Acute Respiratory Distress Syndrome (ARDS)
CPCCRN Protocol Number 079

Collaborative Pediatric Critical Care Research Network
Eunice Kennedy Shriver National Institute for Child Health and Human Development (NICHD)

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This protocol is CPCCRN Protocol Number 079, and has been authored by Anil Sapru, M.D., UCLA, for implementation with the CPCCRN investigators.

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PROTOCOL TITLE:

Acute Respiratory Distress Syndrome

Short Title: ARDS
CPCCRN Protocol Number: 079

Lead Investigator and Author:
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UCLA

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I confirm that I have read this protocol, I understand it, and I will conduct the study according to the protocol. I will also work consistently with the ethical principles that have their origin in the Declaration of Helsinki and will adhere to the Ethical and Regulatory Considerations as stated. I confirm that if I or any of my staff are members of the Institutional Review Board, we will abstain from voting on this protocol, its future renewals, and its future amendments.

Principal Investigator Name: _____

Principal Investigator Signature: _____

Date: _____

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Contents

Abstract

Acute Respiratory Distress Syndrome (ARDS) is a major cause of morbidity and mortality in both adult and pediatric patients, with an incidence of 200,000 patients per year in the United States [1]. The reported mortality among pediatric patients with ARDS varies from 16% to 43% [2]. Most clinical trials have been negative and the only therapy proven to reduce ARDS-related mortality is a lung-protective ventilator strategy [3]. Utilizing markers of underlying biological pathophysiology to inform treatment responsiveness may allow targeted application of therapies and discovery of newer more effective treatments [4],[5].

Preliminary data suggest that mediators in the inflammation and coagulation pathways such as sThrombomodulin (sTM), a biomarker for endothelial damage and loss of TM activity on endothelial cells, and Angiopioietin-2 (Ang-2), a mediator of inflammation, are associated with mortality among patients with ARDS [6, 7]. Our prior published data demonstrate that other mediators in these pathways including PAI-1, IL-6, IL-8, and IL-10 are robust prognostic markers and improve the prognostic value of clinical scores such as PRISM score and OI when used in combination with these [26].

We plan to enroll 300 pediatric patients with ARDS in a prospective longitudinal cohort and collect biological specimens early in the course of illness, clinical data during hospitalization, and follow up post discharge data to characterize long-term morbidity among survivors in order to complete the specific aims outlined in section 1.2 of this protocol.

This prospective longitudinal observational cohort study will validate the association between these biomarkers and mortality risk in children with ARDS and examine relationship of these markers with long-term morbidity and functional outcomes among survivors. Validating these markers (sTM and Ang-2) will lead to these markers informing the design of next generation clinical trials related to ARDS. sTM is available for use as a therapeutic agent for clinical trials and anti-Ang-2 agents are currently being developed and likely to be available soon for therapeutic application.

1 Study Summary

We will examine the relationship between clinical and biological markers with mortality and with long-term outcomes and new morbidities among survivors. This study will characterize prognostic and predictive biomarkers and provide a link between these

biomarkers and the development of clinically relevant clinical outcomes. The molecular phenotypes defined and knowledge acquired will lead to development of precision medicine strategies targeting therapeutic agents to patients with specific molecular phenotypes or biomarker patterns.

1.1 Hypotheses

The hypotheses of this study are:

1. Magnitude and trajectory of change in selected markers of inflammation and coagulation pathways such as (sTM and Ang-2) will be associated with increased mortality and multiple organ dysfunction syndrome (MODS) during hospitalization and with markers of long-term morbidity such as persistent O2-dependence and FSS among survivors post discharge.
2. Common and rare genetic variants in the corresponding genes will be associated with biomarker levels and with mortality and multi-organ failure during hospitalization and with long-term morbidity among survivors.

1.2 Specific Aims

This project has the following Specific Aims:

Specific Aim 1. To determine whether plasma levels of coagulation and inflammation-related biomarkers (e.g., sTM and Ang-2) are associated with increased mortality, MODS, and long-term morbidity among survivors and to identify biomarker cut offs for potential therapeutic trials.

Specific Aim 2. To determine whether genetic variants in genes of coagulation and inflammation related mediators (e.g., sTM and Ang-2) are associated with higher plasma levels of these markers and with mortality, MODS and long-term morbidity among survivors.

Specific Aim 3. To test and validate our previously published prognostic inflammation, coagulation, epithelial, and endothelial ARDS related biomarkers (PAI-1, IL6, IL8, IL10, sTNFr) and published prognostic models such as Latent Class Analysis [26]. We also plan to test additional novel hypotheses related to genomics and biomarkers in ARDS from a rigorously phenotyped cohort with granular clinical data.

1.3 Subject Eligibility, Accrual and Study Duration

Eligible subjects will be identified by on-site study staff.

Inclusion criteria are:

1. Age $>$ 30 days to $<$ 18 years and $\geq$ 36 weeks corrected gestation
2. New or worsening respiratory symptoms within 1 week of a known clinical insult
3. Bilateral opacities on Chest X-ray not fully explained by effusions, lobar/lung collapse, or nodules. Chest X-ray must occur within 24 hours of the first qualifying blood gas (P/F) or saturation (S/F) ratio measurement.
4. Arterial blood gas PaO_2/FiO_2 (P/F) ratios $<$ 200 (or equivalent SpO_2/FiO_2 (S/F) ratios $<$ 221 only if no P/F ratio can be calculated) on two separate occasions at least 6 hours apart while on invasive positive pressure support with a PEEP $\geq$ 5. (P/F and S/F ratios may be adjusted for Denver's altitude)

Exclusion criteria are:

1. Inability to obtain first blood sample within 24 hours of eligibility
2. Family/team lack of commitment to aggressive intensive care as indicated by do not resuscitate orders and/or other limitation of care
3. Exacerbation of underlying chronic lung disease (cystic fibrosis, eosinophilic pneumonia, interstitial pneumonia)
4. Respiratory failure solely due to cardiac failure or hydrostatic pulmonary edema (anuric renal failure only requiring fluid removal)
5. Subject has previously been enrolled in ARDS

2 Rationale and Background

Acute Respiratory Distress Syndrome (ARDS) is a major cause of morbidity and mortality among both adult and pediatric patients. It is a heterogeneous disorder with reported mortality among children ranging from 16% for mild ARDS to 43% for severe ARDS [2] and accounts for 30% of PICU deaths [8]. Biologically, it is characterized by alveolar, epithelial, and endothelial dysfunction in the setting of inflammation and deranged coagulation [9]. Currently, there is no known effective treatment other than use of lung protective ventilation [3].

Multiple clinical trials in critically ill patients with ARDS have been negative, in part

because most of these clinical trials have included patients who are heterogeneous in their underlying biology and disease severity [4]. Utilizing markers of disease severity (prognostic markers) and underlying biological pathophysiology to inform treatment responsiveness (predictive markers) may allow targeted application of therapies and discovery of effective treatments [4, 5, 10]. Retrospective examination of existing pediatric sepsis datasets supports the use of predictive and prognostic enrichment to enhance the efficacy of clinical trials, therefore, there is an urgent need for novel study designs incorporating these strategies to leverage new and potentially effective treatments for ARDS [11].

Previously published data suggests that higher plasma levels of sTM, an endothelial membrane bound activator of Protein C, and genetic variants in the sTM gene are independently associated with increased organ dysfunction and mortality in adults and children with ARDS. The previously published data also reports that plasma levels of Ang-2, a mediator of endothelial permeability that is both a marker, as well as a mediator of endothelial permeability, outperforms other markers of endothelial injury in predicting mortality and multi-organ failure among children with ARDS [6, 7, 12].

Though the evidence describing the role of sTM and Ang-2 in pathogenesis of ARDS and their relationship to multi-organ failure and mortality among patients with ARDS is strong, the results of all observational studies are susceptible to bias due to unmeasured confounding factors and limited sample sizes, and thus must be validated. Both of these predictive markers have potential for clinical translation since sTM is available for clinical trials and anti-Ang-2 agents are currently being developed and likely to be available soon.

In addition, there is limited data among survivors following discharge from the hospital, though preliminary studies suggest that a number of survivors may have significant morbidity upon follow-up [13]. The CPCCRN network has developed and carried out large scale studies using a simple quantitative and reliable tool of measuring functional status scores (FSS) among PICU survivors, and is therefore uniquely poised to carry out studies of long-term functional outcome utilizing FSS [14]. Previous CPCCRN studies have also used the Pediatric Quality of Life Inventory Generic Core Scale (PedsQL) to evaluate health-related quality of life (HRQOL). This scale has been validated in numerous studies, and has shown to be a reliable and effective method of collecting HRQOL data in pediatric patients, regardless if the child self-reports or if parent proxy-report is used [15]. Long-term morbidity and functional outcomes are especially significant among the pediatric population because of lower mortality and a longer life expectancy among survivors as compared to adults. However, despite the significance and relevance of long-term morbidity and functional outcomes in the pediatric population, there is a

paucity of data relating biological markers to long-term morbidity and functional outcomes.

This cohort study of children with ARDS will validate the published bedside model for mortality risk, and confirm the utility of biomarkers with potential therapeutic application [6, 7, 12, 16]. Completion of the study will position CPCCRN to carry out a modern clinical trial based on an intelligent study design utilizing validated clinical and biological markers to stratify patients for targeted therapy that includes measures of long-term morbidity among survivors. This study may also create a baseline cohort study that can be leveraged to carry out additional studies to answer important additional questions in ARDS that are within the scope of strategic plans and priorities of the National Institutes of Health.

3 Study Design and Data Collection

3.1 Participant Screening and Consent

Eligible subjects will be identified by clinical or research staff. Screening will be conducted daily on all potential subjects on invasive positive pressure support in the PICU. Inclusion criteria for oxygenation (P/F or S/F ratio) must be met at two time points, at least 6 hours apart. The first ratio is the qualifying ratio and the second is the confirming ratio. The subject must be on a PEEP of ≥ 5 at during the time both of those ratios were documented. The subject does not have to meet the P/F or S/F for the duration of the 6 hours but they must meet it at two points at least 6 hours apart. Either P/F or S/F may be used at either of those time points for determining eligibility. When both are available, a P/F should always be used over an S/F ratio. For the Denver site, approved adjusted P/F and S/F ratios should be used. The time the subject becomes eligible for the study and blood sampling is whichever occurs later between the confirming oxygenation ratio and the qualifying chest x-ray. The 24 hour window for the first set of samples to be collected begins at the time the subject becomes eligible. The subject should be followed through their entire stay in the ICU to see if they meet all of the eligibility criteria.

To help clarify how to review and apply the eligibility criteria, here are two example situations of how subjects can meet these criteria:

1. Example Situation 1: A potential subject is identified within the specified age range, on invasive positive pressure support with PEEP ≥ 5, with bilateral infiltrates on chest X- ray, and a P/F ratio < 200 (or equivalent S/F ratio). The RC should

reevaluate this potential subject 6 hours later to verify the positive pressure and the P/F or S/F ratio meet the inclusion criteria. If the potential subject does not meet the P/F or S/F eligibility requirements again at the second time point, the potential subject will continue to be monitored by the study team until they either meet eligibility or 12 hours pass from the initial qualifying P/F or S/F ratio. If the potential subject does meet the P/F or S/F ratio at the second time point, the time of that ratio is considered the time of eligibility (if all other eligibility criteria have previously been met) and the RC has 24 hours from that second confirming P/F or S/F ratio to collect the first set of study samples. If the potential subject does not meet the second P/F or S/F ratio within the window, they should still be followed to see if they do meet all of the eligibility criteria at a later time.

2. Example Situation 2: A potential subject within the specified age range, on invasive positive pressure support with PEEP ≥ 5, and a P/F ratio < 200 (or equivalent S/F ratio) is identified. The RC reevaluates this potential subject 6 hours later and verifies their positive pressure and the confirming P/F or S/F ratio meet the inclusion criteria. At that point the subject still does not have a qualifying chest x-ray. If the potential subject has a qualifying chest x-ray within 24 hours after the qualifying P/F or S/F ratio, that x-ray time becomes the time of eligibility and the RC has 24 hours from then to collect the first set of study samples. If the potential subject does not have a qualifying chest x-ray within 24 hours after the qualifying P/F or S/F ratio, they do not meet all eligibility criteria and cannot be enrolled in the study. However, the potential subject should still be followed to see if they do meet all of the eligibility criteria at a later time.

All potential subjects meeting study inclusion criteria will be included in a screening log, regardless if they are enrolled, which will allow for tracking of the entire possible cohort. The subjects enrolled in this prospective cohort study will be recruited from academic center medical/surgical pediatric intensive care units participating in CPCCRN. Eligible potential subjects will be identified and their parents approached by study-trained staff as early as possible, but within 72 hours of becoming eligible for consent to participate in this study. If informed consent is provided, the study team will collect clinical subject data and subsequent study specimens.

The first blood and tracheal aspirate sample must be obtained within 24 hours of meeting all study eligibility criteria. We will request a waiver of consent from the CIRB to collect and save the first blood, tracheal, and urine samples if it is not possible to obtain consent within the first 24 hours. All subjects will be consented within 72 hours of becoming eligible. For participating study sites where local regulations preclude pre-

consent sample collection, consent will be obtained before the first samples are collected within 24 hours of meeting enrollment criteria.

We expect to enroll 300 subjects in this study. It is estimated the study will meet this enrollment goal in under 3 years. The study will collect clinical data for the first 28 days after onset of ARDS/meeting eligibility criteria and follow-up data for up to 10 months. Once enrollment has been completed the lab analysis, data analysis, and manuscript preparation will begin.

3.2 Data Collection

Enrolled intubated pediatric patients with ARDS will have clinical data collected at the time of admission, time of ARDS diagnosis, and daily during hospitalization per the study schedule. For patients discharged alive, we will collect follow up data through phone contact, email, text, or web based surveys. One urine sample will be collected as soon as possible after meeting study criteria, but ideally will be collected within 24 hours of meeting study criteria. Blood and tracheal aspirate samples will be collected on the baseline day (within 24 hours of meeting eligibility criteria) and Day 3 (48-72 hours of meeting eligibility criteria). In the unlikely scenario that a subject is extubated or discharged from the PICU before any blood collection time point, a blood sample will not be collected.

Informed consent will also be obtained from a parent, guardian, or LAR for post discharge follow up in order to obtain detailed baseline and follow-up clinical, PedsQL and FSS data, as well as for obtaining study samples. Subjects who are capable of giving assent and are alert and competent will be asked, following an age-appropriate discussion of risks and benefits, to give assent to the study for the collection of their personality self-assessment, and follow up self-assessment of PedsQL and FSS [14, 17]. If a subject declines to give assent, self-assessment data will not be collected from the subject. Follow up may occur through phone contact, email, text, or web based surveys. The follow up method utilized will be what best suits the needs of the subject and allows for the most secure and reliable means of collection. In an effort to compensate subjects for their time when completing the follow up surveys at the 3 and 9 month timepoints, we will send them a 50 dollar gift card for each set of full surveys completed for a potential total of 100 dollars.

3.3 Baseline Data Collection

Clinical information to be collected from admission and enrollment during PICU stay includes the following:

- Eligibility assessment
- Patient demographics
- Overall clinical status at onset
- Diagnosis associated with the onset of ARDS
- Presence of and/or development of new or progressive multiple organ system dysfunction as defined by Prouix criteria
- Physiologic respiratory data
- Ventilator data
  - PIP, PEEP, MAP, and TV
- Chest radiograph
- PRISM III scores (Collected from ICU admit data and when the subject becomes eligible)
- PCPC, POPC, and FSS scores
- PedsQL - Only collected from English or Spanish speaking subjects

3.4 Daily Hospitalization Data Collection

Clinical information to be collected daily (days 1 to 7) and on day 14, 21 and 28 during PICU stay from the medical record includes the following:

- Presence of and/or development of new or progressive multiple organ system dysfunction as defined by Prouix criteria
- Physiologic respiratory data
- PELOD score
- Ventilator data

  - PIP, PEEP, MAP, and TV

- Chest radiograph

3.5 Day 28 or ICU Discharge Data Collection

Clinical information to be collected at Day 28 or ICU discharge includes the following:

- Admission and discharge dates
- Discharge disposition and survival status (Was subject alive or dead)
- PELOD score
- PCPC, POPC, and FSS scores
- PedsQL - Only collected from English or Spanish speaking subjects

3.6 Status Checks

Site coordinators will perform a status check at day 28 (If the subject was discharged before day 28), 3 months, and 9 months from eligibility for all subjects regardless of ICU discharge status. These checks should be done before the central call center performs a follow up survey. Items reviewed with the subject at the status check will include:

- Survival status (Is subject still living)
- Preferences for follow up contact by central call center
- Verification of contact info

3.7 Follow Up Data Collection

The following information will be collected from English and Spanish speaking survivors at 3 and 9 months from enrollment:

- Survival status
- Verbal assent, consent, and/or parental permission as needed
- Medications
- Hospital visits
- Respiratory symptom questionnaire
- Pediatric Cerebral Performance Category (PCPC) and Pediatric Overall Performance Category (POPC)

- FSS
- PedsQL

ARDS Schedule of Events

Study Activity	Enrollment	Day 1	Day 2	Day 3	Day 4 Through 7	Day 14	Day 21	Day 28, Death, or Discharge[1]	Month 3 and 9[2]
Consent/Assent	X[3]								
Demographics	X								
Follow up contact info	X								
Med history and overall baseline clinical status	X								
Diagnosis associated with the onset of ARDS	X								
Chest x-ray[4]	X	X	X	X	X	X	X	X	
Ventilator data	X	X	X	X	X	X	X	X	
Nutrition data	X	X	X	X	X	X	X	X	
Fluid balance data	X	X	X	X	X	X	X	X	
Vitals and respiratory data	X	X	X	X	X	X	X	X	
Lab and microbiology data	X	X	X	X	X	X	X	X	
Blood sample collection and processing[5]	X[5]			X[5]					
Tracheal aspirate collection and processing[5]	X[5]			X[5]					
Urine sample collection and processing[5]	X[5]								
PRISM III score	X[6]								
PELOD-2		X	X	X	X	X	X	X	
PCPC/POPC	X							X	X
FSS	X							X	X
PedsQL score[7]	X							X	X
Medications	X	X	X	X	X	X	X	X	X
Hospital visits									X
Respiratory symptom questionnaire									X
Status check before follow-up call								X[8]	X

[1]Whichever occurs first.

[2]Questionnaires can be completed -2 weeks to +8 weeks from 3 or 9 month timepoint.

[3]If permission is unable to be obtained at enrollment, it must be collected before the second set of samples is collected. If subject is unable to assent at enrollment, they should be assented as soon as they become capable before any further research specific study activities.

[4]The chest x-ray (CXR) must be collected within 24 hours of the qualifying (1st) blood gas P/F or S/F ratio.

[5]Blood and aspirate samples are collected 24 and 48 to 72 hours from meeting eligibility criteria. The first set of samples (first blood and tracheal aspirate) must be collected within 24 hours of meeting the eligibility criteria. The first set of samples can be collected before permission is obtained if parents/guardian/LAR are unable to provide permission. Permission must be collected before the second set of samples (second blood and aspirate). If permission is unable to be obtained, all research samples collected must be destroyed. The urine sample can be collected anytime within the first 72 hours.

[6]PRISM III will be collected at ICU admission and when the subject meets eligibility.

[7]Only subjects that speak English or Spanish will complete the PedsQL assessment.

[8]All subjects discharged before day 28 will received a status check call from a research coordinator. No follow up surveys will be done at day 28 by the call center.

4 Study Procedures

4.1 Biospecimen Collection and Storage

The research team at each site will collect two blood samples from all research subjects. Samples will be collected on the baseline day (within 24 hours of meeting study eligibility criteria) and Day 3 (48-72 hours after meeting study eligibility criteria).

A total of 3.5cc of blood will be collected at each draw. This blood will be used for our planned analyses. These volumes are adapted for pediatric subjects and are much less than those drawn in adult studies. It is anticipated that this volume will be needed for reliable DNA extraction and provide adequate plasma volume for doing the primary assays including Ang-2 and sTM, as well as previously published prognostic mediators in the inflammation and coagulation pathways such as PAI-1, IL-6, IL-8, and IL10. It will accommodate for assays in duplicate and allowances for potential repeat assays to troubleshoot dilution and other technical issues with optimization of curves, etc. Permission will be obtained to use leftover blood, if any, to validate a prognostic biomarker model based on Latent Class Analysis (sTNFR1) and any additional novel hypothesis related to genomics and biomarkers in ARDS from a rigorously phenotyped cohort with granular clinical data. This will be updated as necessary depending on the evolution of technology and available platforms.

The 7cc blood volume total drawn for this study is much less than IRB limits for research blood collection for even the smallest subjects anticipated to be enrolled in this study. We will collect blood from indwelling catheters or add this study's blood collections to regularly scheduled clinical or other research lab draws in order to limit discomfort to subjects.

500uL of whole blood will be added to 1.4mL of RNA stabilizing solution for RNA extraction. The remaining whole blood samples will be centrifuged to separate plasma from the buffy coat and red blood cell pellet. Plasma, which will be used for biomarker analysis, will be aliquoted into separate microtubes. The buffy coat and red blood cell pellet, to be used for DNA extraction, will be similarly prepared for storage.

Tracheal aspirate samples from clinically indicated suctioning within first 24 hours and 48-72 hours will be collected and saved in RNA preservative solution. These samples will be processed for next generation sequencing of RNA for each lower respiratory specimen. Regularized log-transformed normalized gene expression generated from these analyses

will be used for supervised and unsupervised approaches to identify differentially expressed genes among subsets of subjects.

A 15ml urine specimen will be collected from all research subjects within the first 72 hours in order to better examine the relationship of plasma and urinary biomarkers. The role of environmental factors such as passive cigarette smoke exposure and its interactions with coagulation and inflammation-related mediators and genetic variants will also be studied. Samples will be collected by the bedside nurse using standard clinical procedure. From those children with an existing catheter, the research coordinators may collect directly from the bedside waste container. For children in diapers, cotton balls may be placed in the child′s diaper to collect the urine sample.

Initial processing and aliquoting of all samples will be done at each of the participating institutions. All biospecimens will be assigned a unique, deidentified sample ID and tracked using an automated bar code scanning system. All samples will be batch shipped to UCLA at three month intervals. A detailed standardized operations manual (SOP) describing specimen collection and on-site processing along with supplies necessary for sample collection, processing, coding, storage and transport will be provided to all the participating institutions.

4.2 Sample Processing and Analysis

4.2.1 Biomarker Assay

All measurements will be performed in duplicate and Dr. Sapru will personally evaluate the standard curves and calibration as an additional quality control measure. Details of these assays have been published and represent standard commercially available assays.

Extraction and processing of RNA: Whole blood will be collected in tubes, which contain an RNA preserving agent. RNA will be extracted using the Direct-Zol RNA extraction kit (Zymo, Inc), subjected to DNase and column purification and assessed using the A260/280 ratio of a UV adsorption spectrophotometer.

RNA quality assessment: The 2100 Bioanalyzer system located at the UCLA Center for Systems Biomedicine will be utilized for qualitative and quantitative analysis of RNA. It is a microchannel-based electrophoretic cell that enables rapid, accurate and reproducible analysis of the quality and composition of RNA samples with exceptionally small sample quantities.

We will extract RNA from peripheral blood and tracheal aspirates and perform RNA sequencing in a broad, discovery-based approach to identify novel genes that are differentially expressed in ARDS patients and identify unbiased disease endotypes within ARDS, validating this syndrome as heterogenous, and potentially characterizing subtypes that can be leveraged for better design of targeted therapeutics. Moreover, we will also perform pathway analysis to validate known (i.e., inflammatory, coagulation and endothelial injury pathways) and discover novel pathways that may be dysregulated in ARDS.

RNA extraction: Whole blood will be collected into PAXgene tubes containing an RNA preserving reagent. RNA will be extracted using the PAXgen RNAeasy Blood Extraction Kit (Qiagen). Endotracheal aspirates will be collected in cryovials pre-filled with RNA shield (Zymo). RNA will be extracted using a published protocol from the Zinter/DeRisi lab (65). RNA concentration will be measured using the Qubit High Sensitivity Assay kit (Thermo Fisher) along with the Qubit 4 Fluorometer. RNA quality will be assessed using the High Sensitivity TapeStation Screentape (Agilent) and total RNA will be quality-checked with Agilent 2100 Bioanalyzer. Library preparation: Libraries for RNA-Seq will be prepared with Kapa Hyper Prep Kit for blood and the NEB RNA Ultra II Kit for tracheal aspirates to generate strand-specific RNA-seq libraries. RNA Sequencing: Sequencing will be performed with the Illumina NovaSeq 6000 sequencer on a NovaSeq S4 flow cell to produce 150 base-pair paired-end reads (2 x 150 bp) at the UCLA Center for Genomics for blood and at the UCSF Center for Advanced Technology for tracheal aspirates.

4.2.2 Selection of Genetic Polymorphs

An iterative and stepwise approach will be taken to evaluate the genetic variants. SNPs from the discovery cohort will be analyzed and a comprehensive evaluation among genes of interest by genotyping tag SNPs that represent all SNPs with a minor allele frequency of 1% or more with an R^2 of 0.8 or more will be carried out. Data will be used from the Hapmap, the 1000 Genomes Project, and Seattle SNPs to identify and use Tagger, as implemented in Haploview with functional SNPs forced in. In order to isolate and identify the causal variants, selected regions encompassing the region around the SNPs that have an association with biomarker levels or clinical phenotype will be targeted. In order to avoid confounding due to population admixture, additional SNPs at unrelated loci will be genotyped and used to model for hidden structure, using genomic control [18, 19].

4.2.3 Genotyping

DNA will be extracted from the cell pellets by an experienced technician under Dr. Sapru's supervision using the Genomic DNA Purification Kit (Promega; Madison, WI) and will be stored at -80°C in barcoded vials. Illumina SNP genotyping assays will be used. SNPs that fail the SNPlex or Illumina assays will be genotyped using allelic discrimination based Taqman SNP Genotyping [20]. Genotyping will be blinded to the clinical data and 10% samples will be reassayed.

4.2.4 Target Region Sequencing

Hi-throughput DNA sequencing will be performed on the Illumina 2500 sequencer at the UCLA Genomics Core Facility (GCF) using Agilent SureSelect XT custom targeting panels for sequence enrichment, which utilize hybridization in solution approach with RNA baits to enrich for regions of interest. The UCLA GCF will perform the initial steps of sequencing analysis and deliver data as sequence reads and as VCF files with variants already called by the standard GATK-based pipeline in place.

4.2.5 Tracheal Secretion Transcriptomic Analysis

The current protocol involves ceramic bead-based tissue lysis in RNA-preserving media. This is followed by column-based extraction and purification with an on-column DNase to isolate pure RNA (Zymo). Next, RNA undergoes reverse transcription to form complementary DNA and assessed for quantity and purity using a fluorometer (Qubit). The cDNA libraries then undergo real time PCR amplification and further cleaning. The final libraries then undergo paired-end sequencing at 125 base pair length on an Illumina 4000 platform. Resultant .fastq files will be filtered for minimum quality and complexity cutoffs using PriceSeqFilter (v1.2), followed by alignment to the human genome version 38 using STAR to produce gene counts.

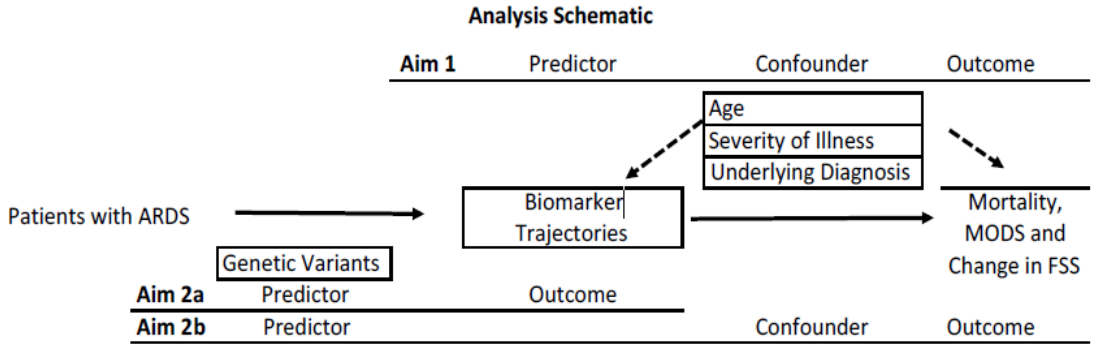
4.2.6 Biomarkers of Cigarette Smoke Exposure

Plasma and urine biomarkers of cigarette smoke exposure (cotinine and NNAL) will be measured using liquid chromatography/tandem mass spectrometry as previously described [21].

5 Data Analysis

The hypotheses of this study are:

1. Magnitude and trajectory of change in selected markers of inflammation and coagulation pathways such as (sTM and Ang-2) will be associated with increased mortality and multiple organ dysfunction syndrome (MODS) during hospitalization and with markers of long-term morbidity such as persistent O2-dependence and FSS among survivors post discharge.
2. Common and rare genetic variants in the corresponding genes will be associated with biomarker levels and with mortality and multi-organ failure during hospitalization and with long-term morbidity among survivors.



5.1 Statistical Considerations

Descriptive measures, including proportions, means, standard deviations or medians, and ranges will be utilized as appropriate to profile variables of interest. Normality of distribution will be assessed using QQ plots, and if the assumptions are not met, alternative or nonparametric methods will be used. For genetic data, SNPs that have call rates less than 95% or that are monomorphic will be removed from analysis. Hardy-Weinberg equilibrium will be evaluated [22]. Genotype calls will be assessed for any significant deviations. A significance level of 0.05 will be used taking into consideration multiple comparisons.

5.2 Specific Aim Analyses

Specific Aim 1. To determine whether plasma levels of coagulation and inflammation-related biomarkers (e.g., sTM and Ang-2) are associated with increased mortality, MODS, and long-term morbidity among survivors and to identify biomarker cut offs for potential therapeutic trials.

Regression models will be used to evaluate the effects of the biomarkers and SNPs on the primary outcome of mortality (logistic regression) and secondary outcomes of morbidity at 1-year (ordinal logistic regression). These models will include terms for clinical covariates as well as markers of interest. Initial evaluation for each marker will construct a model including that marker in additional to the clinical variables. Different formulations of the biomarkers within the models (e.g., baseline, slope of the biomarker over time) will be considered. [23]

In order to estimate cut-off values for biomarker levels to guide therapy the Youden index will be calculated. The Youden index is the sum of the sensitivity and specificity. For a single biomarker evaluation, we will create the ROC curve and then make a choice about what cut-off point to use. Depending on the intended use of the marker there may be a number of ways to choose the cut-off point. A minimum level of the sensitivity or specificity could be decided on and then maximize the other based on that criteria. Maximizing the sum of the sensitivity + specificity (Youden) to get a cut-point that is a bit more balanced and attempts to maximize overall performance will be tried. The CART analysis is a decision tree method that searches for the best possible cut-off point for one or more variables at a time. If a single biomarker is put into a CART analysis, it is likely to come up with the same cut-off point as using the ROC curve and maximizing the Youden index.

Specific Aim 2. To determine whether genetic variants in genes of coagulation and inflammation related mediators (e.g., sTM and Ang-2) are associated with higher plasma levels of these markers and with mortality, MODS and long-term morbidity among survivors.

Regression models will be used to evaluate the effects of the biomarkers and SNPs on the primary outcome of mortality (logistic regression) and secondary outcomes of morbidity at 1-year (ordinal logistic regression). These models will include terms for clinical covariates as well as markers of interest. Initial evaluation for each marker will construct a model including that marker in additional to the clinical variables. Different formulations of the biomarkers within the models (e.g., baseline, slope of the biomarker over time) will be considered. [23] For the SNP models, dominant or co-dominant modes of inheritance will be considered. Benjamini-Hochberg false discovery rate (FDR) will be used to adjust for multiple testing for the SNP associations with the outcomes. Finally, multimarker regression models will be constructed including the significant clinical, biomarker and SNP markers in a single prediction model. AUC will be computed for the logistic model and the c-statistic for the Cox-model to evaluate the overall predictive utility of the markers. 10-fold cross-validation to estimate the effect of model selection on

the performance of the prediction models will also be used.

Specific Aim 3. To test and validate our previously published prognostic inflammation, coagulation, epithelial, and endothelial ARDS related biomarkers (PAI-1, IL6, IL8, IL10, sTNFr) and published prognostic models such as Latent Class Analysis [26]. We also plan to test additional novel hypotheses related to genomics and biomarkers in ARDS from a rigorously phenotyped cohort with granular clinical data.

Comparison of transcriptional pathways to identify differentially expressed gene sets among survivors and non-survivors as well as among patients with different severities and/or etiologies of lung injury.

RNA meta transcriptomic sequencing based assay to characterize bacteria, fungi, and viruses in tracheal aspirates and blood of pediatric ARDS patients

ROC curves will be used to compare the prognostic value of PRISM scores alone with a combined biomarker and PRISM model.

Power Analysis.

We plan to enroll 300 subjects. The primary endpoint will be in-hospital mortality. Based on the inclusion criteria we expect 20% of subjects to die within the hospitalization stay. It is expected that at least 100 subjects from the CPCCRN VAP study will be included in this analysis.

Sample Size	Baseline Mortality Rate	Odds Ratio	Power
400	0.3	1.36	0.8
	0.2	1.42	0.8
	0.15	1.48	0.8
300	0.3	1.43	0.81
	0.2	1.5	0.8
	0.15	1.58	0.81

Biomarker Analysis

Sample Size	Baseline Mortality Rate	Minor Allele Freq	N1 (AA/Aa)	N2 (aa)	Odds Ratio	Power
400	0.30	0.20	40	360	2.3	0.81
		0.10	80	320	2.9	0.83
	0.20	0.20	40	360	2.4	0.8
		0.10	80	320	3.0	0.81
	0.15	0.20	40	360	2.6	0.80
		0.10	80	320	3.2	0.83

Genetic Variants: adjusted for multiple comparison using FDR at 10%

6 Data Management

6.1 Clinical Site Data Management

The investigators and study staff are responsible for maintaining a comprehensive and centralized filling system containing all study-related documentation. Study worksheets are to be completed in a neat, legible manner to ensure accurate interpretation of data. If any corrections or changes must be made to the worksheets, the original entry should be crossed out using a single line, and must be dated and initialed by the individual making the change. The original entry will not be erased or overwritten. Additionally, the Electronic Health Record (EHR) serves as source documentation for this study. Sites are responsible to maintain access or copies of EHR source data in addition to worksheets.

6.2 Electronic Data Capture System

Data for the this study will be entered into an electronic data capture (EDC) system maintained by the Data Coordinating Center (DCC) at the University of Utah. This system provides secure user access via the Internet, and maintains an audit log for all study events and data.

6.3 Study Monitoring

The investigators recognize the importance of ensuring data of excellent quality. Site monitoring is critical to this process. Site monitoring has been a very effective tool for maintaining data quality in previous CPCCRN studies, and this process will be utilized to ensure quality data in the proposed study. The site monitoring plan is designed to

identify problems with sites and methods for handling problems that arise.

Site monitors must be provided with full access to study materials and the medical records for study subjects. If the medical records are in electronic form, the clinical investigator or an authorized individual must provide any assistance necessary to facilitate the site monitor's review of data in the electronic medical record.

6.3.1 Site Monitoring Plan

A supplemental study-specific monitoring plan, separate from the protocol will be completed which outlines specific criteria for monitoring. This plan will include the number of planned site visits, criteria for focused visits, or additional visits, a plan for chart review and a follow up plan for non-compliant sites. The monitoring plan also describes the type of monitoring that will take place (e.g., sample of all subjects within a site; key data or all data), the schedule of visits, how they are reported and a time frame to resolve any issues found. Remote site monitoring schedules will be determined by the Data Coordinating Center in coordination with the study principal investigator.

6.3.2 Clinical Site Monitoring

Site monitoring visits may be performed, on a risk based basis, by a trained site monitor during the study period to ensure regulatory compliance, patient safety, and to monitor the quality of data collected. Essential document binders, regulatory documents and data collection forms may be reviewed. Monitoring visits will take place depending on grant budget, site enrollment, and compliance issues identified. The site monitor will provide each site with a written report, and sites will be required to follow up on any deficiencies. It is anticipated that the study monitoring visits for this protocol will consist of a site initiation visit (prior to patient enrollment), interim visits, and a close out visit. The site initiation may take place as group training made up of site investigators and research assistants.

6.3.3 Remote Monitoring

The Data Coordinating Center primarily uses remote monitoring activities because of the expense of physical site visits. Remote monitoring involves detailed review of the data entered by the Clinical Center and consultations with the Clinical Center investigator and/or research coordinator to review safety and data quality. This may require uploading de-identified copies of specific parts of the medical record, patient study file, regulatory documentation, or other source documents to the Data Coordinating

Center staff, who review those materials against the data recorded in the electronic data capture system. This helps assure protocol compliance and accurate data collection. The Data Coordinating Center may conduct more remote monitoring activities early in the study to assure protocol compliance and identify any training issues that may exist. Remote monitoring the documents will be retained in accordance with federal requirements. Safety of subjects will be monitored and ensured in accordance with the Data and Safety Monitoring Board (DSMB) plan.

6.4 Data Coordinating Center

6.4.1 Data Center Description

The Data Coordinating Center (DCC) in the Department of Pediatrics at the University of Utah School of Medicine provides data coordination and management services for a variety of national research networks. Anchoring these services is a new state-of-the-art, energy efficient data center completed in 2013. The data center facility supports more than 1400 users around the world and provides a secure, reliable, enterprise-wide infrastructure for delivering critical DCC systems and services. The new data center was built using high industry standards and energy efficient cooling solutions. The data center is cooled by Rittal's LCP inline cooling technology, providing efficiency, redundancy and modularity. Cooling is based upon a hot/cold aisle design that allows for even air distribution with minimal hot spots. The data center electrical power system contains a redundant Mitsubishi uninterruptible power system (UPS) with a diesel backup generator. The data center is protected with a FM200 fire suppression system, early warning smoke detectors and a heat detection warning system to act as a secondary system to the smoke detectors. Security guards are on-site conducting access control and rounds 24/7/365. Entry into the data center is restricted by card access and layered security measures and controls. The data center and external building access points are monitored with video surveillance.

In 2011 the data center began a large scale VMware server virtualization deployment. Currently, the data center has virtualized about 99% of its environment. The virtual environment consists of more than 200 virtual servers. The data center's virtualization solution provides key advantages:

- high availability – in the event of hardware failure, virtual servers automatically go back online in a seamless process.
- flexible infrastructure – disk storage, memory and processor capacity can be increased or reallocated at any time.

- rapid deployment – servers can be provisioned on-demand with minimal waiting on hardware of software.

The data center also enhanced its storage resources by implementing a networked storage system to support its virtualized environment. The data center currently manages over 50 terabytes of data. The storage solution consists of Dell's EqualLogic PS Series Storage system for providing a virtualized storage area network (SAN). Some of the benefits that are realized through this technology are:

- storage architecture is no longer be a bottleneck for IT services;
- performance is better than with the previous architecture;
- tiered storage is now possible;
- provisioning and reclamation of SAN disk will be much easier; and most important,
- the new architecture includes a redesign of the SAN fabric to include complete redundancy.

Production servers running critical applications are clustered and configured for failover events. Servers are backed up with encryption through a dedicated backup server that connects across an internal 10 gigabit network to a tape drive. DCC storage area networking (SAN) applications, clusters, and switch-to-switch links are also on a 10 gigabit network. Incremental backups occur hourly Monday through Friday from 6 am to 6 pm. Incremental backups also are performed each night with full system backups occurring every Friday. Tapes are stored in a fireproof safe inside the data center facility, and full backups are taken off site on a weekly basis to an off-site commercial storage facility.

In the event of catastrophic failure, such as a fire in the server facility, daily backups would probably survive because of the fire suppression system and fireproof safe, but there would be obvious delay in re-establishing data center function because the servers will not survive such a disaster. Total destruction of the data center facility could cause the loss of up to one week's data. In future investments, the data center is making co-location, disaster recovery and business continuity solutions a top priority.

DCC information systems are available 24 hours a day, 7 days a week to all users unless a scheduled maintenance interruption is required. If this occurs, we notify all users of the relevant systems, and data entry can be deferred until after the interruption is over. Critical systems availability has exceeded 99.9% for the past two years, and there has been no unscheduled downtime in over five years.

6.4.2 Security and Confidentiality

The data center coordinates the network infrastructure and security with the Health Sciences Campus (HSC) information systems at the University of Utah. This provides us with effective firewall hardware, automatic network intrusion detection, and the expertise of dedicated security experts working at the University. Network equipment includes four high-speed switches. User authentication is centralized with two Windows 2012 domain servers. Communication over public networks is encrypted with virtual point-to-point sessions using transport layer security (TLS) or virtual private network (VPN) technologies, both of which provide at least 128 bit encryption. All of our Web-based systems use the TLS protocol to transmit data securely over the Internet. Direct access to data center machines is only available while physically located inside our offices, or via a VPN client.

All network traffic is monitored for intrusion attempts, security scans are regularly run against our servers, and DCC IT staff is notified of intrusion alerts. Security is maintained with Windows 2012 user/group domain-level security. Users are required to change their passwords every 90 days, and workstations time out after 5 minutes of inactivity. All files are protected at group and user levels; database security is handled in a similar manner with group-level access to databases, tables, and views in Microsoft SQL Server. Finally, all laptop computers in use in the DCC or in the Department of Pediatrics are whole-disk encrypted.

The data center uses control center tools to continuously monitor systems and failure alerts. Environmental and network systems are also monitored to ensure up time. Highly trained system administrators on staff are available to respond in high risk emergency events.

All personnel involved with the DCC have signed confidentiality agreements concerning data encountered in the course of their daily work. All personnel (including administrative staff) have received Human Subjects Protection and Health Information Portability and Accountability Act (HIPAA) education. All users are required to sign specific agreements concerning security, confidentiality, and use of DCC information systems, before access is provided.

6.5 Record Access

The medical record and study files (including informed consent, permission, and assent documents) must be made available to authorized representatives of the Data Coordinating Center, upon request, for source verification of study documentation. In addition, medical

information and data generated by this study must be available for inspection upon request by representatives (when applicable) of the Food and Drug Administration (FDA), NIH, other Federal funders, and the Institutional Review Board (IRB) for each study site.

7 Protection of Human Subjects

7.1 Institutional Review Board (IRB) Approval

The Data Coordinating Center and each clinical center must obtain approval from their respective IRB prior to participating in the study. The Data Coordinating Center will track IRB approval status at all participating centers and will not permit subject enrollment without documentation of initial IRB approval and maintenance of that approval throughout subsequent years of the project.

7.2 Informed Consent

Parental, Legal Guardian, or Legally Authorized Representative (LAR) Permission

Subjects who are eligible for this study are under 18 years of age, and written permission from a parent, legal guardian, or legally authorized representative (LAR) will be required for participation. The site investigator or designee will approach the parent or legal guardian to offer participation for their child in the study within 72 hours of becoming eligible. For participating study sites where local regulations preclude pre-consent sample collection, consent will be obtained before the first samples are collected within 24 hours of meeting enrollment criteria. The parent or legal guardian will be informed about the objectives of the study and the potential risks and benefits of participation. A waiver of consent will be used to collect and save the first blood, urine, and tracheal aspirate samples in the event that consent is unable to be obtained within the first 24 hours of meeting eligibility. Whenever the first set of samples is collected before obtaining consent, a parent/guardian/LAR will be approached for consent before the second set of samples is collected and within 72 hours of becoming eligible. If consent is denied, then the samples will be discarded and the subject will be removed from the study.

This study meets criteria for waiver of consent as per OHRP 45 CFR 46.116(d)

1. The research involves no more than minimal risk to the subjects;

- There will be two minimally invasive blood draws, which involve no more than minimal risk to the subjects. The 3.5cc blood samples are within limits for minimal risk criteria. The urine and tracheal aspirate samples are collected as part of standard clinical care and will be given to the study team as a study sample rather than being discarded.

2. The waiver or alteration will not adversely affect the rights and welfare of the subjects;

   - The research team will approach a parent, guardian, or LAR to obtain consent before the second set of blood samples are collected and within 72 hours of meeting study criteria. We will destroy the study samples of any subject whose parent, guardian, or LAR does not agree to their child′s participation.

3. The research could not practicably be carried out without the waiver or alteration;

   - The initial blood sample must be collected within 24 hours of meeting ARDS eligibility. It is crucial to collect the sample within 24 hours because biomarker levels fluctuate within the first few days of ARDS onset, and because the purpose of this study is to use biomarkers to guide targeted therapy, biomarker level measurements are needed early in the duration of illness. There may be limited opportunities for consenting the family during this narrow crucial window of time due to the physical availability of parents and guardians, the ability to meet with the research team, and the sensitive and tumultuous emotional frame of mind and potential for additional emotional stress and anxiety to the family. Not having access to all potential subjects would bias the results and limit generalizability of results.

4. Whenever appropriate, the subjects will be provided with additional pertinent information after participation.

   - The parent or guardian will be approached within 72 hours and provided all the pertinent information and the right to allow or refuse their child′s participation in the study.

Child Assent

Subjects who are eligible for this study are receiving positive pressure support, and child assent will not be possible at the time of study enrollment. If positive pressure support is no longer needed while the child is undergoing study procedures, subjects who are capable

of providing assent will be invited to participate in the study by providing self-report data for the follow-up questionnaires. Questionnaires will only be administered to the parent/guardian/LAR and to subjects who are 8 to 18 years old. Subjects 0 to 7 years old will not be asked to complete the self-report questionnaires. For subjects who are not able to be assented before they leave the ICU, assent will be verbally or electronically collected and documented at the beginning of the questionnaires for all subjects. If the subject does not provide assent, the subject questionnaires will not be collected but the parent/guardina/LAR will still be collected.

Subject Consent

Subjects who are eligible for this study are under 18 years of age. If a subject attains the age of 18 years during the study period, then informed consent becomes applicable. If this occurs, 18 year old subjects who are alert and competent and capable of giving consent will be asked, following an appropriate discussion of risks and benefits, to give consent to the study for further study procedures. Subject consent will be waived if the subject has a severely reduced mental age, decreased level of consciousness, psychological problems, or other legitimate reasons as judged by the Institutional Review Board at each site. For subjects who are not able to be consented before they leave the ICU, consent will be verbally or electronically collected and documented at the beginning of the questionnaires for all subjects. If the subject does not provide consent, the subject questionnaires will not be collected but the parent/guardina/LAR will still be collected.

Consent for Specimen Banking

Enrolled subjects will be offered the option to allow research blood, urine, and tracheal aspirate samples to be banked for future use beyond the end date of the study. This will be included in the informed consent document. If the subject denies having research specimens banked, the samples will be destroyed after the analysis phase of the study has been complete.

Withdrawal of Consent

Parents or research subjects may withdraw permission for further sampling at any time. The subject may also be discontinued from the study at the discretion of the Investigator to protect the subject for reasons of safety or for administrative reasons. The study withdrawal will be documented.

7.3 Potential Risks

Urine Collection. A single urine specimen will be collected from subjects and potential subjects as soon as possible after intubation. Urine will be collected non-invasively from indwelling Foley catheters (if in place as part of clinical care), bed pans or other urine receptacles, or cotton balls placed in the diaper. Sample collection poses no added risk to participants.

Blood Draw. One blood sample will be collected within 24 hours of meeting study criteria, and a second sample will be collected within 48-72 hours of meeting study criteria. The risks of a blood draw are minor discomfort, bruising, and infection. Samples will be drawn from an existing intravenous line or at the time of a medically necessary venipuncture so participants will not need to have any additional needle sticks for research purposes. Further samples will not be drawn for the study if the subject′s doctor indicates that taking the extra blood for research might be unsafe.

Risk of Loss of Privacy. There is a minor risk of loss of confidentiality. This risk is significantly mitigated by the items discussed in section 6.4.2 of this protocol.

7.4 Protections Against Potential Risks

All study information obtained from the subject will be kept in a secure location. Data from this study will be entered into an electronic data capture (EDC) system used by the Data Coordinating Center (DCC) at the University of Utah. This system provides secure user access via the Internet, and maintains an audit log for all study events and data. All electronic information will be stored on secure password protected servers. Publications generated by this research will not include identifying data.

7.5 Potential Benefits

Both the risks and benefits for each individual subject are minimal. The overall benefit includes validation of mortality risk models and biomarkers with potential therapeutic application, which may help future patients.

8 Study Training

8.1 Study Training

A formal training program for investigators and research staff will be held prior to the start of enrollment. The training program will cover regulatory topics and Good Clinical Practice. The training will also provide in depth explanations regarding study procedures, clinical care, data entry procedures, quality assurance, site monitoring, and the informed consent process. A manual of operations will be provided to each investigator and research staff prior to the start of enrollment. The manual will detail specific information about the study procedures, regulatory information, safety reporting, and other necessary information. Updates and revisions to the manual will be made available electronically. The DCC, in collaboration with the study investigator (Dr. Sapru), will be the main contact for study questions.

9 Regulatory Issues

9.1 Health Insurance Portability and Accountability Act

Data elements collected include the date of birth and date of admission. Prior to statistical analyses, dates will be used to calculate patient age at the time of the study events. The final data sets (used for study analyses and archived at the end of the study) will be de–identified, and will exclude these specific dates.

Data elements for race, ethnicity, and gender are also being collected. These demographic data are required for Federal reporting purposes to delineate subject accrual by race, ethnicity, and gender.

For purposes of the DCC handling potential protected health information (PHI) and producing the de–identified research data sets that will be used for analyses, all study sites have been offered a Business Associate Agreement with the University of Utah. Copies of executed Business Associate Agreements are maintained at the DCC.

9.2 Inclusion of Women and Minorities

There will be no exclusion of patients based on gender, race, or ethnicity.

9.3 ClinicalTrials.gov Requirements

This study will not be registered at ClinicalTrials.gov as it is not an interventional trial.

9.4 Retention of Records

For federally funded studies subject to the Common Rule, records relating to the research conducted shall be retained for at least 3 years after completion of the research. Completion of the research for this protocol should be anticipated to include planned primary and secondary analyses, as well as subsequent derivative analyses. Completion of the research also entails completion of all publications relating to the research. All records shall be accessible for inspection and copying by authorized representatives of the regulatory authorities at reasonable times and in a reasonable manner [45 CFR §46.115(b)].

9.5 Public Use Data Set

After subject enrollment and follow up have been completed, the DCC will prepare a final study database for analysis. A releasable database will be produced and completely de-identified in accordance with the definitions provided in the Health insurance Portability and Accountability Act (HIPAA). Namely, all identifiers specified in HIPAA will be recoded in a manner that will make it impossible to deduce or impute the specific identity of any patient. The database will not contain any institutional identifiers.

The DCC will also prepare a data dictionary that provides a concise definition of every data element included in the database. If specific data elements have idiosyncrasies that might affect interpretation or analysis, this will be discussed in the dictionary document. In accordance with policies determined by the investigators and funding sponsors, the releasable database will be provided to users in electronic form.

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