found evidence for ARDS endotypes in our preliminary cohort of 67 subjects, we anticipate adequate numbers for endotype discovery. As a comparison, in adult sepsis, 4 endotypes were discovered using whole blood gene expression from 306 subjects [50].

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## **7 SAFETY MANAGEMENT**

### **7.1 Clinical Adverse Events**

Clinical adverse events (AEs) will be monitored throughout the study.

### **7.2 Adverse Event Reporting**

Since the study procedures are not greater than minimal risk, SAEs are not expected. If any unanticipated problems related to the research involving risks to subjects or others happen during the course of this study (including SAEs) these will be reported to the IRB in accordance with CHOP IRB SOP 408: Unanticipated Problems Involving Risks to Subjects. AEs that are not serious but that are notable and could involve risks to subjects will be summarized in narrative or other format and submitted to the IRB at the time of continuing review.

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## 8 STUDY ADMINISTRATION

### 8.1 Data Collection and Management

For patients who meet eligibility criteria, the parents or legal guardians will be approached for study enrollment. The site PIs or trained staff members will engage them in a discussion regarding reasons for the study, the study procedures, and the risks and benefits and answer all questions. Due to the critical nature of the patients' conditions in the PICU, this discussion may take place at the patient's bedside or in an alternative location (e.g., family conference room) at the parent/guardian's option and the study investigator's discretion. All reasonable safeguards to ensure patient privacy will be taken. Parents/guardians will be given sufficient time (i.e., up to several hours) to decide whether or not to participate in this study. Following the above conversation seeking informed consent from each patient's parent/guardian and obtaining agreement from the parent/guardian to participate in this study, we will provide a copy of the combined consent-HIPAA authorization document to the parent/guardian and patient.

We also have a waiver of assent for all patients. Given the inclusion criteria of invasive mechanical ventilation, and the necessary associated sedation, we do not anticipate any instances of subjects being able to assent.

Paper versions of all CRFs will be kept on-site in a locked office. Data will be entered into web-based online CRF (Redcap) that will be password-protected. Only study personnel authorized by the IRB will have access to study-related data and files, and have password access.

Confidentiality will be assured by keeping the list containing personal health identifiers (PHI) and subject ID restricted to the individual sites, such that the only data submitted to CHOP is limited in PHI (site, dates of ARDS, demographic information) and coded with a subject ID number. Furthermore, all downloaded study-related data will be kept in encrypted, password-protected files on the PI's desktop computer in a locked office, with password-protected back-ups on the Departmental share drives for research. Dr. Yehya (PI) and his research coordinator will have access to this data.

Specific consent will be obtained to store blood and use data for future research, which will be explicitly recorded. There will be plans to share this data with the research community using the Biologic Specimen and Data Repository Information Coordinating Center (BioLINCC) program at NHLBI, and we will follow suggested guidelines for de-identifying the data (coding of site IDs, conversion of dates to study days).

Pursuant to the NIH position that sharing of resources (such as this study) is an important means to enhance the value of NIH-sponsored research, all data obtained from this proposal will be made readily available for research by qualified individuals within the scientific community after publication of the proposed studies. Gene expression data from Aims 2 and 3 will be made available simultaneous with publication of the results of these aims by uploading to the Gene Expression

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Omnibus, consistent with the NIH Genomic Data Sharing Policy. For dissemination of plasma biomarker results and the associated clinical dataset, we will use the Biologic Specimen and Data Repository Information Coordinating Center (BioLINCC) program at NHLBI, with release of a de-identified dataset with appropriate untraceable identifiers within 2 years of publication of the main manuscript, alongside a detailed mechanism of how to request data samples. As we intend to use a layered consent, with an option to opt out of future studies, we will ensure this information is conveyed to BioLINCC.

### **8.1.1 Data sources**

Data will be collected by trained research coordinators from the patient's inpatient chart. In the majority of cases, we anticipate this being an electronic medical record, rather than a paper chart.

## **8.2 Confidentiality**

All data and records generated during this study will be kept confidential in accordance with Institutional policies and HIPAA on subject privacy. The Investigator and other personnel will not use such data and records for any purpose other than conducting the study.

Confidentiality will be assured by keeping the list containing personal health identifiers (PHI) and subject ID restricted to the individual sites, such that the only data submitted to CHOP is limited in PHI (site, dates of ARDS, demographic information) and coded with a subject ID number. Furthermore, all downloaded study-related data will be kept in encrypted, password-protected files on the PI's desktop computer in a locked office, with password-protected back-ups on the Departmental share drives for research. Dr. Yehya (PI) and his research coordinator will have access to this data.

No identifiable data will be used for future study without first obtaining IRB approval. The investigator will obtain a data use agreement between provider (the PI) and any recipient researchers (including others at CHOP) before sharing a limited dataset (demographics, dates).

## **8.3 Regulatory and Ethical Considerations**

### **8.3.1 Data and Safety Monitoring Plan**

All data and records generated during this study will be kept confidential in accordance with Institutional policies and HIPAA on subject privacy and that the Investigator and other site personnel will not use such data and records for any purpose other than conducting the study. Confidentiality will be assured by keeping the list containing personal health identifiers (PHI) and subject ID restricted to the individual sites, such that the only data submitted to CHOP is limited in PHI (site, dates of ARDS, demographic information) and coded with a subject ID number. Furthermore, all downloaded study-related data will be kept in encrypted, password-protected files on the PI's desktop computer in a locked office, with password-

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protected back-ups on the Departmental share drives for research. All study-related files and drives will be password-protected.

All blood specimens will be collected and stored using tubes labeled with a study ID number. Any remaining blood specimens following study measurements will be stored in a coded manner after obtaining specific informed consent to store these specimens and use for future research purposes. No identifiable data or PHI will be used for future study without first obtaining IRB approval.

### **8.3.2 Risk Assessment**

The risks for patients participating in this study are not greater than minimal risk and include those related to 1) blood collection for study measurements and 2) breach of confidentiality. There is no other practical way to collect data for the proposed research.

Risks related to blood collection: The volume of blood collection will be limited to 6.1 mL per one-time draw in all subjects. In any patient whose clinical condition might be adversely affected by removal of the stated blood volume for this study (e.g., patients with significant anemia or compromised cardiac output), we will discuss further limiting the volume of blood withdrawn for research purposes with the patient's attending physician. Any patient concurrently enrolled in another study for whom enrollment in this study would mean the combined blood volume collection would exceed IRB regulations will be excluded from this study and the reason for exclusion noted. Finally, since patients with ARDS typically undergo multiple blood draws per day and enrollment is restricted to those with indwelling blood-drawing catheters, whenever possible blood sampling for study purposes will be timed with other clinically indicated lab draws in order to limit access to indwelling vascular catheters.

Risks related to breach of confidentiality: Extensive efforts will be taken to protect this information including storage of downloaded data in an encrypted, password-protected desktop, and keeping the majority of the PHI at the respective sites, with only a limited dataset utilizing unique study IDs used for the majority of data analyses. PHI will be removed as soon as is practical after data collection. If families choose not to participate, data will not be collected for study purposes. The CHOP IRB requires annual updates for continuing approval for all studies, and the CHOP Office of Research Compliance and Regulatory Affairs performs routine audits of all protocols.

### **8.3.3 Potential Benefits of Study Participation**

Pediatric ARDS carries substantial morbidity and mortality, with no directed therapies available. Guidelines for management are often extrapolated from adult ARDS, despite the distinct epidemiology and outcomes in pediatric ARDS. Studies specifically investigating the mechanisms underlying pediatric ARDS are needed. The proposed studies will lay the foundation for improved risk stratification of pediatric ARDS, and potentially for the identification of specific sub-populations which may benefit from trials of targeted therapies. While there are no direct

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benefits to patients as a result of participation in this study, these indirect benefits for future pediatric patients with ARDS may lead to improved outcomes.

#### **8.3.4 Risk-Benefit Assessment**

Given the minimal risk profile associated with this study, the overall benefits to science, pediatric medicine, future generations of children with ARDS, and society as a whole clearly outweigh the risks.

#### **8.4 Recruitment Strategy**

Potential study patients will be recruited from the PICU population. All intubated, mechanically ventilated patients will be eligible for screening. Reviewing inclusion and exclusion criteria and confirming eligibility as part of recruitment will involve querying the medical records and discussion with the patient's care team *only*. Potential subjects will not be asked screening questions prior to obtaining informed consent. Since all inclusion and exclusion eligibility criteria will be determined based on clinical documentation in the medical record and no further information about the prospective participant will be required to determine if they are eligible for the research, consent for the recruitment and screening process is not required.

For patients who meet eligibility criteria, the parents or legal guardians will be approached for study enrollment. The site PIs or trained staff members will engage them in a discussion regarding reasons for the study, the study procedures, and the risks and benefits and answer all questions. Due to the critical nature of the patients' conditions in the PICU, this discussion may take place at the patient's bedside or in an alternative location (e.g., family conference room) at the parent/guardian's option and the study investigator's discretion. All reasonable safeguards to ensure patient privacy will be taken. Parents/guardians will be given sufficient time (i.e., up to several hours) to decide whether or not to participate in this study. Following the above conversation seeking informed consent from each patient's parent/guardian and obtaining agreement from the parent/guardian to participate in this study, we will provide a copy of the combined consent-HIPAA authorization document to the parent/guardian and patient.

#### **8.5 Informed Consent/Assent and HIPAA Authorization**

For patients who meet criteria, the parents or legal guardians will be approached for study enrollment. The site PI, co-investigators, or trained staff members will engage them in a discussion regarding reasons for the study, the study procedures, and the risks and benefits and answer all questions. Due to the often critical nature of the patients' conditions in the PICU, this discussion may take place at the patient's bedside or in an alternative location (e.g., family conference room) at the parent/guardian's option and the study investigator's discretion. Also, since it is common for parents/guardians to be away from the bedside, we anticipate the need to seek consent over the phone for many patients. Regardless of the where this discussion takes place (i.e., in person or via telephone), all reasonable safeguards to ensure patient privacy will be taken. Parents/guardians will be given sufficient time (i.e., up to several hours) to make a decision to participate in this study.

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Following the above conversation seeking informed consent from each patient's parent/guardian and obtaining agreement from the parent/guardian to participate in this study, we will provide a copy of the combined consent-HIPAA authorization document to the parent/guardian and patient.

We will also seek a waiver of assent for all patients, in accordance with regulations 45 CFR 46.408(a).. Given the inclusion criteria of invasive mechanical ventilation, and the necessary associated sedation, we do not anticipate any instances of subjects being able to assent.

### **8.5.1 Waiver of Consent**

We are seeking a waiver of documentation of consent and a waiver of documentation of HIPAA authorization only in cases consented over the phone, under 45CFR46.117(c)(2) because this study presents no more than minimal risk of harm to patients and involves no procedures for which written consent is normally required outside of the research context. In accordance with 45CFR46.117(c)(2), we will document verbal consent and verbal HIPAA Authorization on the consent form and a note written in the patient's medical record.

If the parent/guardian consents to participate in the study, further verbal consent will be sought to store any remaining blood specimens following completion of study measurements for possible use in future research. Consent to store blood for future use will be indicated on the same consent form (layered consent) by the person obtaining informed consent.

For non-English speaking patients, at sites where the short form consent process is approved, the short form will be read to the potential subjects before the summary document is translated; the interpreter will sign the short form and summary document to document that this has been done. The person obtaining consent shall sign the summary document, and the consenting party will sign the short form (the latter only if written consent/HIPAA authorization are obtained). Each site that uses the short form consent process to enroll LEP subjects will abide by local policies and procedures pertaining to the process (both in person and/or via phone, as applicable).

### **8.5.2 Waiver of Assent**

We will also seek a waiver of assent for all patients, in accordance with regulations 45 CFR 46.408(a).. Given the inclusion criteria of invasive mechanical ventilation, and the necessary associated sedation, we do not anticipate any instances of subjects being able to assent.

### **8.5.3 Waiver of HIPAA Authorization**

We are seeking a waiver of documentation of consent (see above) and a waiver of documentation of HIPAA authorization only in cases consented over the phone, under 45CFR46.117(c)(2) because this study presents no more than minimal risk of harm to patients and involves no procedures for which written consent is normally required outside of the research context. In accordance with 45CFR46.117(c)(2), we will document verbal consent and verbal HIPAA

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Authorization on the consent form and a note written in the patient's medical record.

### **8.6 Payment to Subjects/Families**

No monetary or physical reimbursement will be provided to patients or their families for participation in this study. The cost of all study measurements will be covered by the investigational team.

## **9 PUBLICATION**

This study will be carried out with the goal of publication of results in a peer-reviewed journal. Only de-identified aggregate data will be published.

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