The APS Phenotyping Study Protocol A (full protocol)

Protocol Full Title:	The ARDS, Pneumonia, and Sepsis (APS) Consortium: A Prospective Observational Study to Evaluate Phenotypes (Protocol A – full protocol)
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Clinical Center Hub Institutions:	(1) University of California, San Francisco (2) Intermountain Medical Center (3) University of Colorado (4) Vanderbilt University Medical Center (5) University of Michigan (6) University of Pennsylvania
Coordinating Center & Single IRB:	Vanderbilt University Medical Center
Investigators:	See section 1
Corresponding Investigator:	Wesley H. Self, MD, MPH wesley.self@vumc.org
Purpose:	This protocol outlines study procedures for the collection of data and biospecimens to establish and build the APS phenotyping cohort. This protocol (Protocol A) is the full study protocol that describes all study procedures and governs study conduct for participants who have completed informed consent for study participation. A companion protocol (Protocol B) describes alteration of informed consent procedures and outlines study procedures that may be completed with alteration of informed consent. Appendices in this protocol (Protocol A) outline specific uses of the data and biospecimens by APS investigators for Consortium-wide studies.

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1. Investigators

Table 1. APS Consortium investigators

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UCSF	Stanford University	Joseph Levitt, MD, MS
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2. Protocol Notes

2.1 Protocol Revision History

Protocol Version Number	Protocol Version Date	Summary of Revisions
1.0	January 7, 2024	Original protocol submitted to sIRB
1.1	March 18, 2024	Revision submitted to sIRB, with splitting of protocol documents into two protocols: Protocol A (full protocol), and Protocol B (alteration protocol)
1.2	March 20, 2024	Revision submitted to sIRB, with the addition of this sentence to section 13.2.1: Remote consent must use a procedure compliant with Title 21 CFR Part 11, such as signature through Adobe Acrobat Sign or DocuSign.
1.3	March 26, 2024	Revised section 14.2.3 to change the time frame of reporting adverse events to the sIRB from within 14 days to within 7 days of site awareness of event.
2.0	April 13, 2024	Protocol version used to initiate study enrollment (approved by single IRB at Vanderbilt University Medical Center). (1) Revised sections 9.2 and 10 to include data collection on whether a participant is pregnant at 3-month, 6-month, and 12-month surveys. (2) Revised section 13.2.4 to note that, "If a participant attends a long-term outcome in-person visit without having previously provided informed consent for study participation, informed consent for study participation will be obtained at that time using ICD#1."
2.1	June 6, 2024	- Section 8.1.1: This item removed from in-hospital interview: Cognitive function (PROMIS Cognitive Function Short Form 8a) - Section 9: clarified that study day number started at enrollment (study day 0) and not hospital admission. - Table 5: corrected typographical error - Table 7: corrected typographical error - Throughout the document, especially section 13, language was revised to more precisely outline the use of identifiable information. Enrolling sites will enter identifiable information, such as date of hospital admission and date of birth, into the study's database. These data will be visible by APS coordinating center personnel. De-identified datasets will be created for long-term storage of data outside the APS Consortium.

Protocol Version Number	Protocol Version Date	Summary of Revisions
2.2	July 13, 2024	- Section 1: Added Xiaoli Zhao as a consortium investigator. - Sections 9.2.2 and 13.2.4: Added language outlining study procedures for the long-term outcome surveys when the patient had previously been participating in the study via surrogate consent. In this situation, at the 3-month phone call, the study team will seek consent for study participation from the patient with waiver of documentation of signature prior to administering survey questions via phone call. If the patient is unable to provide consent for study participation, a limited number of survey questions may be administered to the surrogate.
3.0	August 5, 2024	- Sections 7.1.3, 9.3, and 10 and Table 8: The protocol was altered so that pregnant participants only undergo study procedures that are no greater than minimal risk. - Section 8.2: Language added to clarify that aliquots of biospecimens collected on this research protocol may be used for clinical laboratory testing if the local team judges such tests to be potentially beneficial for the patient.

2.2 Abbreviations

Table 2. Abbreviations

Abbreviation	Full term
AE	Adverse Event
AESI	Adverse Event of Special Interest
APS	ARDS, Pneumonia, and Sepsis
ARDS	Acute Respiratory Distress Syndrome
AUDIT-C	Alcohol Use Disorder Identification Test – Concise
CC	Clinical Center
CCC	Consortium Coordinating Center
CNS	Central Nervous System
COI	Covariate of Interest
COPD	Chronic Obstructive Pulmonary Disease
COS	Core Outcomes Set
COX-IV	Cytochrome c oxidase subunit IV
CPT	Cell Preparation Tube
CT	Computed Tomography
CXR	Chest X-Ray
DAMP	Damage-Associated Molecular Pattern
ddPRC	Droplet digital Polymerase Chain Reaction
DICOM	Digital Imaging and Communications in Medicine
DLCO	Diffusing Capacity of the Lungs for Carbon Monoxide
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
EHR	Electronic Health Record
ELISA	Enzyme-Linked Immunosorbent Assay
EQ-5D-5L	EuroQol 5 Dimensions 5 Level
FVC	Forced Vital Capacity
GDF-15	Growth differentiation factor 15
HADS	Hospital Anxiety and Depression Scale
HME	Heat Moisture Exchanger
ICAM-1	Intracellular adhesion molecule 1
ICAP-Revised	Inventory for Client and Agency Planning-Revised

Abbreviation	Full term
ICC	Intraclass correlation coefficient
ICU	Intensive Care Unit
IES-6	Impact of Event Scale-6
IQ-CODE	Informant Questionnaire on Cognitive Decline in the Elderly
IRB	Institutional Review Board
Katz ADL	Katz Index of Independence in Activities of Daily Living
LAR	Legally Authorized Representative
Lawton IADL	Lawton Instrumental Activities of Daily Living scale
LCA	Latent class analysis
lpm	liters per minute
LRM	Logistic-Regression Model
LTO	Long Term Outcome
MCP1	Monocyte chemoattractant protein 1
mMRC dyspnea scale	Modified Medical Research Council dyspnea scale
mNGS	metagenomic Next Generation Sequencing
MODS	Multiple Organ Dysfunction Syndrome
mtDNA	Mitochondrial DNA
NBBAL	Non-Bronchoscopic Bronchoalveolar Lavage
ND-1	NADH dehydrogenase 1
NET	Neutrophil-Endothelial Traps
NHATS	National Health and Aging Trends Study
NHLBI	National Heart, Lung, and Blood Institute
NIGMS	National Institute of General Medical Sciences
NIH	National Institutes of Health
OSMB	Observational Study Monitoring Board
PAI-1	Plasminogen activator inhibitor 1
PEEP	Positive End-Expiratory Pressure
PERMANOVA	Permutational multivariate analysis of variance
P:F ratio	Partial pressure of oxygen: Fraction of inspired oxygen ratio
PFT	Pulmonary function testing
PICS	Post-Intensive Care Syndrome
PRC	Protocol Review Committee
PTSD	Post-Traumatic Stress Disorder

Abbreviation	Full term
qPCR	Quantitative Polymerase Chain Reaction
RAGE	Receptor for advanced glycation end-products
RALE score	Radiographic Assessment of Lung Edema score
RBM	Rules-Based Model
RNA	Ribonucleic acid
rRNA	ribosomal RNA
SAE	Serious Adverse Event
SC	Steering Committee
sIRB	Single Institutional Review Board
SIRS criteria	Systemic Inflammatory Response criteria
SMS	Short Message Service
SOFA score	Sequential Organ Failure Assessment score
SP-D	Surfactant protein D
SPPB	Short Physical Performance Battery
STAR	Spliced Transcripts Alignment to a Reference
sTM	Soluble thrombomodulin
TA	Tracheal aspirate
TNFR1	Tumor necrosis factor receptor 1
UMAP	Uniform Manifold Approximation and Projection
UP	Unanticipated Problem
WHODAS-12	World Health Organization Disability Assessment Schedule 12-item version

2.3 List of Tables and Figures

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3. Purpose of this Protocol / Program Overview

3.1 Scope of Study

This protocol describes the study activities that the ARDS, Pneumonia and Sepsis (APS) Consortium will complete together as a group, termed "Consortium-wide study procedures" and "Consortium-wide science." The main body of this protocol is a *Master Protocol* and includes the overall rationale for the APS program, and the procedures for enrolling participants, collecting data, and collecting biospecimens.

Data and biospecimens collected using this master protocol will be used for three types of scientific studies:

i. **APS Consortium-Wide Science:** Studies the APS Consortium investigators will complete and disseminate together using data and biospecimens collected under this master protocol. This science is described in the appendices of this protocol. Each of the 7 appendices describes one aim (study) of the APS Consortium-wide science. Consortium-wide science is funded by the NIH as part of the APS program.

ii. **APS Clinical Center-Specific Studies:** Studies that individual Clinical Centers within the APS program will complete with data and biospecimens collected under this master protocol. The science of these studies will be completed without involvement of the full APS consortium and is not governed by this protocol. Use of data and biospecimens in Clinical Center-specific studies will be governed by other protocols, as necessary. Clinical Center-specific studies are funded by NIH as part of the APS program through grants to individual Clinical Centers.

iii. **Ancillary Studies:** Studies that use banked data and biospecimens collected under this master protocol for conducting science not part of the APS Consortium-wide science nor the APS Clinical Center-specific studies. Use of data and biospecimens in ancillary studies will be governed by other protocols, as necessary. Ancillary studies are not funded as part of the APS program. Ancillary studies may be completed by APS Consortium investigators or investigators from outside the APS Consortium.

In summary, this protocol governs the collection of data and biospecimens for Consortium-wide study procedures and, in the appendices, describes the use of some of these data and biospecimens for consortium-wide scientific studies. Other studies, including Clinical Center-specific studies and Ancillary Studies, that will use data and biospecimens banked from this master protocol will be governed by other protocols managed by the investigators leading those studies.

3.2 Protocol Documents

The APS Consortium study is governed by two study protocols:

- Protocol A (full protocol): This document is Protocol A. It describes all study procedures that a participant may complete during the course of the APS study and governs study procedures for participants who have completed informed consent for research participation. Protocol A contains two parts:
  - o Master protocol: Study procedures that govern enrollment, data collection, and biospecimen collection. The master protocol is described in the main text of this document.
  - o Consortium-wide science: specific analyses that will use data and biospecimens collected in this study and will be undertaken by the APS Consortium investigators collaboratively across the entire consortium. Consortium-wide science is described in appendices to this document.

- Protocol B (alteration protocol): This is a separate document not contained in the document presented herein. It describes a procedure for participation in the APS Consortium study with alteration of informed consent. Protocol B will be used for participants for whom informed consent for research cannot be obtain via the participant or a legally authorized representative prior to initiation of study procedures. Minimal risk procedures within the APS study may be completed using alteration of informed consent. Protocol B outlines the minimal risk procedures that may be completed with alteration of informed consent.

Participants who have provided informed consent will complete study procedures described in Protocol A (this document), and participants who have not provided informed consent will complete study procedures described in Protocol B (a separate document). Protocol B includes a subset of study procedures in Protocol A and does not include any study procedures not in Protocol A. Hence, patients participating under alteration of informed consent will complete a subset of study procedures (Protocol B) completed by participants with informed consent for study participation (Protocol A). Participants who enter the study on the alteration of informed consent protocol (Protocol B) will be iteratively approached for consent; if and when informed consent for participation in the APS Study is obtained, the participant will be moved from Protocol B to Protocol A. For participants who started study participation under alteration of informed consent, greater than minimal risk procedures may be completed after informed consent is obtained. Data and biospecimens collected under Protocol A and Protocol B will be pooled for storage and analysis.

4. Master Protocol Synopsis

This master protocol presented in the main text of this document describes the enrollment of participants into the APS Consortium Cohort and the collection of data and biospecimens from these participants. Eligible hospitalized adult patients ≥18 years old will be identified and consented for participation in a multi-site cohort study that seeks to understand risk factors, molecular phenotypes, and illness trajectory during acute and recovery phases for ARDS, pneumonia, and sepsis as common and potentially overlapping critical illness syndromes. Baseline and longitudinal clinical data, questionnaires, and biospecimens from approximately 4000 participants will be collected during the index hospitalization and post-hospitalization periods. Attempts will be made to contact all surviving participants for post-hospitalization assessments, including up to 600 participants who will return for additional in-person study visits and biospecimen collection.

Site investigators will adjudicate for the presence of ARDS, pneumonia, and sepsis syndromes among enrolled participants by reviewing the electronic health records (EHR) and other available data during a defined period of the index hospitalization. The APS Consortium anticipates issuing periodic guidance that seeks to ensure adequate enrollment among the following subgroups: (1) racial/ethnic/sex subgroups, (2) at least 1000 patients with ARDS, 2000 patients with pneumonia, and 2000 patients with sepsis in the final cohort (these syndromes are not mutually exclusive), (3) patients invasively mechanically ventilated (and thus eligible for sampling of the lower respiratory tract).

Clinical data and biospecimens will be shared with the Consortium Coordinating Center (CCC) for cataloging, analysis to complete the Consortium-wide scientific aims, and distribution to other investigators for use. Data and biospecimens will ultimately be deposited in the NHLBI BioDataCatalyst and BioLINCC respectively, to support future use among APS Consortium and non-Consortium researchers.

5. Introduction

5.1 Background

ARDS, pneumonia, and sepsis are common critical illness syndromes inflicting significant morbidity and mortality, both during the acute phase and in the years following the initial illness.[1] Hallmarks of APS include high mortality; complexity of illness; a high burden of pre-existing chronic illness; the possibility of rapid shifts in clinical status; a plethora of adverse events resulting from multiple, simultaneous interventions that can exacerbate and confound the acute disease state; and long-term physical, psychological, and cognitive morbidity.

There is substantial heterogeneity in each of these syndromes – ARDS, pneumonia, and sepsis – both in terms of the insults that incite them and in patients' physiological and biological responses.[1] Currently, there are no host-targeted pharmacotherapies with established efficacy against ARDS, pneumonia, or sepsis and their sequelae.[2] One of the leading theories as to why pharmacotherapy development has been unsuccessful in this area is due to the heterogeneity of these syndromes.[2] Study treatments applied homogenously in heterogeneous populations, as has historically been done in most APS clinical trials, may have concealed important benefits in specific sub-phenotypes of APS patients.[2] Indeed, increasing evidence has suggested the presence of distinct clinical and biological phenotypes within critical illness syndromes in which differential treatment responses are expected.[2] The presence of significant heterogeneity within each syndrome, combined with the overlapping phenotypes among syndromes, suggests that study of common critical illness syndromes in an interrelated manner may improve the understanding of mechanisms that determine short- and long-term outcomes and enhance the identification of sub-phenotypes across syndromes.[2]

The overarching goal of the APS Consortium is to support the development of deeper mechanistic understandings of critical illness syndromes to facilitate precision-based therapies that will curtail the devastating morbidity and mortality caused by ARDS, pneumonia and sepsis.[1] The goals of the APS Consortium were established by the NIH/NHLBI in the request for applications (RFA) soliciting applications for the clinical centers and are described in detail at (https://grants.nih.gov/grants/guide/rfa-files/RFA-HL-23-001.html). In brief, the Consortium seeks to understand the heterogeneity and underlying mechanisms of critical illness syndromes and recovery in adults with ARDS, pneumonia, and/or sepsis, as well as the relationship and biological overlap among these syndromes, through a prospective, longitudinal observational study with common data and biospecimen collection. The scientific focus of the consortium is on identifying novel phenotypes of critical illness, describing the clinical and biological features that define these phenotypes, establishing their prognostic and clinical value, and identifying their fundamental mechanisms; in addition, as set out in the RFA, the Consortium will generate a richly characterized clinical dataset and biobank for future investigations.

The ultimate goal of this research endeavor is to advance our ability to deliver precision critical care by more precisely matching interventions to specific phenotypes that are more likely to respond to those interventions, following the precision medicine paradigm of "right drug, right patient, right time." At the end of the Consortium, we aspire to have identified tangible novel phenotypes with strong evidence for their specific mechanistic underpinnings and practical methods for identification in clinical practice so as

to lay the foundation for precision-targeted clinical trials. We anticipate that by combining careful clinical characterization according to currently accepted syndromic definitions with the novel deep molecular characterization will allow us to describe the relationship of novel phenotypes to existing syndromes, and potentially may refine clinical syndromes to those that are more biologically homogeneous.

By focusing on the recovery post-ARDS, pneumonia, and sepsis in a large and rigorously characterized population, we expect to inform clinicians about which baseline characteristics and dynamic features predict favorable or delayed recovery in specific domains, helping clinicians better prognosticate and helping future trials identify the highest risk patients to enroll for new tested interventions. Finally, we believe that some aspects of our clinical and/or molecular characterization for the APS consortium may display enough utility to spur development as clinically certified tests that are adopted into clinical care in the future.

To achieve these goals, the Consortium will conduct a longitudinal cohort study of approximately 4,000 adults hospitalized in the United States with ARDS, pneumonia and/or sepsis, and collect multidimensional data and biospecimens for up to one year from the time of index hospitalization. These data and biospecimens will be used both within the Consortium and by others to enhance our understanding of the mechanistic underpinnings of ARDS, pneumonia and sepsis.

5.2 Study objectives

5.2.1 Master protocol objectives

The central objective of Consortium-wide procedures described in this master protocol is to generate a deeply phenotyped cohort of participants with ARDS, pneumonia, and/or sepsis patients, and create a comprehensive, longitudinal biobank of specimens collected from these patients during acute illness and recovery that will be available to investigators both inside and outside the original APS Consortium investigator group.

5.2.2 Consortium-wide science objectives

The APS Consortium Investigators will use some of the data and biospecimens collected under the master protocol to complete scientific aims (Consortium-wide science). At initiation of this protocol, the APS Consortium Investigators have outlined the following 7 scientific aims they will complete. Details of each of these aims are contained in appendices to this protocol. Additional consortium-wide scientific aims may be added in the future via additional protocol appendices.

The initial 7 consortium-wide scientific aims are:

1. To determine and interrogate the role of microbiota (pathogen and microbiome) in the clinical and biological heterogeneity of ARDS, pneumonia, and sepsis, and their long-term outcomes.

2. To determine the host response phenotypes that most strongly associate with organ failure and 3, 6, and 12-month health status, and test the incremental utility of markers of vascular dysfunction, plasma DAMPs, and leukocyte expression for APS short- and long-term outcomes.

3. To test the hypothesis that sampling the airspaces of mechanically ventilated patients with ARDS, sepsis, and pneumonia will uncover novel APS phenotypes and endotypes and inform our understanding of central mechanisms that drive short- and long-term outcomes, including pulmonary dysfunction.

4. To determine trajectories of established phenotypes over time and utilize longitudinal data to identify novel phenotypes of APS during the acute phase and recovery.

5. To determine and interrogate the role of patient comorbidities, exposures, and biophysical constitution in the clinical and biological heterogeneity of ARDS, sepsis, and pneumonia.

6. To develop approaches for translating phenotypes to the bedside to enable follow-on precision clinical trials.

7. To determine whether APS phenotypes identify differences in multiple organ dysfunction syndrome (MODS).

6. Study Design

This is a multicenter observational cohort study enrolling participants with acute cardiovascular or pulmonary organ dysfunction in the context of ARDS, pneumonia, sepsis, or a condition at high risk to progress to one of these syndromes. Acute cardiovascular and pulmonary dysfunction are highly relevant clinical manifestations of ARDS, pneumonia, and sepsis, and hence the target of enrollment strategies. Targeted sample size for enrollment is 4,000 participants. Baseline and longitudinal molecular samples including blood, respiratory samples, circulating blood cells, urine, stool, and oral, nasal, and rectal swabs will be collected. Rich clinical data will be collected from the EHR, and participants will be asked to complete surveys and tests that will be used to phenotype multiple domains of health. Following discharge, participants will be asked to complete surveys and a subset will be asked to return for in-person assessments that will include biospecimen sampling (blood, stool, microbiome swabs) and assessments of physical, cognitive, and emotional health. Following the index hospitalization, participants will have up to 3 in-person study visits at 3, 6, and 12 months.

7. Participant Enrollment

7.1 Eligibility Criteria

7.1.1 Inclusion Criteria

To be eligible for enrollment, a patient must meet all the following inclusion criteria at the time of the first study-specified biospecimen collection (Time 0):

1. Age ≥ 18 years old.

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2. Admitted (or planned to be admitted) to an ICU or other in-patient hospital location where IV vasopressors or advanced respiratory support (invasive mechanical ventilation, non-invasive ventilation, or high flow nasal cannula) are routinely provided (referred to as an "eligible unit.")

3. Acute cardiovascular or pulmonary organ dysfunction defined by meeting at least one of the two criteria below:
   i) New receipt of invasive mechanical ventilation, non-invasive ventilation, high flow nasal cannula, or supplemental oxygen at a flow rate of $\geq$ 6 lpm for acute hypoxemia.
      → Patients who use chronic oxygen therapy are eligible to participate if they are receiving at least 6 lpm higher than their baseline oxygen requirement (e.g., a patient on 3 lpm O2 at baseline is eligible if they require ≥9 lpm for hypoxemia) or are started on advanced respiratory support (invasive mechanical ventilation, non-invasive ventilation, or high flow nasal cannula).
   ii) Receipt of intravenous infusion of a vasopressor medication for at least one hour.

4. Acute cardiovascular or pulmonary organ dysfunction (inclusion criterion #3) is attributed to an acute inflammatory condition, including but not limited to any of the following:
   i) Any infection including pneumonia.
   ii) Aspiration pneumonitis.
   iii) Pancreatitis.
   iv) Auto-inflammatory condition such as:
      a. Hemophagocytic lymphohistiocytosis.
      b. Suspected acute rheumatologic or auto-immune disease with pulmonary or cardiovascular manifestations.
      c. Suspected cryptogenic organizing pneumonia presenting acutely.
      d. Suspected diffuse alveolar hemorrhage.
      e. Suspected acute anaphylaxis.
      f. Suspected acute pulmonary drug toxicity.

7.1.2 Exclusion Criteria

To be eligible for enrollment, a patient must not meet any of the following exclusion criteria at the time of the first study-specified biospecimen collection (Time 0):

1. Patient/LAR declines participation.

2. Acute cardiovascular or pulmonary organ dysfunction (inclusion criterion #3) has been present for > 48 hours.

3. Patient has been in an eligible unit (inclusion criterion #2) for more than 120 hours (five days).

4. Patient is no longer expected to meet the acute cardiovascular or pulmonary organ dysfunction inclusion criterion (inclusion criterion #3) 24 hours after enrollment.

5. Patient desires comfort measures only.

6. Patient is a prisoner.

7. Patient had out-of-hospital cardiac arrest leading to this hospitalization.

8. Residence immediately before this hospitalization in a long-term acute care facility.

9. Presence of tracheostomy for respiratory failure.

10. Home invasive mechanical ventilation or non-invasive ventilation (except patients with non-invasive ventilation prescribed as a treatment for a sleep disorder may participate).

11. Suspected cause of the patient's acute cardiovascular and/or pulmonary dysfunction (inclusion criterion #3) is an alternative condition (not ARDS, pneumonia, or sepsis), including but not limited to the list below:

    i)   Drug overdose (without aspiration, lung injury, pneumonia, or infection).
    ii)   Trauma (without aspiration, pneumonia, or infection).
    iii)   Chronic lung disease without suspected infection, aspiration, or inflammation.
    iv)   Asthma, COPD, sarcoidosis, interstitial lung disease, neuromuscular respiratory failure.
    v)   Status epilepticus.
    vi)   Acute pulmonary embolism.
    vii)   Acute decompensated heart failure.
    viii) Diabetic ketoacidosis.
    ix)   Acute stroke or intracranial hemorrhage.
    x)   Acute bleeding (GI bleeding, post-procedural bleeding, hemolysis).
    xi)   Cytokine release syndrome due to chemotherapy.

12. Inability or unwillingness to complete study-specified blood draws, for example, due to local policies about hemoglobin thresholds for research blood draws.

7.1.3 Rationale for eligibility criteria

The goal is to enroll patients with acute cardiopulmonary dysfunction that is due to either (a) ARDS, pneumonia, or sepsis, or (b) an acute inflammatory condition that places patients at high risk for short-term development of ARDS, pneumonia, or sepsis. Patients with common critical care conditions that may result in acute cardiopulmonary dysfunction, but which have established pathophysiologic mechanisms distinct from ARDS, pneumonia, and sepsis will be excluded. This approach will optimize the ability to understand the pathophysiology of ARDS, pneumonia, and sepsis, whereas an approach that limited enrollment to patients who meet the historical syndrome definitions would limit our ability to identify new, meaningful phenotypes. In addition, this approach recognizes the difficulty in making definitive clinical diagnosis of historical syndromes (ARDS, pneumonia, and sepsis) at the time of enrollment among critically ill patients. By taking this approach to inclusion criteria, the APS Consortium is expected to further improve our ability to make diagnoses in real time.

Potential participants of all sexes, genders, races, ethnicities, language proficiencies, and nationalities are invited to participate. Enrollment in the cohort will not be limited by the language(s) spoken by the

participant. Individual components of APS studies, such as long-term outcome surveys, may be limited to participants who speak certain languages to ensure adequacy of data collection for those components. All adult patients across the age span are invited to participate; pediatric participants are not the focus of this project and will not be enrolled.

Pregnant individuals are eligible to participate in the APS cohort because pregnant patients experience APS syndromes at rates higher than the general population of similar age, and there is no strong rationale to exclude such participants. The risks of the procedures in this study are not greater for pregnant participants than for other participants. Due to potential risks to a fetus posed by study procedures that are greater than minimal risk, study procedures for pregnant participants will be limited to procedures that are not greater than minimal risk. The altered schedule of procedures for pregnant participants is detailed in the schedule of events section of this protocol.

Prisoners are excluded because there is concern that their participation may not be fully voluntary.

Patients who are unable to consent for themselves due to acute illness, cognitive impairment, or psychological impairment are eligible to participate. Details of inclusion of these patients are included in the Human Subjects section of this protocol.

Pediatric participants <18 years old are excluded because they are frequently admitted to different, specialized pediatric hospitals, the tools for assessing organ dysfunction and molecular phenotyping are different for children, and the focus of this study is on adults.

7.2 Anticipated Study Population

7.2.1 Enrollment targets

The APS Consortium investigators recognize that phenotyping efforts require adequate numbers of patients within specific groups for which phenotyping is desired. Additionally, care will be taken to promote representation among multiple sociodemographic groups. The APS Consortium recognizes three key priorities for representation: (1) sociodemographic representation, (2) distribution across the historical syndromes of ARDS, pneumonia, and sepsis, and (3) patients in whom deep respiratory samples are available (patients ventilated through an endotracheal tube).

The APS Consortium Steering Committee will regularly monitor participant characteristics of the overall cohort and the subset with in-person long-term outcome visits to promote adequate representation of several groups. The Steering Committee will work to achieve the following goals:

i.   Adequate race, ethnicity, and sex representation, including inclusion of participants reporting Black race or Hispanic ethnicity at or above percentages reported for these groups in US census data;
ii. Adequate representation from rural areas, based on urban-rural classifications by the US Census Bureau following the 2020 census. A rural area is defined as any area in the US outside the 2,646 recognized urban areas. An urban area is defined as a census area with >2,000 housing units

or >5,000 population. A list of urban areas is available upon request. US Census Bureau definitions of urban and rural are available at: https://www.census.gov/programs-surveys/geography/guidance/geo-areas/urban-rural.html;

iii. Adequate representation from the historical ARDS, pneumonia, and sepsis categories, with at least 1000 patients with ARDS, 2000 patients with sepsis, and 2000 patients with pneumonia in the overall cohort, recognizing that a given patient may count toward multiple categories;

iv. Adequate numbers of patients who received invasive mechanical ventilation in the overall cohort (target: ≥1000 patients) and long-term outcome subset (target: ≥150 patients).

The APS Steering Committee will review participant characteristics of the enrolled cohort overall and by site no less frequently than every 3 months (quarterly) during the enrollment period. This review will include data tables that display participant characteristics for the following categories:

- Sociodemographics, including race, ethnicity, and sex
- Home location (zip code used for rural/urban classification)
- Distribution of historical syndromes (ARDS, pneumonia, sepsis)
- Use of invasive mechanical ventilation during the index hospitalization

After reviewing these quarterly data, the APS Steering Committee will develop an action plan for enrollment for the next quarter (Table 3). Specific action plans will be developed in response to data on characteristics of participants enrolled during the last quarter and overall in the study, and the effect that prior action plans had on enrollment. Data tables showing participant characteristics and the APS Steering Committee action plan for enrollment in the next quarter will be submitted to the APS Observational Study Monitoring Board (OSMB) each quarter. After reviewing the data tables and action plan, the OSMB may issue further guidance and/or call an ad hoc meeting among the OSMB, NIH, and APS Consortium investigators to discuss and revise the action plan.

Table 3. Potential action plans to alter enrollment practices in response to observed quarterly enrollment data.

Specific action plans will be developed by the APS Steering Committee in response to data. Hypothetical examples are shown in this table.

Observed Data	Potential Action Plan
The proportion of participants with Black race is below the proportion of adults in the US census with Black race.	Provide study teams at sites with a high proportion of Black patients with additional training on consent practices and enrolling patients with alteration of informed consent procedures. If it is found that patients with Black race report an aversion to particular study procedures (such as stool samples or post-discharge surveys), the steering committee may consider dropping these study procedures. The APS Coordinating Center will work with the Clinical Center principal investigator for sites with a large population of Black patients to try to ensure funds are available for optimizing staffing of the enrollment teams at those sites. Adding sites to the consortium that care for a large number of Black patients could be an option if additional funds become available.
The proportion of participants with Hispanic ethnicity is below the proportion of adults in the US census with Hispanic ethnicity.	Provide study teams at sites with a high proportion of Hispanic patients with additional training on consent practices and enrolling patients with alteration of informed consent. Study materials, including consent forms and surveys, will be available in Spanish. The coordinating center will ensure study teams understand these Spanish language documents. If it is found that patients with Hispanic ethnicity report an aversion to particular study procedures (such as stool samples or post-discharge surveys), the steering committee may consider dropping these study procedures. The APS Coordinating Center will work with the Clinical Center principal investigator for sites with a large population of Hispanic patients to try to ensure funds are available for optimizing staffing of the enrollment teams at those sites, including the availability of Spanish interpreters. Adding sites to the consortium that care for a large number of Hispanic patients could be an option if additional funds become available.
The proportion of participants with a rural home zip code is below the proportion of adults in the US census with a rural home zip code.	Provide study teams at sites with a high proportion of rural patients with additional training on consent practices and enrolling patients with alteration of informed consent procedures. If it is found that patients from rural zip codes report an aversion to particular study procedures (such as stool samples or post-discharge surveys), the steering committee may consider dropping these study procedures. The APS Coordinating Center will work with the Clinical Center principal investigator for sites with a large rural population to try to ensure funds are available for optimizing staffing of the enrollment teams at those sites. Adding sites to the consortium that care for a large number of rural patients could be an option if additional funds become available.
Less than 25% of enrolled participants have ARDS.	Change eligibility criteria so fewer patients with mild lung injury and no lung injury are enrolled. Potential protocol changes include requiring hypoxemia for enrollment (removing the path for eligibility based on vasopressor use only without hypoxemia) and increasing the threshold for supplemental oxygen for eligibility (for example, increasing from 6 lpm to 10 lpm).

7.2.2 Anticipated enrollment by APS categories

Most patients enrolled according to the eligibility criteria of this study will have ARDS, pneumonia, and/or sepsis according to current diagnostic criteria for these syndromes at some point during the index hospitalization.[3-8] Eligibility criteria for this study require participants to have evidence of acute cardiopulmonary organ dysfunction, operationalized as receiving vasopressors, new substantial oxygen therapy (at least 6 liters per minute of supplemental oxygen over baseline), or new respiratory support (high-flow nasal cannula, non-invasive ventilation, or invasive ventilation).

Figure 1 displays anticipated enrollment numbers by syndrome. Importantly, there is substantial overlap among ARDS, pneumonia, and sepsis, such that many enrolled patients will have more than one of these syndromes. The number of participants in each category cannot be anticipated perfectly. Throughout the enrollment period of the study, the APS Consortium Steering Committee will issue guidance to enrolling sites to help ensure the final cohort includes at least 1000 patients with ARDS, 2000 patients with pneumonia, and 2000 patients with sepsis.

Eligibility criteria for this study limit enrollment of patients with pneumonia to those on vasopressors or newly treated with ≥6 liters per minute of supplemental oxygen or respiratory support. Therefore, nearly all patients enrolled with pneumonia are expected to have a Sequential Organ Function Assessment (SOFA) score ≥2 via cardiovascular dysfunction (vasopressor use) or respiratory dysfunction (P:F ratio <300),[9] and therefore also meet the Sepsis-3 criteria for sepsis.[8] The enrolled pneumonia cohort will include patients with pneumonia causing acute cardiovascular or respiratory organ dysfunction.

The most common cause of sepsis in this cohort is anticipated to be pneumonia. Patients with sepsis without pneumonia (non-pulmonary sepsis) meeting the eligibility criteria will also be enrolled. The enrolled sepsis cohort will include patients with sepsis causing acute cardiovascular or respiratory organ dysfunction, regardless of the etiology of sepsis. Patients who have suspected infection and a SOFA score ≥2 (a common operationalization of the Sepsis-3 definition) without acute cardiovascular or respiratory dysfunction will not be enrolled. Sepsis with a SOFA score ≥2 can occur without cardiovascular or respiratory dysfunction if organ dysfunction is present in the central nervous system (GCS ≤12), coagulation system (platelets <100,000 /mcl), liver (serum total bilirubin ≥2.0 mg/dl), or kidneys (creatinine ≥2.0 mg/dl).

Patients with ARDS meeting study eligibility criteria will be enrolled regardless of whether the etiology of ARDS is pneumonia (anticipated to be most common), non-pulmonary sepsis (anticipated to be second most common), or an insult other than infection (anticipated to be least common).

Figure 1. Anticipated overlap of ARDS, pneumonia, and sepsis among participants enrolled in the APS Consortium cohort study. Displayed numbers are projections based on published cohorts and investigator experience; actual enrollment numbers may differ and will be followed closely throughout the recruitment period to ensure adequate numbers of patients with ARDS, pneumonia, and sepsis.

Projected Enrollment (n)
- Total participants = 4000

Enrollment by syndrome (not mutually exclusive):
- ARDS: 1500
- Pneumonia: 2750
- Sepsis: 3750

Enrollment by mutually exclusive category:
- ARDS + Pneumonia + Sepsis: 1000
- ARDS + Pneumonia (w/o sepsis): 0
- ARDS + Sepsis (w/o pneumonia): 250
- Pneumonia + Sepsis (w/o ARDS): 1750
- ARDS alone: 250
- Pneumonia alone: 0
- Sepsis alone: 750
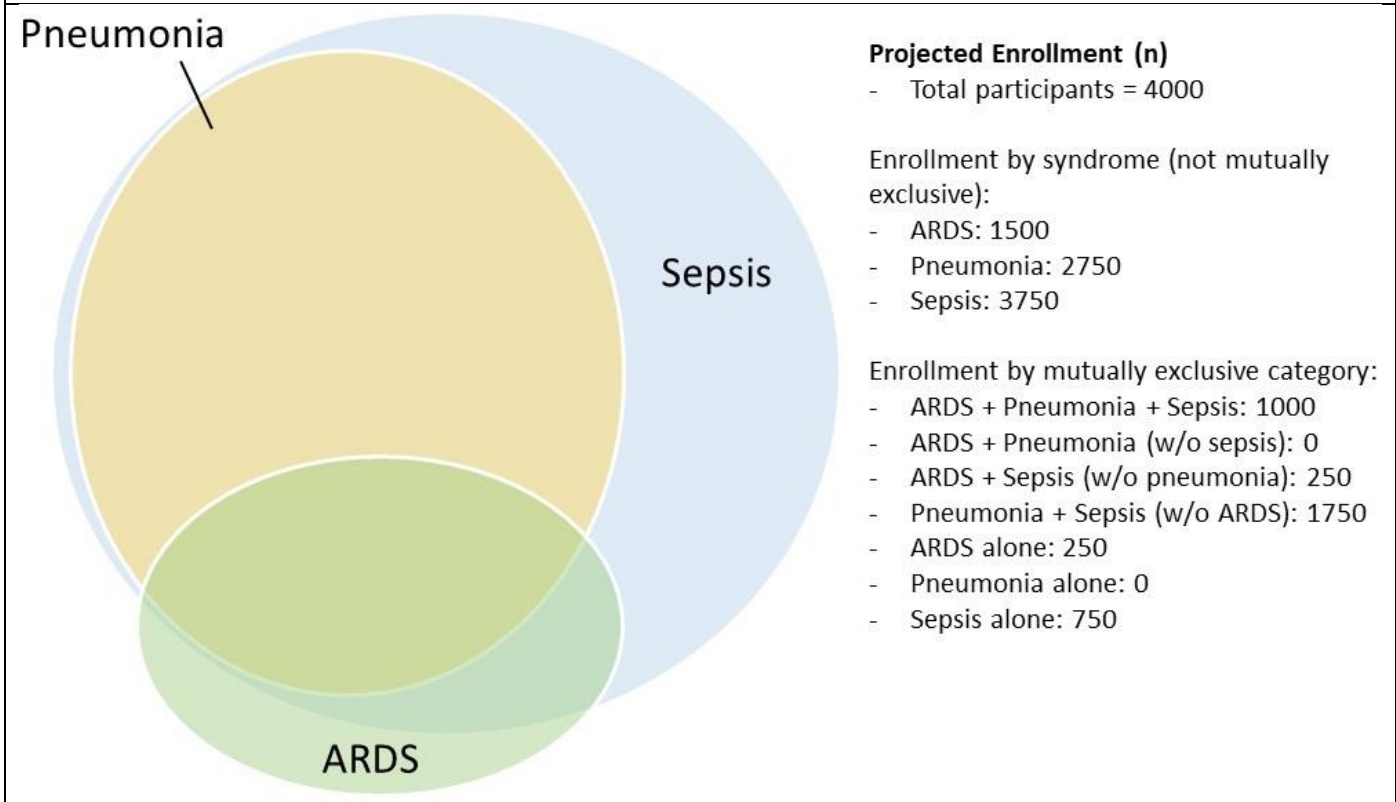
7.3 Definition of Enrollment Time Zero

The calendar day of the first study-specified biospecimen collection is defined as "Day 0." The study schedule of events follows "study day" nomenclature, with enrollment day (the day of the first biospecimen collection) identified as Day 0. Study Day 1 is the calendar day following enrollment. Study Day -1 is the calendar day before enrollment. In some circumstances, consent for participation may be obtained after Day 0 (such as on Day 1) or prior to Day 0 (such as Day -1).

Time zero for enrollment is defined as the time of the first biospecimen collection for the study, regardless of the timing of hospital admission, consent, and development of ARDS, pneumonia, or sepsis. A patient is considered enrolled when the first study-specified biospecimen collection occurs. Time stamps will be collected for key events, such as hospital admission, meeting study eligibility criteria, and collection of biospecimens, so that analyses can evaluate the time between these events.

8. Study Procedures During Index Hospitalization

8.1 Data collection during index hospitalization

Data collection approaches within the APS Consortium are designed to optimize: (a) participant experience; (b) the scientific integrity of the Consortium-wide scientific aims; and (c) a robust and useable biobank for future research that is consistent with applicable regulations, guidance, and ethical norms.

8.1.1 Participant/surrogate interview

Attempts will be made to interview enrolled participants (or a surrogate) during the index hospitalization. The purpose of the interview (which may be performed with the participant's surrogate if the participant is unable to participate in the interview) is to obtain contact information (which has been demonstrated to improve cohort retention), to assess baseline health and health behaviors, to assess race/ethnicity and skin tone, to understand social drivers of disease at baseline, and to assess for preexisting comorbidities and frailty. Domains of data collection via interview include:

- Contact information of patient and family members
- Skin color (Monk skin tone scale)
- Self-assessment of overall health (5-point Likert scale from Health & Retirement Study)
- Smoking status (current/former/never; pack-years)
- Alcohol use (AUDIT-C)
- Health-related quality of life (EQ-5D-5L)
- Frailty (Clinical Frailty Scale)
- Functional Limitations (Katz ADL/Lawton IADL)
- Disability (WHODAS-12)
- Employment status (ICAP-Revised)
- Cognitive function (IQ-CODE)
- Social isolation (NHATS-6)
- Engagement with healthcare (National Health Interview and All of Us Surveys)
- Education (Health and Retirement Study)
- Housing instability (Council of State Governments)
- Current/former opioid misuse (ARDS Network Long Term Outcomes Study)

8.1.2 Medical record data abstraction

Medical record data abstraction, elements of which may be confirmed via patient/surrogate interview, may occur at varying times during the hospitalization based on operationalization at a given site. These data, whether extracted at the time of a given study visit or near or after hospital discharge, will be tied to

the schedule of events. Features of data abstraction will be extracted to correspond with the baseline visit as well as subsequent visits. Domains of data collection via medical record review include:

- Contact information of patient and family members, including home address and type of residence
- 9-digit zip code. This allows for linkage to other datasets to capture measures of social vulnerability (e.g., Social Vulnerability Index, Social Deprivation Index, Area Deprivation Index), as well as healthcare availability and rurality. Note: to avoid risk of re-identification of study subjects, zip code will be stripped from shared datasets; data on social vulnerability, healthcare availability, rurality, and other factors obtained by linkage to 9-digit zip code will be retained as categorial variables in shared datasets.
- Demographics
- Home medications
- Biometrics (height, weight)
- Language fluency
- Place of residence
- Chronic health conditions (including tobacco use, alcohol misuse, and opioid misuse)
- Health insurance status
- Prior hospitalizations
- Clinical laboratory results, medications, and vital signs, including SOFA score elements
- Therapies administered (including respiratory support therapies)
- Discharge disposition

8.1.3 Acute Respiratory Distress, Pneumonia, and Sepsis Syndrome Classification

Enrolled participants will be evaluated for ARDS, pneumonia, and sepsis utilizing detailed, investigator driven review and application of published and accepted clinical criteria for each syndrome. To evaluate for ARDS, pneumonia, and sepsis, investigators will evaluate data and the clinical record for each participant for Study Day -2 through Day 7 while the patient is hospitalized (the phenotyping observation window). Individual criteria for each syndrome will be collected and recorded separately allowing for patients to be classified as ARDS, Sepsis, and/or Pneumonia using multiple published criteria (e.g., for sepsis using both "sepsis-2" and "sepsis-3" criteria). These data will then be used to code variables in our dataset that classify patients according to syndrome definitions. Standard operating procedures will be provided to each site to ensure accuracy and consistency of syndrome adjudication.

ARDS will be identified based on the Berlin Definition with the additional modifications proposed in the New Global Definition of ARDS.[3,4] Trained physician investigators will determine the presence of potential precipitating factors for ARDS (e.g., sepsis, aspiration, pancreatitis). Chest radiographs and chest computed tomography scans conducted for clinical purposes during the observation window will be reviewed by trained physician investigators for bilateral infiltrates consistent with ARDS. The type of chest imaging, time and date of imaging acquisition, and bilateral infiltrates present, absent, or equivocal will be recorded in case report forms. The presence or absence of pleural effusions will also be recorded. Arterial blood gas (ABG) values drawn for clinical purposes and corresponding fraction inspired oxygen percentage (FiO2) will also be recorded in case report forms. If an ABG is unavailable on a given day,

SpO2 and corresponding FiO2 will be recorded to calculate the S/F ratio. Invasive and non-invasive oxygenation and ventilation parameters will also be extracted from the medical record. We will apply strict physiologic criteria in ARDS definitions to determine the presence or absence of ARDS daily, timing when ARDS criteria are met, and the severity of ARDS based the level of hypoxemia (P:F and S:F ratios).

Participants will be assessed for the presence of pneumonia based on radiographic, clinical, and laboratory criteria, including those published by CDC and the Infectious Disease Society of America/American Thoracic Society.[5,6] Chest imaging conducted for clinical purposes during the phenotyping observation window will be reviewed by trained physician investigators for new/persistent/progressive infiltrates, consolidation, or cavitation consistent with a pneumonia event. Clinical signs and symptoms potentially indicating a respiratory infection will be identified, including vital signs, laboratory results, and clinical status. Assessment of clinical status will include: 1) new onset purulent sputum or change in character of sputum, increasing respiratory sections, or increasing suctioning requirements, 2) new onset or worsening cough, dyspnea, or tachypnea, 3) rales or bronchial breath sounds, and 4) worsening gas exchange. These data will form the core dataset for classifying the presence and absence of pneumonia.

Sepsis will be identified based on the Sepsis-2 and Sepsis-3 definitions of sepsis, severe sepsis and septic shock.[7,8] Trained physician investigators will review the medical record to determine if an infection is confirmed, suspected, not suspected, or unknown daily for the observation window. The presence of positive culture will not be required to identify the confirmed or suspected presence of an infection; however, positive cultures can be used to help determine the presence of sepsis. Suspected sources of infection will be adjudicated by the physician investigators. The physiology of sepsis and septic shock will be classified based on the change in Sequential Organ Function Assessment (SOFA) scores, the presence or absence of the systemic inflammatory response (SIRS) criteria, the presence or absence of vasopressors, and values for lactate. These data will be used to facilitate sepsis classification by the investigator adjudicators.

8.2. Biospecimen collection during index hospitalization

Aliquots of biospecimens collected on this research protocol may be sent for clinical laboratory testing if such testing is judged to be of potential benefit to the patient. Clinical laboratory testing is optional and may be completed at the discretion of the local site team. If clinical laboratory testing is completed, results may be delivered to clinicians caring for the patient according to local practice patterns.

8.2.1 Blood specimen collection during index hospitalization

During the index hospitalization, blood will be collected from patients at multiple timepoints, collected in EDTA or sodium citrate tubes (for protein or metabolite measurements), PAXgene tubes (for nucleic acids), CPT tubes (for peripheral blood mononuclear cells), and sodium heparin tubes (for whole blood CyTOF and flow cytometry). Blood will be collected at the timepoints indicated in the schedule of events for biospecimen collection (Section 10). Participants known to be pregnant will only have blood collected

during the index hospitalization on Day 0 and Day 2, ensuring <50 ml of blood volume collected in any 8-week period and ≤2 blood draws in any 1-week period for research purposes, which is considered to be no greater than minimal risk in guidance from the Office Human Research Protections (OHRP) [https://www.hhs.gov/ohrp/regulations-and-policy/guidance/categories-of-research-expedited-review-procedure-1998/index.html].

8.2.2 Respiratory specimen collection during index hospitalization

During the index hospitalization, the following respiratory specimens will be collected: 1) nasal swabs to facilitate both microbiome profiling and pathogen detection, 2) oral swabs, 3) tracheal aspirate (TA) from patients receiving invasive mechanical ventilation, 4) heat moisture exchanger (HME) filter fluid from patients receiving invasive mechanical ventilation, 5) non-bronchoscopic bronchoalveolar lavage (NBBAL) fluid among a subset of patients receiving invasive mechanical ventilation.

Description of the NBBAL procedure in this study:
The NBBAL procedure will be completed in a subset of participants undergoing invasive mechanical ventilation after a safety screen for the procedure (see below) and consent is obtained. The NBBAL will be performed as soon as possible (and no later than 96 hours) after initiation of invasive mechanical ventilation. The NBBAL procedure will entail a catheter being inserted into the endotracheal tube and the catheter tip being wedged into a distal airway. Serial aliquots of sterile saline are instilled and aspirated with gentle suction (maximum volume instilled, 120ml). The aspirated fluid will be collected and processed as a biospecimen for the study.

Safety Screen for NBBAL in this study:
Participants meeting any of the following criteria will not undergo NBBAL for research purposes in this study:

1. Known pregnancy
2. FiO2 ≥ 0.80.
3. FiO2 increased by > 0.1 in the prior hour.
4. PEEP ≥ 12 cm H2O.
5. PEEP increased by > 2 cm H2O in the prior hour.
6. Intracranial pressure > 15 or known labile intracranial pressure.
7. Open external ventricular drain (EVD).
8. Known pneumothorax.
9. Uncontrolled shock or rapidly escalating vasopressor requirements.
10. Unstable dysrhythmia.
11. Scheduled bronchoscopy.
12. Current therapeutic anticoagulation.
13. Known INR > 2.0.
14. Platelets < 50,000 /mcL.
15. Frank hemoptysis.
16. Partial lung resection or pneumonectomy.
17. Known pulmonary hypertension with pulmonary artery systolic pressure (PASP) >55 cm H2O.
18. Expected extubation attempt in the next 6 hours.
19. Clinical provider or site investigator judgement that procedure would not be safe.

8.2.3 Other specimens during the index hospitalization

Participants will have rectal swabs and stool samples serially collected for the primary purpose of microbiome profiling and urine collected for future use.

8.3. Imaging studies during index hospitalization

During the index hospitalization, investigators will review chest imaging completed clinically during the observation window for clinical phenotyping (Study Day -2 through Day 7) and enter key findings into the study data collection forms. Additionally, as detailed in the schedule of events section, serial chest x-ray (CXRs) and CT scan images that were performed as part of the participant's clinical care will be collected and uploaded to a common, secure site, with eventual deposition into BioData Catalyst. The initial goal is to upload up to 3 CXRs and one CT scan (chest CT preferred, abdominal CT acceptable when no chest CT is available) completed during the index hospitalization as part of clinical care. The number of images uploaded per patient may change over time. These stored images will be used by the APS investigators and will be available in the APS databank for future work.

9. Study Procedures during Post-Hospital Assessments

9.1 Schedule of post-hospital study assessments

As specified in the Post-Hospital schedule of assessments (**Error! Reference source not found.**, in S chedule of Events section below), multiple study procedures will be used to collect data on participants following the hospitalization in which patients were enrolled in this study. Data collection timepoints will be approximately 3, 6, and 12 months after enrollment. Data will be collected post-hospitalization remotely for all surviving patients, and a subset of patients will undergo additional study procedures via in-person visits at the site where they were enrolled, a nearby participating site, or at home.

Post-hospital procedures include:
1. Chart reviews
2. Short message service (SMS) ("text")/Email follow-up surveys
3. Telephone follow-up surveys (for patients who do not complete SMS/Email surveys)
4. In-person assessments/biospecimen collection/imaging in approximately 600 patients
5. At-home stool collection in approximately 600 patients

9.2 Data collection during post-hospital study visits

9.2.1 Post-hospital medical record reviews

Personnel will complete a medical record review that includes data generated after the hospitalization in which the patient was enrolled. These chart reviews will be collected at approximately 3, 6, and 12 months after enrollment. The chart review will include review of the site's electronic health record, as well as any other health records viewable. The 3-month chart reviews will be completed for all patients who were discharged alive. The 6-month and 12-month chart reviews will be completed for participants not known to be dead through the 3-month chart review and 6-month chart review, respectively. Key data collected by chart review will include:

1. Mortality (yes/no); if yes: date of death
2. Re-hospitalization: if yes: Hospitalizations since last contact (capture admit date, discharge date, admitting diagnosis, ICU admission (yes/no), invasive mechanical ventilation(yes/no) for each admission

In addition to the chart review, personnel will conduct a Google search for public obituary information to confirm status for any patients not identified as deceased in the medical record and not reached for follow-up.

9.2.2 Email/SMS/Telephone Surveys

Following the chart review at each follow-up time point (3, 6, and 12 months), patients who have provided consent for study participation themselves (not through a surrogate or via alteration), are not known to be dead and not in hospital will be sent a link by email or SMS ("text") message to a REDCap survey. The survey will be available in English and Spanish.

Patients who do not complete the online survey, either due to no response or because they did not previously provide consent themselves for study participation, will be contacted by telephone by study personnel. During this phone contact, patients who had not provided consent for study participation themselves (that is, who had been participating via surrogate consent or alteration of informed consent) will be given an opportunity to consent for the study themselves (see section 13.2.4). After consent procedures, surveys for data collection will be administered over the phone. Only patients who provide consent for themselves will be asked long-term outcome survey questions. If the patient lacks capacity for consent at the 3-month phone call, and a surrogate is available, a limited battery of questions may be administered to the surrogate over the phone. Survey phone calls will be completed in English or Spanish.

For each survey completed, patients may be reimbursed by the local Clinical Center/site in compensation for their participation (suggested amount: $40). Reimbursement may be provided by gift card, electronic gift card, cash, or ClinCard, as determined by the local Clinical Center/site.

Surveys will include the following assessment domains/measures:

1. Reconfirm Contact Info: Email, Cell Number (Telephone survey only)
2. Pregnancy status (not known to be pregnant vs known to be pregnant)
3. Health related quality of life (EQ5D-5L)
4. Mental health, PTSD (IES-6)

5. Mental health, anxiety/depression (HADS)
6. Feedback questions related to ImproveLTO core outcome set surveys [www.improveLTO.com]
7. Recovery from acute illness (1-item Recovery Question from Long-COVID COS)
8. Dyspnea/pulmonary function (mMRC dyspnea scale)
9. Frailty (Clinical frailty scale)
10. Functional limitations: Abridged I/ADL limitations (toileting, transferring, feeding, telephone, med management, financial management)
11. Disability (WHODAS 2.0, 12-item)
12. Cognitive decline (PROMIS Cognitive Function Short Form 8a, 8-item)
13. Return to work (4 custom questions, used in prior research)
14. Financial toxicity (3 custom questions, used in prior research)
15. Health insurance (1 custom questions, used in prior research)
16. Healthcare utilization (Hospitalizations since last contact (capture admit date, discharge date, admitting diagnosis, ICU admission (yes/no), mechanical ventilation via endotracheal tube (yes/no) for each admission)
17. Housing instability (2 questions, from NHIS and All of Us Survey)

9.2.3 Mental health safety plan

Our mental health safety plan follows standard procedures used in prior cohort studies with longitudinal telephone follow-up. We will not directly ask patients about suicidality as part of the surveys, but there may be rare instances where patients express thoughts about suicide. In these instances, study personnel will direct participants to contact their psychiatrist or primary care provider (if available) or instruct them to call the National Suicide Line (988), which will connect them with local resources. Such instances will be discussed with the local investigator overseeing long-term follow-up. Additionally, the APS Consortium will have an on-call clinician who has experience with mental health emergencies. For a high-risk situation where there is uncertainty about the appropriate next steps, the local site investigator and study team can contact the on-call clinician for guidance. The on-call clinician can also do remote well checks.[10-14] Study personnel conducting phone surveys will be trained to assess suicide risk using the Columbia Suicide Severity Rating Scale.[15-19]

9.2.4 Post-hospital in-person visits

Expanded biospecimen, imaging, and data collection will be completed for a subset of enrolled participants. Post-hospital in-person visits may occur in a variety of settings, including but not limited to a hospital, clinic, or the participant's home. Participants who live within a reasonable drive time (to be determined by the local site) of a follow-up visit location and are fluent in either English or Spanish will be eligible to participate in in-person follow-up visits. Participating in the in-person long-term outcome visits is limited to patients fluent in English or Spanish because information gathered during these visits will be paired with survey data and the participating sites have capacity to administer the surveys only in English or Spanish. Enrollment for in-person follow-up will close after approximately 600 patients have completed a 3-month in-person follow-up visit.

The first 600 participants to complete a 3-month in-person study visit will constitute the in-person long-term outcome cohort. The 600-participant in-person long-term outcome cohort will be filled on a "first come" basis with eligible, consenting participants.

Eligibility for the in-person long-term outcome cohort:

1. Enrolled in the APS Consortium Study during the index hospitalization (all participants in the in-person long-term outcome cohort must also be in the primary APS study cohort).
2. Survived to hospital discharge
3. Fluent in English or Spanish
4. Live within a reasonable distance of the in-person follow-up visit location to enable completion of 3 in-person visits without undue burden on the participant or study personnel (the threshold distance will be defined at each site based on local context)
5. Consent for the primary APS Study, including the long-term outcome surveys (informed consent document #1)
6. Consent for the in-person long-term outcome follow-up visits (informed consent document #2)

Study Procedures at in-person long-term outcome follow-up visits:

In-person follow-up will include the following assessments at 3, 6, and 12 months:

1. Short physical performance battery (SPPB), which includes balance test, timed 4-meter walk test, and chair stand test
2. Handgrip strength (dominant hand only; unless that hand is impaired)
3. CNS-Vital Sign (computerized test that assesses cognitive function)
4. Dominant hand side quadriceps muscle ultrasound for cross-sectional area and muscle thickness with banking of the DICOM images (at a subset of sites due to specialized equipment and training needs)
5. Dominant hand side quadriceps muscle strength using dynamometer (at a subset of sites due to specialized equipment and training needs)

Additionally, in-person follow-up will include the following assessments at 12 months only:

1. Spirometry and DLCO (lung function tests) in patients who have not had clinically obtained spirometry and DLCO in the prior 6 weeks.
2. Chest CT scan (see section 9.4)

The local clinical center/site may provide reimbursement to patients for completing in-person visits (recommended amounts: $200 for the 3-month and 6-month visit, and $250 for the 12-month visit). Reimbursement will be provided by the clinical center/site and may be dispensed as a gift card, electronic gift card, ClinCard, or cash, as determined by the clinical center/site.

9.3 Biospecimen collection during post-hospital study visits

Biospecimen collection will occur during in-person follow-up visits. Specimen collections are described below.

9.3.1 Blood specimen collection during post-hospital study visits

Blood will be collected from patients in plasma EDTA tubes (for protein measurements) and PAXgene tubes (for RNA extraction and transcriptomics) at the 3-, 6-, and 12-month post-hospital study visits. Blood will also be collected in CPT tubes (for peripheral blood mononuclear cells) at the 3-month follow-up visit.

9.3.2 Respiratory specimen collection during post-hospital study visits

Nasal and oral swabs will be collected at the 3-, 6-, and 12-month post-hospital study visits.

9.3.3 Other specimen collection during post-hospital follow-up

Patients participating in the in-person visits and 3-months, 6-months, and 12-months will be asked to provide a stool sample for each visit. These stool samples may be collected at the time of the in-person visit or collected by the patient at home and shipped to the study team. For patients who prefer to collect stool at home, the study team will provide a home collection kit and return mailer.

9.4 Imaging studies during post-hospital study visits

At the 12-month follow-up visit, patients will undergo a study-specified thin-cut chest CT scan. This CT scan will be obtained on study protocol and funded with study budget. (Patients who have had a similar CT scan completed in the prior 6 weeks will have the clinically obtained scan uploaded rather than undergoing an additional CT scan.) Patients who are pregnant at the time of the 12-month visit will not undergo the research CT scan. Pregnancy testing prior to the 12-month CT scan will be undertaken according to the relevant policies and/or practices of the radiology center performing the CT scan. This 12-month visit research CT scan may be completed on the same day as other 12-month visit study procedures or on another day within the 12-month visit time window. The CT scans will be interpreted by radiologists. Site investigators will be responsible for disclosing clinically important findings on these research-dedicated CT scans to participants.

Additionally, patients who complete the 6-month and/or 12-month follow-up visit will have up to 1 clinically obtained CT scan after hospital discharge uploaded into the study database. A chest CT scan is preferred. In the absence of a chest CT, an abdominal CT scan can be used. If multiple clinically obtained CT scans are available, the scan closest to 6 months will be used.

Images from both the study-specified and clinically obtained scans will be uploaded to the study database for banking and future use.

10. Schedule of Events

Study procedures, including collection of data, biospecimens, and radiographic images, are detailed in tables within this section.

Table 4. Schedule of Events: Data collection for in-hospital component of the study.

Event [a]	Time Point					
	Study Day (in-hospital only)				**At Discharge**	**Hospital Summary**
	-2	**-1**	**0** [b]	**1 - 7**		
A. Eligibility and Baseline Data						
Eligibility Criteria			X			
Informed consent (main study)			X [c]			
Informed consent (LTO in-person visits)					X [d]	
Baseline History			X			
Demographics			X			
Chronic Medications			X			
Admitting Diagnoses			X			
B. Participant/Surrogate Interview [e]						
Contact Information			X			
Skin Color			X			
Baseline Health Assessments			X			
Alcohol Use			X			
Smoking Status			X			
C. Data Collection						
Daily Data						
- Vital Signs	X	X	X	X		
- Laboratory Values	X	X	X	X	X	
- Ventilator Data	X	X	X	X		
- Select Medications	X	X	X	X	X	
- Fluid Balance	X	X	X	X		
- Cumulative IV Sedation [f]	X	X	X	X		
- Sedation/Delirium	X	X	X	X		
- Blood Products	X	X	X	X		
APS Classification	X	X	X	X		
Outcome Data						
- Mortality						X
- Discharge location					X	
- Organ Failures	X	X	X	X	X	X
- ECMO / Prone position						X

Table 4 Footnotes

a. The data collection schedule of events displays the study days from which data will be collected. The collected data will reflect patient status on those study days. The data may be collected later in a respective fashion.

b. Study Day 0 is anchored on when the first study-specific biospecimen is collected. The time of first biospecimen collection is termed Time 0 and denotes the time a patient is enrolled into the study. Per study eligibility criteria, enrollment must occur within 48 hours of meeting inclusion criteria.

c. Informed consent for the main study is listed in this table as a Day 0 procedure because most participants are expected to have informed consent obtained on Day 0. However, patients who enter the protocol on Protocol B using alteration of informed consent procedures may have consent obtained on a later day.

d. Informed consent for the long-term outcome in-person visits is a separate consent process from consent for the main study. Informed consent for long-term outcome visits is listed as a hospital discharge procedure in this table because this consent is anticipated to be completed prior to hospital discharge for many patients. However, this consent may be completed at any time prior to initiation of in-person long-term outcome visit study procedures, including after hospital discharge. Long-term outcome surveys, which do not require in-person visits, may be completed without separate consent for the in-person long-term visits.

e. The participant/surrogate interview aims to obtain information about participants' pre-hospital/baseline health and can occur at any time during index hospitalization, with completion as soon as possible after enrollment preferred.

f. Cumulative amounts of continuous IV administration of select sedation agents will be collected from Study Day -2 to 7.

Table 5. Scheule of Events: Timing of post-hospital study visits**.**

Event	Study Day window for completing Event, by time point		
	3-month	**6-month**	**12-month**
A. Events for all survivors			
Medical record review	Day 53-67	Day 143-157	Day 329-337
SMS/email survey invite	Day 68-70	Day 158-160	Day 338-340
SMS/email reminder 1	Day 75-77	Day 165-167	Day 345-347
SMS/email reminder 2	Day 82-84	Day 172-174	Day 352-354
Telephone survey	Day 85-135	Day 175-240	Day 360-450
B. Events for LTO in-person subset			
In-person visit (details in Table 6)	Day 75-135	Day 150-240	Day 330-450
Stool collection (mailer, if needed)[a]	Day 75-135	Day 150-240	Day 330-450

Table 5 Footnotes

a. Stool will be collected from participants at in-person visits at 3 months, 6 months, and 12 months. Stool may be collected at the visits or stool can be mailed by the participant to the consortium coordinating center.

Table 6. Schedule of Events: Data collection during post-hospital long-term outcome assessments.

Event	Time Point (see Table 5 for windows for each event)		
	3-month	**6-month**	**12-month**
A. Chart review			
Mortality	X	X	X
Re-hospitalization	X	X	X
B. Participant survey (online or phone) [a]			
Pregnancy status	X	X	X
Health related quality of life	X	X	X
Mental health, PTSD	X	X	X
Mental health, anxiety/depression	X	X	X
Feedback questions related to ImproveLTO core outcome set surveys	X	X	X
Recovery from acute illness	X	X	X
Dyspnea/pulmonary function	X	X	X
Frailty	X	X	X
Functional limitations	X	X	X
Disability	X	X	X
Cognitive decline	X	X	X
Return to work	X	X	X
Financial toxicity	X	X	X
Health insurance	X	X	X
Healthcare utilization	X	X	X
C. In-person assessments [b]			
Short physical performance battery	X	X	X
Handgrip strength	X	X	X
CNS-Vital Sign	X	X	X
Dominant hand side quadriceps muscle ultrasound [c]	X	X	X
Spirometry [d]			X
DLCO [d]			X
Chest CT scan [e]			X (not completed on pregnant participants)

Table 6 Footnotes

a. All surviving patients will be sent a link to complete the survey online, patients who do not complete the online survey within 3 weeks of receiving the link will be contacted via telephone to complete the survey through a phone interview.

b. Enrollment for in-person follow-up will close after approximately 600 patients have completed a 3-month in-person follow-up visit.

c. Quadriceps ultrasound and strength testing will be conducted at only a subset of sites due to requirements for specialized equipment and in-person training.

d. Spirometry and DLCO will only be collected in patients who have not had clinically obtained spirometry and DLCO in the 6 weeks prior to the 12-month visit.

e. A chest CT scan will only be completed in patients who have not had a clinically obtained chest CT scan in the 6 weeks prior to the 12-month visit. CT scans will not be completed for research purposes on participants known to be pregnant.

Table 7. Schedule of Events: Biospecimens collected during index hospitalization and long-term follow-up visits for participants who are <u>not known to be pregnant</u>.

This table shows the maximum number and volume of biospecimens collected per participant. All biospecimens will not be collected on all participants.

Participants Not Known to be Pregnant

Event	Time Point								
	Index Hospitalization (Study Day)						Long-Term Visits (Study Month)		
	Day 0[a]	NBBAL [b]	Day 2	Day 4[c]	Day 6	Day 14	3 mons	6 mons	12 mons
Acceptable study days for collection	Day 0-1	+96 hrs from intubation	Day 2-3	Day 3-5[c]	Day 6-8	Day 14-17	Day 75-135	Day 150-240	Day 330-450
Max Total Blood Volume (ml)[d]	33.2	16	15.2	6	27.2	8.5	28.5	12.5	12.5
- EDTA tubes (total mL)	2x 6ml (12)		1x 10ml (10)	1x 6ml (6)	1x 6ml (6)	1x 6ml (6)	1x 10ml (10)	1x 10ml (10)	1x 10ml (10)
- Extra EDTA tube if CPT not collected (total ml)[e]	1x6ml (6)				1x6ml (6)		1x6ml (6)		
- RNA Paxgene tubes (total mL)	1x 2.5ml (2.5)		1x 2.5ml (2.5)		1x 2.5ml (2.5)	1x 2.5ml (2.5)	1x 2.5ml (2.5)	1x 2.5ml (2.5)	1x 2.5ml (2.5)
- CPT tubes (total mL)[f]	2x 8ml (16)	2x 8ml (16)			2x 8ml (16)		2x 8ml (16)		
- Na citrate tubes (total mL)	1x 2.7ml (2.7)		1x 2.7ml (2.7)		1x 2.7ml (2.7)				
- Buffy coat from EDTA	From EDTA								
- RBCs	From EDTA						From EDTA	From EDTA	From EDTA
Urine tubes (total mL)	1x 5ml (5)								
Oral swab	x		x		x		x	x	x
Nasal swab	x		x		x		x	x	x
Rectal swab	x		x		x				
Stool	x		x		x		x	x	x
HME filter fluid[g]	x		x	x	x	x			
Tracheal aspirate[g]	x		x	x	x	x			
NBBAL[g]		x							

Table 7 Footnotes

a. Study Day 0 is anchored as the calendar day when the first study-specified biospecimen is collected. By study eligibility criteria, this must occur within 48 hours of the patient meeting eligibility criteria. The first biospecimen collected will, by definition, be collected on Day 0. Other biospecimens scheduled for collection on Day 0 can be collected as late as Day 1.

b. The NBBAL time point is anchored on the time of completion of a research-dedicated NBBAL and may occur on any study day between Study Day 0 and Study Day 8. The NBBAL time point specimens should only be collected once and should be collected as soon as possible after intubation and the patient passes the NBBAL safety screen. Two specimen types are collected at the NBBAL time point: NBBAL fluid and CPT tubes (two 8 ml tubes). The window for collecting NBBAL fluid is between intubation and 96 hours later. Do not complete a NBBAL more than 96 hours after intubation. The CPT collection should occur as soon as possible after NBBAL fluid collection. For the NBBAL time point, only collect CPT tubes for patients who have NBBAL fluid collected. CPT collection for the NBBAL time point is in addition to the CPT tubes collected for other time points. The NBBAL time point may occur on the same study day as specimen collection for other time points. For example, if a patient has a NBBAL completed on Day 0, the specimens for both Day 0 and the NBBAL time point may be collected on Day 0, which would result in the collection of four 8ml CPT tubes on Study Day 0.

c. Day 4 specimens are only collected from participants on invasive mechanical ventilation (IMV). Day 4 specimen collection is preferred on Study Day 4 or 5 but may be collected on Day 3 if Day 2 specimens were collected on Day 2 (instead of Day 3).

d. The approach to blood volume collection is governed by 3 principles: collection of <50 mL combined on Day 0 and Day 2 to facilitate minimal risk blood collection volumes through day 2 in the alteration of informed consent protocol (Protocol B); collection of < 150 mL total blood volume during the index hospitalization; collection of <50 mL per timepoint at long-term outcome visits. The maximum blood volume listed in this table is the maximum volume any one participant will have taken. The volume of blood collected is variable among participants but will not exceed the volumes listed here.

e. Participants who do not have blood collected for cellular analyses (CPT tubes) will have an additional 6 ml tube of EDTA collected at the study visit on Day 0, Day 6, and 3 months. Participants who do have blood collected for cellular analyses (CPT) will not have this extra tube of EDTA collected.

f. During processing of CPT tubes, plasma will be saved and stored when possible.

g. HME filter fluid, tracheal aspirate fluid, and NBBAL only collected from participants on invasive mechanical ventilation (IMV). For additional information about NBBAL specimen collection, please see footnote b.

Table 8. Schedule of Events: Biospecimens collected during index hospitalization and long-term follow-up visits for participants who are <u>known to be pregnant</u>.

Compared to the biospecimen collection schedule for non-pregnant participants, pregnant participants do not undergo the NBBAL procedure and do not have blood collected on Day 4, Day 6, or Day 14.

This table shows the maximum number and volume of biospecimens collected per participant. All biospecimens will not be collected on all participants.

Participants Known to be Pregnant

Event	Time Point							
	Index Hospitalization (Study Day)					**Long-Term Visits (Study Month)**		
	Day 0[a]	**Day 2**	**Day 4[b]**	**Day 6**	**Day 14**	**3 mons**	**6 mons**	**12 mons**
Acceptable study days for collection	Day 0-1	Day 2-3	Day 3-5	Day 6-8	Day 14-17	Day 75-135	Day 150-240	Day 330-450
Max Total Blood Volume (ml)[c]	33.2	15.2	0	0	0	28.5	12.5	12.5
- EDTA tubes (total mL)	2x 6ml (12)	1x 10ml (10)				1x 10ml (10)	1x 10ml (10)	1x 10ml (10)
- Extra EDTA tube if CPT not collected (total ml)[d]	1x6ml (6)					1x6ml (6)		
- RNA Paxgene tubes (total mL)	1x 2.5ml (2.5)	1x 2.5ml (2.5)				1x 2.5ml (2.5)	1x 2.5ml (2.5)	1x 2.5ml (2.5)
- CPT tubes (total mL)[e]	2x 8ml (16)					2x 8ml (16)		
- Na citrate tubes (total mL)	1x 2.7ml (2.7)	1x 2.7ml (2.7)						
- Buffy coat from EDTA	From EDTA							
- RBCs	From EDTA					From EDTA	From EDTA	From EDTA
Urine tubes (total mL)	1x 5ml (5)							
Oral swab	x	x		x		x	x	x
Nasal swab	x	x		x		x	x	x
Rectal swab	x	x		x				
Stool	x	x		x		x	x	x
HME filter fluid[f]	x	x	x	x	x			
Tracheal aspirate[f]	x	x	x	x	x			

Table 8 Footnotes

a. Study Day 0 is anchored as the calendar day when the first study-specified biospecimen is collected. By study eligibility criteria, this must occur within 48 hours of the patient meeting eligibility criteria. The first biospecimen collected will, by definition, be collected on Day 0. Other biospecimens scheduled for collection on Day 0 can be collected as late as Day 1.

b. Day 4 specimens are only collected from participants on invasive mechanical ventilation (IMV). Day 4 specimen collection is preferred on Study Day 4 or 5 but may be collected on Day 3 if Day 2 specimens were collected on Day 2 (instead of Day 3).

c. For participants known to be pregnant, the approach to blood volume collection ensures less than 50 ml of blood collected in any 8-weeks and a maximum of 2 blood draws in any 1-week period.

d. Participants who do not have blood collected for cellular analyses (CPT tubes) will have an additional 6 ml tube of EDTA collected at the study visit on Day 0 and 3 months. Participants who do have blood collected for cellular analyses (CPT) will not have this extra tube of EDTA collected.

e. During processing of CPT tubes, plasma will be saved and stored when possible.

f. HME filter fluid and tracheal aspirate fluid only collected from participants on invasive mechanical ventilation (IMV).

Table 9. Schedule of Events: Approximate number of participants who will have each biospecimen collected.

In-hospital biospecimen collection will only occur if the patient is alive and remains in the hospital on that day. Additionally, some samples will be collected only for a selected subset of participants. This table estimates the number of patients who will have samples collected based on these principles. The description of which patients will have specimen collection is shown in parentheses in each cell. These estimates are not exact. The final number of participants with each biospecimen type will depend on several factors that cannot be predicted exactly.

| Event | Approximate number of patients (description of patients) with biospecimen collected at time point | | | | | | | | |
| | Index Hospitalization (Study Day) | | | | | | Long-Term Visits (Study Month) | | |
	Day 0	NBBAL[a]	Day 2	Day 4	Day 6	Day 14	3 mons	6 mons	12 mons
EDTA blood	4,000 (all)		3700 (all still in hospital)	800 (on IMV)	1800 (all still in hospital)	500 (all still in hospital)	600 (all who attend visit)	550 (all who attend visit)	500 (all who attend visit)
Extra EDTA tube if CPT not collected	3,000 (no CPT)				1350 (still in hospital, no CPT)		300 (attended visit, no CPT)		
RNA Paxgene blood	4,000 (all)		3700 (all still in hospital)		1800 (all still in hospital)	500 (all still in hospital)	600 (all who attend visit)	550 (all who attend visit)	500 (all who attend visit)
CPT blood	1000 (first 1000 patients)	700 (patients with NBBAL)			450 (first 1000 patients still in hospital)		300 (50% subset who attend visit)		
Na citrate tubes blood	4,000 (all)		3700 (all still in hospital)		1800 (all still in hospital)				
Buffy coat from EDTA	4,000 (all)								
RBCs from EDTA	4,000 (all)						600 (all who attend visit)	550 (all who attend visit)	500 (all who attend visit)

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Event	Approximate number of patients (description of patients) with biospecimen collected at time point								
	Index Hospitalization (Study Day)						Long-Term Visits (Study Month)		
	Day 0	NBBAL[a]	Day 2	Day 4	Day 6	Day 14	3 mons	6 mons	12 mons
Urine	3500 (all who can produce urine)								
Oral swab	4,000 (all)		3700 (all still in hospital)		1800 (all still in hospital)		600 (all who attend visit)	550 (all who attend visit)	500 (all who attend visit)
Nasal swab	4,000 (all)		3700 (all still in hospital)		1800 (all still in hospital)		600 (all who attend visit)	550 (all who attend visit)	500 (all who attend visit)
Rectal swab	4,000 (all)		3700 (all still in hospital)		1800 (all still in hospital)				
Stool	4,000 (all)		3700 (all still in hospital)		1800 (all still in hospital)		600 (all who attend visit)	550 (all who attend visit)	500 (all who attend visit)
NBBAL		700 (intubated, passed safety screen, consented)							
HME filter fluid	1333 (on IMV)		1233 (on IMV)	800 (on IMV)	600 (on IMV)	200 (on IMV)			
Tracheal aspirate	1333 (on IMV)		1233 (on IMV)	800 (on IMV)	600 (on IMV)	200 (on IMV)			

Table 9 Footnotes

a. The NBBAL time point is anchored on the time of completion of a research-dedicated NBBAL and may occur on any study day between Study Day 0 and Study Day 8. The NBBAL time point specimens should only be collected once and should be collected as soon as possible after intubation and the patient passes the NBBAL safety screen. Two specimen types are collected at the NBBAL time point: NBBAL fluid and CPT tubes (two 8 ml tubes). The window for collecting NBBAL fluid is between intubation and 96 hours later. Do not complete a NBBAL more than 96 hours after intubation. The CPT collection should occur as soon as possible after NBBAL fluid collection. For the NBBAL time point, only collect

CPT tubes for patients who have NBBAL fluid collected. CPT collection for the NBBAL time point is in addition to the CPT tubes collected for other time points. The NBBAL time point may occur on the same study day as specimen collection for other time points. For example, if a patient has a NBBAL completed on Day 0, the specimens for both Day 0 and the NBBAL time point may be collected on Day 0, which would result in the collection of four 8ml CPT tubes on Study Day 0. NBBAL time point specimen collection may only occur for participants with consent for study procedures (that is, NBBAL time point specimen collection may not be performed under waiver of informed consent). Participants known to be pregnant do not undergo the NBBAL procedure.

Table 10. Schedule of events: Radiology and pulmonary function tests during index hospitalization and long-term follow-up visits.

Images from the following radiographic studies will be collected by study personnel and uploaded into the study database. Results of pulmonary function tests will be recorded by study personnel into the study database.

Imaging type	Procedures	Estimated number of images
In-hospital clinically obtained CXRs	Upload up to 3 CXRs per participant. Details of which CXRs to upload will be outlined in study operating procedures. Initially, CXRs targeted for uploading will be CXRs completed closest to 8:00am on Day 0, Day 2, and Day 6.	3 per participant for all enrolled participants [number of x-rays =12,000 if all 4,000 patients each have 3 uploaded CXRs]
In-hospital clinically obtained CT scans	Upload up to 1 scan per participant. If multiple scans are available, select the scan completed closest to 8:00am on Day 0. Chest CT preferred, abdominal CT acceptable if no chest CT performed during hospitalization.	1 per participant for all enrolled participants [number of scans = 4,000 if all 4,000 patients have a clinical CT scan in hospital]
Post-discharge clinically obtained CT scans	Upload up to 1 scan per participant. If multiple scans are available, select the scan closest to 6 months. Chest CT preferred, abdominal CT acceptable if no chest CT performed between hospital discharge and 12 months.	1 per participant among those in the LTO cohort [number of scans = 600 if all 600 patients in the LTO cohort have a clinical CT scan after hospital discharge]
Study-specified Chest CT scan at 12-month visit (Not completed on pregnant participants)	Upload up to 1 scan per participant. Scheduled study-protocol thin-cut chest CT scan. Funded by study budget. If a similar scan has been completed clinically in the prior 6 weeks before 12-month visit, upload the clinical scan and do not repeat as a research procedure.	1 per participant among those who complete the 12-month follow-up visit [number of scans = 500]
Study-specified pulmonary function tests (spirometry and DLCO) at 12-month visit	Record results of up to 1 pulmonary function test battery per participant. Scheduled study specified spirometry and DLCO. Funded by study budget. If similar pulmonary function tests have been completed clinically in the prior 6 weeks before 12-month visit, record the results of those tests and do repeat as a research procedure.	1 per participant among those who complete the 12-month follow-up visit [number of PFT batteries = 500]

11. Use of Data and Biospecimens

Data, biospecimens, and radiographic images collected in the APS Consortium will be used for 3 purposes:

1. Completion of APS Consortium-wide science as outlined in appendices to this protocol (funded by the APS Consortium budget).

2. Completion of APS Clinical Center-specific science as outlined in grants proposals from Clinical Centers investigators and selected for funding via the NIH peer review process (funded by the APS Consortium budget).

3. Banking for use in future ancillary studies conducted by investigators both inside and outside the APS Consortium (biobanking is funded by the APS Consortium budget but ancillary studies for use of bio-banked data and specimens is not funded by the APS Consortium budget).

During conduct of the APS Consortium study procedures outlined in this master protocol, data, biospecimens, and radiographic images will be collected by enrolling sites and transmitted to the APS Consortium Coordinating Center at Vanderbilt University Medical Center. Data and biospecimens will be organized and catalogued at the coordinating center. The coordinating center will distribute data and biospecimens as needed to complete Consortium-wide science and Clinical Center-specific science. Additionally, the coordinating center will periodically transmit data and biospecimens to repositories managed by the National Heart, Lung, and Blood Institute (NHLBI) -- BioData Catalyst (data repository) and BioLINCC (biospecimen repository).

During the period of performance for the APS Consortium, requests for data and/or biospecimens for use in ancillary studies will be reviewed for approval by the APS Steering Committee. After the completion of the APS Consortium period of performance, requests for data and/or biospecimens for use in ancillary studies will be governed by NHLBI through the BioData Catalyst and BioLINCC programs.

Table 11 outlines principles of governance for APS Consortium-wide science, APS Clinical Center-specific science, and ancillary studies.

Table 11. Use of data and biospecimens.

Program Component	Consortium-Wide Study funded under APS Consortium	Clinical Center-Specific Projects funded under APS Clinical Center Grants	Investigator initiated ancillary study (from investigators inside or outside of APS)
Data	Data available includes all elements in the APS Consortium-wide CRF/EDC. The CCC oversees data management. Data will be deposited to BioData Catalyst by the Consortium Coordinating Center.	Data includes all elements from Consortium wide CRF/EDC plus any data collected independently by the Clinical Center. Data collected independently will be managed by the Clinical Center and deposited to BioData Catalyst as necessary by the Clinical Center.	Data available include the data approved for sharing by the APS Steering Committee (during the Consortium) or available in BioData Catalyst (after the Consortium). New data/results generated by an ancillary study are the responsibility of the ancillary study investigators, including transmission to BioData Catalyst if necessary.
Specimens	Specimen collection detailed in the Consortium-wide mast protocol. Eligible specimens will be deposited to BioLINCC by the Consortium Coordinating Center.	May use specimens captured via APS master protocol for the Consortium plus any biospecimens collected independently by the Clinical Center. Specimens not outlined in the APS Consortium master protocol are the responsibility of the Clinical Center investigators, including collecting specimens, shipping, and depositing in BioLINCC (as necessary).	Biospecimens available include the biospecimens approved for sharing by the APS Steering Committee (during the Consortium) or available in BioLINCC (after the Consortium). Once biospecimens are transferred to the ancillary study investigators, all biospecimen responsibilities lie with the ancillary investigators, including transmission to BioLINCC as necessary.
Protocol	Protocol written by APS Consortium steering committee and managed by the APS Consortium Coordinating Center.	Protocol written by and managed by Clinical Center investigators.	Protocol written by and managed by ancillary study investigators.
Consent Documents	Consent documents for the Consortium-wide protocol, which manages Consortium-wide science, is managed by the CCC and approved by the Consortium sIRB	If necessary, separate consent procedures and documents are the responsibility of the Clinical Center.	If necessary, separate consent procedures and documents are the responsibility of the ancillary study investigator.
IRB	Vanderbilt University Medical Center serves as the single sIRB. The Consortium Coordinating Center manages regulatory approvals.	As needed, the Clinical Center determines overseeing IRB and manages regulatory approvals.	As needed, the ancillary study investigator determines overseeing IRB and manages regulatory approvals.

12. Terminology and Statistical Considerations

12.1 Phenotyping Terminology Considerations

Several categories of phenotypes will be evaluated in APS consortium analyses. This section outlines principles for how the different categories of phenotypes will be considered in the consortium's work (Table 12).

The first category of "phenotype" is the historical clinical syndromes that the APS consortium seeks to target – namely, ARDS, pneumonia, and sepsis. These syndromic phenotypes will be defined as previously stated (Section 8.1.3) using clinically established and published definitions.

The second category of "phenotype" to be considered is previously defined clinical, biologic, and/or physiologic phenotypes that were described in acute illness phenotyping literature prior to launch of the APS consortium. Examples of these types of phenotypes include: (i) the latent class analysis (LCA)-derived "hyperinflammatory" and "hypoinflammatory" phenotypes of ARDS and sepsis defined by clinical features and plasma protein biomarkers; (ii) the SRS1 and SRS2 phenotypes of sepsis defined by whole blood transcriptomic profiles, (iii) high/low elastance phenotypes of ARDS and pneumonia described by particular respiratory physiology parameters. Several of the consortium-wide scientific aims plan to incorporate these previously defined phenotypes in their analyses to place the Consortium's work in context with previous literature and/or as a contrast for the novel phenotypes to be identified within the Consortium (see protocol appendices, section 16).

The third category of "phenotype" to be considered is novel phenotypes identified within the APS Consortium using unsupervised and/or supervised clustering methodologies. Developing and validating these novel phenotypes will be key scientific goals of the Consortium as detailed in the appendices to this protocol (section 16).

Table 12. Phenotyping categories consider in the APS Consortium.

Phenotype category	Examples
1. Historical clinical syndromes	ARDS, pneumonia, sepsis
2. Previously defined phenotypes	i. hyper- vs hypo-inflammatory ii. SRS1 vs SRS2 iii. High elastance vs low elastance
3. Novel phenotypes	To be determined based on new findings in the APS Consortium

12.2 Statistical Considerations

12.2.1 Global considerations

The APS Consortium has seven consortium-wide aims, within which there are a combination of discovery and hypothesis testing analyses proposed (see appendices, Section 16). The aims are developed at a time when computational power is increasing, analytical strategies are developing, and the field of data science is evolving more rapidly than ever before. Moreover, as data are accrued more will be learned about the data quality, completeness and fitness for purpose. To respect the emerging technologies that will be available by the time digital data are available for an analysis and with an understanding of data quality, availability, and completeness, we will fix the statistical analysis plan immediately prior to embarking on an analysis, as discussed below. A global statistical analysis plan (SAP) will be developed for each APS aim, and each manuscript or clinical question will also have a pre-specified SAP. The purpose of this section is to lay out general considerations and principles that will guide development of SAPs and conduct of analyses.

SAPs will be developed with close collaboration among biostatisticians, bio-informaticists, and APS subject matter experts, while keeping aims somewhat broad to encourage innovation and exploration of novel ideas and hypotheses. Biomedical knowledge will inform analysis strategies to the extent possible. Analytical methods will be chosen to make efficient use of the data; when a singular approach cannot be established due to competing methods, the alternative approaches should be discussed and opportunities to compare approaches considered for inclusion in the SAP. Any method selected ideally makes full use of available raw data, including raw longitudinal data. Explanatory and exploratory analyses will be distinguished in SAPs and reporting of results. Both unsupervised and supervised learning techniques are expected to play major roles. Strong emphasis will be given to validation of both pattern discoveries and estimation/predictive instruments.

12.2.2 Statistical Analysis Plans (SAPs)

All significant projects will develop and file SAPs before commencing the primary analyses. A central APS file structure will be used to hold all SAPs and to date-stamp changes to them as well as to name responsible analysis designers. SAPs should be specific enough to reproduce analyses with minor assistance from the project's statisticians/bio-informaticians in combination with reproducible analysis scripts. Unsupervised (to patient outcomes) preprocessing should be described and included in the ultimate SAP, recognizing the preprocessing steps may commence before SAPs are completed. SAPs may be changed after analyses commence but reasons for changes must be documented in the SAP document.

12.2.3 Reproducible research

All analytical steps must be scripted in an accessible programming environment. The general approach will be to embed analysis code into a report document so that statistical reports can be recreated by issuing a single command, using software such as Quarto (which is programming language agnostic) or RMarkdown or equivalents in other languages. Program and package versions will be documented when running the final script and should match published results or discrepancies should be explained. Statistical code will be shared. More background information and some resources may be found [here].[20]

12.2.4 Validation

When patterns or structures are discovered through data analysis, these identified patterns should be validated in the sense of demonstrating sturdiness to small changes in the dataset used to find the structures. Predictive and estimation instruments should also be validated. Split-sample validation (validating on a holdout sample) is often used and may be appropriate in some instances. Repeating the process of splitting into derivation and validation samples a few times can reveal volatility of the split-sample approach when the sample is of insufficient size, and splitting the sample reduces the derivation sample size. When the statistical approach allows, cross-validation and bootstrap validation should be considered in order to maximize use of information and to optimize the reliability of the validation. Carefully repeating, inside each resampling loop, all learning/data analysis steps can reduce overfitting.

Validation analyses should include an assessment of the stability of discovered patterns, feature selection, etc. In high-dimensional situations in which "winning" features are sought, it is important to validate the ability of the data to find such "winners." This can be done by, for example, computing bootstrap confidence intervals for importance rankings of competing features.[21,22] Human supervision of feature selection may be important and adopted in specific cases depending on individual project goals. When the goal is to make absolute predictions (outcome risks, life expectancy, etc.), validations should include information on the absolute predictive accuracy (e.g., overfitting-corrected smooth calibration curves).

When patient clustering or any method that categorizes patients is used, additional validation should be considered. For example, when the goal is to predict patient outcome or to estimate differential treatment effect, an approach to validating patient clustering might be:

- Start with cluster indicator variables as the only variables (other than background covariates that may be included in all models) in the model predicting patient outcome, or as main effect and interaction-with-treatment effects in an analysis of treatment effect heterogeneity. Measure the predictive information in the categorical cluster indicators.

- Verify that clusters are sufficiently homogeneous by adding distances from cluster centers (when cluster centers can be defined) in the second stage; if distance from centers provides additional predictive information over cluster indicators, then the clusters are not compact, and distances might be preferred predictors over categories.

- In the third stage, add pre-specified prognostic variables (including variables used to derive the clusters) to the cluster variables (indicators or distances). If the prognostic variables add more information than the cluster variables alone, then the use of clustering should be reconsidered.

12.2.5 Considerations for particular analytic procedures

When there are missing data in variables being analyzed, case-wise deletion of partial patient records should be avoided. Multiple imputation or other missing data procedures should be considered. When using imputation procedures, leveraging longitudinal data should be considered. In some settings, for example estimation of correlation matrices and principal components, pairwise deletion of missing values may be a viable technique.

When fitting statistical models for longitudinal data it is often important to consider a realistic correlation structure. Where random intercepts and slopes fail to result in a well-fitting correlation structure, serial correlation structures (e.g., AR1 or Markov models) should be considered. When using generalized estimating equations (GEE) to correct for actual correlation patterns, the accuracy of GEE approximations and efficiency should be reported, and notice should be taken that GEE may not be robust to missing values that are not missing completely at random.

When considering cluster analysis outputs as explanatory variables, or effect modifiers, uncertainty in cluster membership should be carried forward through to final statistical inference. For example, if a cluster analysis also provides information on the probability of membership to each cluster for each individual, Bayesian regression models can incorporate this information in subsequent analyses.

Any special sampling or sub-sampling used in the study design phase should be considered in the analysis phase.

As APS is a multi-site study, adjusting for the multi-level structure of the data may be important when fitting statistical models. An exception occurs when performing single-site analyses or when patient-level characteristics already capture potential variation across sites. Accounting for multi-level structure may also be important in multiple imputation.

As a general principle, treatments received will be considered in analyses; treatments may affect biomarkers more than they affect patient outcomes. Adjusting biomarkers for treatments received ahouls be considered. Consideration will also be made of whether treatments alter cluster membership. Potential for confounding should be considered when estimating treatment effects on cluster membership or trajectory, as well as heterogeneity of treatment effect across clusters. When allowing for cluster membership to evolve over time, special consideration should be given to the potential for time-varying confounding if modeling cluster effects on outcomes.

Some consortium-wide science specific aims will use multiple-phase analytic approaches - needed because of the complexity of data and analytical methods. Although the statistical analysis procedures used in any one stage may be tried and tested in the statistical literature, the performance of all analytical phases in tandem may not have been validated for stability and accuracy. In those situations, simulation studies should be considered to check the performance of the multi-phase procedure; these will be

considered on a case-by-case basis as SAPs are filed depending on complexity and review of relevant methods literature. The structure of such studies may be specific to the analytic approach but might generally include two key investigations. First, datasets that resemble the dimensions of the real data but for which the relationship/association/effect of interest is entirely noise, should be generated and the multi-phase procedure run on these random datasets to make sure that noise is not incorrectly identified to be statistical signals. Second, a simulation of a very large dataset in which real signals are present can be performed, making sure that the true signals are the ones found by the proposed analytic procedure. Then the sample size can be gradually reduced to find the breaking point where results are no longer reliable. Users of the procedure then make sure that the available sample size is higher than the identified breaking point.

12.2.6 Statistical considerations summary

As laid out above, we have developed a broad approach for each statistical analysis plan, and we have described robust methods to achieving reproducible findings. To demonstrate the range of statistical methods that might be used, we have described some analytical approaches within individual aims (described in the appendices, Section 16). Regardless of the details, every analysis will proceed as follows:

i)   The analysis plan should be pre-specified, and a system to review, approve and file the SAPs will be implemented.
ii)   When a preferred statistical approach cannot be easily identified or agreed upon, multiple approaches may be specified and comparisons among approaches will be incorporated into the SAP.
iii)   The SAP should specify the approach to missingness.
iv)   The SAP should specify if and how the multi-level data structure will be considered in the analysis.
v)   The SAP should specify the approach to evaluating validity, including addressing the propagation of uncertainty through a complex or multi-phase analysis pipeline.
vi)   Statistical code used to generate results will be made available, and deviations from the SAP should be transparently documented.

13. Human Subjects

13.1 Risks and benefits

This master protocol for the APS Consortium describes an observational prospective cohort study with longitudinal collection of data and biospecimens with methods routinely used in current routine medical practice.

The risk to participants in the APS Consortium study are detailed in Table 13. The primary risks are related to the potential of disclosure of private health information and complications from biospecimen collection procedures. These risks will be minimized by using a secure REDCap data collection system for data entry and storage, maintaining good clinical practice procedures at all sites for handling of private health data, and training study teams on best practices for biospecimen collection.

Samples taken as part of this study may be used to evaluate human genetics in the future. Genetic testing results will not be linked to identifiable patients nor placed in the medical record. Inadvertent disclosure of genetic testing results could influence insurance policies or future employment.

Participants are not expected to receive direct personal benefits for participating in the APS Consortium study. On a societal level, benefits of participants joining the APS Consortium study include contributing to increased knowledge about ARDS, pneumonia, and sepsis, which could lead to medical advances that ultimately decrease morbidity and mortality from these syndromes.

Table 13. Potential risks to participants in the APS Consortium study by study procedure.

Study procedure	Potential risks	Study methods to minimize risks
Collection of personal data	Inappropriate disclosure of private health information (loss of confidentiality/privacy).	Only study personnel trained in good clinical practice for clinical research studies will be involved in data handling. Each enrolling site will maintain appropriate training and certification in protection of human participants for all study personnel involved in interacting with patients and/or handling data. Data will be entered into and stored within REDCap, a secure internet-based data collection tool. REDCap employs several layers of security, including authentication of end-users, automatic user logout after 30 minutes of inactivity, a time-stamped audit trail, encrypted web-based information transmission, and firewall protection of uploaded documents. Additional information on REDCap is available at: www.project-redcap.org.
Blood collection (phlebotomy)	Pain at phlebotomy site, infection at the phlebotomy site, bleeding, damage to surrounding nerves, transient light-headedness, fainting/syncope.	Only study or clinical personnel trained in phlebotomy will collect blood for this study. Patients will be positioned in a safe location for phlebotomy, such as supine in a bed or sitting in a chair. When possible, blood will be collected from pre-existing vascular catheters and timed with blood draws for clinical care to avoid additional phlebotomy.
Urine collection	No plausible risks identified.	Only study or clinical personnel trained in the collection of urine will collect urine for this study. Urine will be collected via patient voiding or collection from pre-existing urinary catheters. No invasive procedures will be initiated in the study to collect urine.
Collection of oral, nasal, and rectal swabs	Localized transient irritation, pain, and/or bleeding may occur at the swab site.	Only study or clinical personnel trained in the collection of oral, nasal and rectal swabs will collect swabs for this study. Personnel will be trained to hold pressure on the swab site if it bleeds.
Stool collection	No plausible risks identified.	Stool will only be collected via participants freely stooling or via stool collection systems in place for clinical care (e.g., rectal tube, fecal incontinence bag). No invasive procedures will be initiated by the study to collect stool.
HME (ventilator) filter collection	Inadvertent disconnection of ventilator tubing for longer than necessary to collect the filter, which could rarely result in low oxygen levels, organ damage, and death if not recognized. The risk of patient injury from HME (ventilator) filter collection is considered very low.	Only study or clinical personnel trained in the collection of HME (ventilator) filters will collect the filters. Ventilators at all study sites have alarms that alert clinical teams when ventilator tubing is disconnected. HME filters are routinely used for clinical care. Patients receiving invasive mechanical ventilation are routinely disconnected from the ventilator in clinical care daily. When possible, HME filters will be collected during a disconnection of the ventilator that is occurring as part of clinical care.
Tracheal aspirate fluid collection	Bleeding, localized pain, drop in oxygen levels (hypoxemia), and dislodgement of the tracheal tube. A drop in oxygen levels from tracheal aspirate collection is very unlikely to cause patient harm.	Only study or clinical personnel trained in the collection of tracheal aspirate fluid will collect this specimen. Tracheal suctioning is a routine clinical procedure and tracheal aspirates for this protocol will be done at times that tracheal aspirates are being collected as part of clinical care, whenever possible.
NBBAL procedure	Bleeding, localized pain, dislodgement of the tracheal	Only study or clinical personnel trained in the NBBAL procedure will complete this procedure for the study. NBBAL

Study procedure	Potential risks	Study methods to minimize risks
	tube, and drop in oxygen levels (hypoxemia), which if severe and uncorrected, can lead to organ injury and death. Serious injury from a NBBAL is rare (<1%).[23]	is a common clinical procedure in some hospitals. Only patients who are intubated will undergo the NBBAL procure. Only patients who pass a pre-procedural safety screen under the supervision of the site investigator will undergo a NBBAL. Hypoxemia temporally related to a study NBBAL procedure will be collected as an AESI and reported to the sIRB and OSMB.
Long-term outcome surveys (via phone, SMS, or email)	No plausible physical risks. Participants might experience embarrassment or emotional discomfort.	Participants do not have to answer any questions that make them feel too uncomfortable. An on-call mental health specialist will be available to talk with patients that have severe emotional stress or suicidal thoughts.
Short physical performance battery (SPPB) [at in-person LTO visits]	SPPB assesses lower extremity functioning. It includes a balance test, a timed 4-meter walk test, and chair stand test. There is a small risk of falling. Activities may increase heart rate.	The testing will be performed by trained study staff. Movements are completed in a standard order, starting with easier movements. Participants will be able to skip any movement that they cannot do or feel would be unsafe to try.
CNS-Vital Signs [at in-person visits]	CNS vital signs is a computer based cognitive assessment. Participants might experience embarrassment or emotional discomfort from not being able to answer some questions.	Participants do not have to answer any questions that make them feel too uncomfortable.
Handgrip strength [at in-person LTO visits]	No plausible risks identified.	Participants may skip this procedure if they do not feel comfortable completing it.
Muscle ultrasound and strength testing [at in-person LTO visits]	No plausible risks identified.	Participants may skip this procedure if they do not feel comfortable completing it.
Pulmonary function testing [at 12-month LTO visit]	Pulmonary function testing is a common procedure in clinical medicine and has very low risks. Participants may feel dizzy, lightheaded, or tired or cough during or shortly after the procedure. These symptoms should go away shortly after completion of the testing.	Pulmonary function testing, including spirometry and diffusing capacity of the lungs for carbon monoxide (DLCO), will be done using standard clinical procedures. Participants will be allowed to rest or stop the testing if they feel dizzy, lightheaded, or tired. Participants may skip any portion of testing they do not feel comfortable completing.
Chest CT scan [at 12-month LTO visit]	Chest CT involves exposure to a small dose of radiation. At doses much higher than participants will receive for the study CT scan, radiation is known to increase the risk of developing cancer after many years. At the doses participants will receive for the study CT scan, it is unlikely to cause any adverse effects.	The amount of radiation used in chest CTs is low (approximately 10 mSv); this amount of radiation from on chest CT scan is similar to the background radiation dose experienced by people in the United States over one year.[24,25] Participants with a recent chest CT scan obtained clinically will not undergo a research CT scan. Participants are able to skip any portion of testing they do not feel comfortable completing.

Study procedure	Potential risks	Study methods to minimize risks
Storage of biospecimens for future genetic testing	Biospecimens will be placed in long-term storage, where they may be retrieved for future studies involving human genetic testing. Inadvertent disclosure of genetic testing results could cause loss of privacy and in severe cases affect insurance policies and employment opportunities.	Biospecimens for future genetic testing will be deidentified before they are stored. These biospecimens will be labeled with a study number and the link between that study number and the patient's identity will not be provided to long-term storage facilities. Results of genetic testing will not be placed in the medical record. We cannot guarantee that no one will ever be able to use genetic information to identify participants.

13.2 Informed Consent

13.2.1 Approach to informed consent for this study

Study procedures described in this protocol (APS Study Protocol A – full protocol) will be completed after written informed consent for the study has been obtained from the participant or a legally authorized representative (LAR)/surrogate decision maker. Consent may be obtained using a paper document or remotely with electronic/e-consent procedures. Remote consent with an LAR/surrogate is possible using electronic procedures as long as the risks and benefits of the study are discussed with the consenting study personnel and the LAR/surrogate has an opportunity to ask questions. Remote consent must use a procedure compliant with Title 21 CFR Part 11, such as signature through Adobe Acrobat Sign or DocuSign. In situations when the patient/LAR/surrogate does not speak English, a short-form consent document and qualified interpreter may be used to facilitate an informed consent discussion and documentation.

If a patient or LAR/surrogate declines study blood draws or research genetic testing during an informed consent discussion, the patient should not be enrolled.

13.2.2 Consent through a Legally Authorized Representative (LAR)/surrogate

Some patients who are eligible for this study are likely to not possess decision making capacity. In these cases, consent for study participation may be obtained through a legally authorized representative (LAR) or other surrogate decision maker.

When a patient is identified as eligible for study enrollment, the study team will assess the patient's capacity to make decisions about participation in research. This assessment is completed by a study team member trained in good clinical practice for the conduct of clinical research who is in direct contact with the patient. If the patient cannot meaningfully engage with the study team member (such as, a state of intubation, chemical sedation, and/or not responsive to verbal stimuli), the study team member may consider the patient to lack decision making capacity for consent. If the patient can meaningfully engage

with the study team member, the study team member will explain the APS study to the patient and review the informed consent document with the patient. After this explanation, if the patient cannot describe the basic elements of the study, including risks and benefits of study participation, the study team member may consider the patient as not possessing decision making capacity for consent. The study team member will document the process and outcome of capacity assessment in the APS Study Electronic Data Capture instrument.

If an eligible patient lacks decision making capacity to consent for study participation, the study team may obtain consent for study participation through an alternative decision maker. In this study, we use the term "LAR/surrogate" to indicate the alternative decision maker who is making decisions for the patient while the patient lacks capacity. The US Code of Federal Regulations (CRF) section 46.102(i) defines a legally authorized representative (LAR) as: "an individual or judicial or other body authorized under applicable law to consent on behalf of a prospective subject to the subject's participation in the procedure(s) involved in the research. If there is no applicable law addressing this issue, *legally authorized representative* means an individual recognized by institutional policy as acceptable for providing consent in the nonresearch context on behalf of the prospective subject to the subject's participation in the procedure(s) involved in the research." The term "LAR/surrogate" is applied to the person making medical decisions for the patient, either as an explicit legally authorized representative or through local policy and custom. The study team should follow local laws and policies when identifying an alternative decision maker for consent for study participation.

If consent for study participation is obtained via an LAR/surrogate, the study team will iteratively assess the patient to evaluate whether the patient has gained decision making capacity. These assessments will be made through hospital discharge or Study Day 14 (whichever occurs first). If the patient is identified as regaining capacity, the study team will obtain consent for study participation from the patient at that time by having the patient sign the consent form that has already been signed by an LAR/surrogate. At that time, the patient may decline to continue participation in future study procedures or withdraw informed consent; details of procedures for withdrawal of informed consent are outlined below.

13.2.3 Rationale for two study protocols with different approaches to informed consent

A key objective of this study is to rigorously phenotype ARDS, pneumonia, and sepsis, among severely and critically ill patients throughout their entire course of illness, beginning immediately when a patient meets eligibility criteria through 12 months later. To achieve this objective, patients must be enrolled as early as possible in their course of illness. Early biospecimens are crucial for the objectives of the APS Consortium. Up to 75% of patients in the ICU experience delirium or altered mental status prohibiting their ability to provide informed consent.[26] Frequently, LARs for critically ill adults are not available to provide written informed consent for research participation in a timely fashion. For example, prior work has shown that at a publicly-funded hospital, 18% of eligible patients for ARDS clinical trials are not enrolled due to the patient not having capacity for consent and no LAR/surrogate being available.[27]

An accompanying protocol (APS Study Protocol B – alteration protocol) will be used when consent for study participation cannot be obtained before study entry. These protocols are linked. Protocol B

describes minimal risk study procedures that may be completed with alteration of informed consent and no written informed consent for study participation. The minimal risk study procedures in Protocol B are identical to study procedures in Protocol A. Protocol B contains a subset of study procedures from Protocol A that are minimal risk.

An approach of using two protocols to enroll participants – one that governs consented patients (Protocol A) and one that governs patients participating under alteration of informed consent (Protocol B) – is being utilized to minimize selection bias that would occur if only patients who were able to immediately provide written informed consent were enrolled. Outlining minimal risk procedures from the APS Study schedule of events in a separate protocol (Protocol B) enables participants without consent to begin minimal risk study procedures using an alteration of informed consent process. Meanwhile, participants with consent completed may engage in all study procedures, including both minimal risk procedures and greater than minimal risk procedures.

Goals of the study team include:
- Early enrollment of a population that represents the patient population of those suffering from ARDS, pneumonia, and sepsis, including those who are critically ill and often unable to rapidly consent for research.
- Obtain informed consent for study participation prior to initiation of study procedures for as many participants as possible.
- For participants who enter the study with alteration of informed consent, obtain written informed consent as soon as possible.

13.2.4 Description of consent procedures for Protocol A and Protocol B

Protocol A (full protocol): This document contained herein is Protocol A. It describes all study procedures that a participant may complete during the course of the APS study and governs study procedures for participants who have completed informed consent for research participation (either directly or through an LAR/surrogate). Protocol A has two accompanying informed consent documents (ICDs):

- ICD #1 (primary study): Informed consent document for the primary APS study, including in-hospital study procedures and long-term outcome surveys. ICD #1 contains two part--part 1, which includes information shared across all sites participating in the study and part 2, which contains local context information for a particular site and signature lines. Participants who entered the study with alteration of informed consent may consent for the full primary study at any time through the 3-month follow-up time point. While the participant is in the hospital, consent will be obtained via ICD #1. If a participant enters the study via alteration of informed consent (Protocol B) and is discharged from the hospital without informed consent being obtained, the study team will attempt to contact the participant at the 3-month study time point to obtain consent. If this contact at the 3-month time point is made via telephone, consent may be obtained with waiver of documentation of signature. Similarly, if a participant had previously been participating in the study via surrogate consent, the patient will be given the opportunity to consent for themselves at the 3-month phone call ("reconsent") with waiver of documentation of signature. Waiver of documentation of signature will use the following procedures:

    i.   study team member explains the study to the participant;
   ii.   study team member reviews ICD #1 with the participant;
  iii.   study team member provides opportunity for participant to ask questions and answers those questions;
  iv.   participant provides verbal confirmation of wanting to participate in the study;
   v.   study team member documents this consent process in the Study Electronic Data Capture instrument.

If a participant attends a long-term outcome in-person visit without having previously provided informed consent for study participation, informed consent for study participation will be obtained at that time using ICD#1.

- ICD #2 (LTO in-person visits): Informed consent document for long-term outcome in-person visits. ICD #2 contains two part--part 1, which includes information shared across all sites participating in the study and part 2, which contains local context information for a particular site and signature lines. Participants who attend in-person long-term follow-up visits will complete informed consent for the in-person long-term outcome study procedures prior to initiation of those procedures. This informed consent document for in-person long-term outcome visits (ICD #2) is separate from the informed consent document for the primary study (ICD #1).

Protocol B (alteration protocol): Protocol B is described separately in a dedicated protocol outside this document. It describes a procedure for participation in the APS Consortium study with alteration of informed consent. Protocol B will be used for participants for whom informed consent for the study participation cannot be obtain via the patient or LAR/surrogate prior to initiation of study procedures. Minimal risk procedures within the APS study may be completed using alteration of informed consent. Protocol B outlines the minimal risk procedures that may be completed without informed consent for research. Protocol B has no informed consent document but includes a patient information sheet to help notify patients and others about enrollment in the study.

Participants with informed consent will complete study procedures described in Protocol A (this document), and participants who have not provided informed consent will complete study procedures described in Protocol B (a separate document). Participants without informed consent for study participation will complete a subset of study procedures (described in Protocol B) completed by participants with informed consent for research (described in Protocol A). Participants who enter the study on the alteration of informed consent protocol (Protocol B) will be iteratively approached for consent; if and when informed consent for participation in the APS Study is obtained, the participant will be moved from Protocol B to Protocol A (Figure 2).

Greater than minimal risk procedures may be completed after informed consent is obtained among participants who started study participation under alteration of informed consent procedures. Patients who move from Protocol B to Protocol A will seamlessly continue on the APS Study schedule of events and maintain the same Study Day structure initiated at study entry. Study days continue through the transition from Protocol B to Protocol A; that is, if a patient provides informed consent on Day 4, study procedures

completed on Protocol B for Day 0 through Day 3 will remain unchanged and Study Day 4 procedures will pick up on Protocol A.

For participants who enter the study with alteration of informed consent, study team members will attempt to obtain informed consent while the patient is in the hospital through Study Day 14 and at the 3-month time point.

Data and biospecimens collected under Protocol A and Protocol B will be pooled for storage and analysis.

13.2.5 Withdrawal of informed consent

If a participant or LAR/surrogate indicates an intention to withdraw informed consent for study participation or forego future study procedures, the study team will clarify the wishes of the participant/LAR/surrogate and categorize the request into one of the following categories. Steps to honor wishes of the participant/LAR/surrogate are outlined for each category.

(i) Category 1 Request: Complete withdrawal of consent, including destruction of identifiable data and biospecimens and no participation in any future study procedures.

Action: The study team will note in the study data capture instrument that the participant or LAR/surrogate withdrew consent. An attempt will be made to understand and document why the participant withdrew consent. No additional study procedures will be performed. Identifiable participant data will be removed from the data capture instrument. Biospecimens that are stored at the enrolling site and APS Coordinating Center will be destroyed. Deidentified data and biospecimens that have been transferred to NIH repositories or to laboratories for analysis may not be able to be destroyed. Completed works, such as manuscripts, that used the participant's data and/or biospecimens will not be revised or retracted and data to support those works will be retained.

(ii) Category 2 Request: Previously collected data and biospecimens may be retained but desire expressed to not participate in future direct-contact study procedures.

Action: The study team will note in the study data capture instrument that the participant or LAR/surrogate maintains consent for the study but requested no additional direct contact study procedures. The study team will attempt to understand and document why the participant requested to stop additional direct-contact study procedures. The study team will not perform any additional procedures that involve direct contact between study personnel and the participant, including interviews, biospecimen collection, long-term outcome surveys, and in-person long-term outcome visits. Medical record data capture will continue. All collected data and biospecimens will be used.

(iii) Category 3 Request: No participation in some specific study procedures but willingness to participate in other study procedures.

Action: The study team with work with the participant/LAR/surrogate to understand what specific procedures the participant/LAR/surrogate wants to avoid in the future. The study team will note in the data capture instrument which study procedures will not be completed and why the participant/LAR/surrogate requested to opt out of those procedures. The study team will avoid the specific study procedures as requested, such as future blood draws, future swabs, future interviews/surveys, or future in-person visits. All collected data and biospecimens will be used.

Figure 2. Flow diagram of informed consent procedures.

Eligible Patient for the APS Phenotyping Study

Participants for whom written informed consent cannot be obtained at time of study entry

Participants for whom written informed consent (ICD #1) is obtained at the time of study entry

Study Protocol B: Alteration of Informed Consent Procedures

- Participants for whom consent cannot be obtained at time of study entry may undergo minimal risk study procedures described in Protocol B using an alteration of informed consent process.
- Once consent is obtained, Protocol A procedures are followed moving forward (study days are not restarted upon moving from Protocol B to Protocol A).
- Protocol B governs study procedures prior to consent; Protocol A governs study procedures after consent.
- <u>Not</u> permittable with alteration of informed consent (not permitted on Protocol B): Greater than minimal risk procedures, LTO surveys, In-person LTO visits.

Consent for study participation may be obtained between study entry and the 3-month study time point, which triggers a move from Protocol B to Protocol A

Study Protocol A:
Full In-Hospital Study Component

- Participants who have ICD #1 signed at study entry immediately start on Protocol A.
- Participants who have ICD #1 signed after study procedures have started on Protocol B move to Protocol A after ICD#1 is signed.
- After ICD #1 is signed, Protocol A governs study procedures, including in-hospital procedures and follow-up LTO surveys.

LTO Surveys

- Participants may be contacted for LTO surveys if they previously completed ICD #1 or may complete informed consent at the first LTO contact (3-month time point).
- LTO surveys are usually completed remotely.
- LTO surveys are scheduled at 3 months, 6 months, 12 months.

Selected participants for whom a second LTO-specific written informed consent (ICD #2) is obtained

LTO In-person Visits

- A selected subset of participants (n~600) will complete in-person LTO visits.
- ICD #2 (consent for in-person LTO visits) must be completed to participate in in-person LTO visits.
- Once ICD #2 is signed, Protocol A governs study procedures for in-person LTO visits at 3 months, 6 months & 12 months.

13.3 Human subjects considerations for data and biospecimen banking and sharing

13.3.1 Disclosure of research results to participants

Results for CT scans completed at the 12-month visit for research purposes will be disclosed to participants via site investigators. The investigators do not intend to disclose the results of other research testing to participants.

13.3.2 Storage of data and biospecimens

Data collected as part of the Consortium-wide study, including those captured from the EHR and other hospital databases, will be transferred into the study database via standardized electronic case report forms (eCRFs), which will reside in a centralized database located on secure servers. Study data will be entered and accessed via a secure, password-protected REDCap database website wherein all web-based information is encrypted. REDCap was developed specifically around HIPAA Security guidelines and is recommended by both the Vanderbilt University Privacy Office and Institutional Review Board. REDCap is available to all sites participating in the APS Consortium.

Data transferred to the APS Coordinating Center will include dates (for example, hospital admission date, and date of birth) and contact information to facilitate post-hospital follow-up visits. Biospecimens will be labeled with a study identification number without patient name, medical record number, or date of birth. Access to personal health information in study databases will be limited to only those individuals requiring that level of access.

Study data and biospecimens will be stored for an indefinite period of time for future use. Deidentified data and biospecimens will be shared with researchers outside the APS Consortium. For long-term storage of data, personal identifiers will be removed once quality assurance has been confirmed and prior to data lock. All research records will be accessible for inspection by authorized representatives of the IRB, federal regulatory agency representatives, and NIH representatives.

13.3.3 Sharing data and biospecimens

The APS Consortium will develop data management and sharing plans consistent with NIH policies (https://sharing.nih.gov/).

APS Consortium investigators will be permitted to access and use data and biospecimens for the purpose of achieving the Consortium-wide and center-specific project aims directly from the Consortium Coordinating Center. Consortium-wide biospecimens and data will be sent to the central biorepository and study database housed at the Consortium Coordinating Center. Investigators seeking to perform approved ancillary studies with data and/or biospecimens collected by the APS Consortium may request data and biospecimens from the Consortium Coordinating Center before data and biospecimens are deposited in BioData Catalyst and BioLINCC.

Ultimately, the Consortium Coordinating Center will deposit de-identified data and biospecimens in BioData Catalyst and BioLINCC. Once data and biospecimens reach BioData Catalyst and BioLINCC, they will be available to investigators through the governance of BioData Catalyst and BioLINCC without involvement of the Consortium Coordinating Center.

13.3.4 Co-enrollment with other studies

The APS Consortium steering committee, OSMB, and NHLBI will agree on co-enrollment procedures before participants in the APS study are co-enrolled with other studies. Principles for co-enrollment will include the following:

- Co-enrollment should not affect the scientific goals of the APS Consortium. Co-enrollment will not be permitted if it compromises the scientific integrity and/or statistical power of APS Consortium studies.
- Co-enrollment will be compliant with NIH and NHLBI guidelines and policies.
- Co-enrollment will only be allowed when study procedures for the APS Consortium studies can be achieved. Co-enrollment in the APS Consortium will not be permitted when study procedures in a co-enrolled study would prevent completion of data or biospecimen collection for the APS Consortium study.
- Safe blood collection procedures for critically ill patients will be followed as detailed by the PETAL Network Investigators.[28]

14. Adverse Events and Safety Monitoring

14.1 Overview of Safety Monitoring

In this observational study that uses routine techniques for data and biospecimen collection, substantial numbers of serious, study-related adverse events are not anticipated. The study will not be overseen by the US Food and Drug Administration (FDA). Safety monitoring will be performed by the investigators, the study's single IRB at Vanderbilt University Medical Center, and an NHLBI-appointed Observational Safety Monitoring Board (OSMB).

14.2 Adverse Events

14.2.1 Paradigm for collecting Adverse Events

In this study, investigators will collect and report adverse events (AEs) that are classified as serious and related to study procedures, those prespecified as adverse events of special interest (AESIs), and those that potentially change the risk: benefit balance for patient participation (Unanticipated Problem (UP)). AEs that meet at least one of the following three criteria will be collected in this study:

1) both serious and study related;
2) AESI;
3) UP.

AEs that do not meet any of these criteria will not be collected.

Events that meet criteria for an AE in the population enrolled in this study will be numerous due to the severe medical conditions these patients have. The proportion of AEs experienced by the study population that are related to study procedures is anticipated to be extremely small. Thus, the paradigm of AE collection and reporting in this study was designed to capture all the events that could potentially represent a safety concern for the study while avoiding overly burdensome AE monitoring for participants, study teams, the IRB, and OSMB.

14.2.2 Definitions for Adverse Events

(i) Adverse Event

An AE is defined as any untoward medical occurrence.

(ii) Serious Adverse Event (SAE)

A serious AE (SAE) is an untoward medical occurrence that directly causes at least one of the following in the judgement of the study team:

- Death
- Life-threatening condition that places the participant at immediate risk of death
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions or a congenital anomaly/birth defect.
- An important medical event not meeting the criteria for one of the outcomes above but, based on medical judgment, jeopardized participant safety or required medical or surgical intervention to prevent one of the outcomes listed in this definition.

(iii) Relatedness of SAEs

SAEs will be evaluated for relatedness to study procedures using the definitions below:

- Definitely Related: The adverse event meets all three of the following criteria: (a) a temporal sequence from study procedure to the adverse event suggests relatedness, (b) the event cannot be explained by the known characteristics of the participant's clinical state or therapies, and (c) evaluation of the participant's clinical state indicates to the study team the experience is definitely related to study procedures.

- Possibly Related: In the study team's opinion, the adverse event has a reasonable possibility of being related to study procedures but one or more of the above criteria for "Definitely Related" are not met.

- Probably Not Related: The adverse event occurred at a time when it could have been caused by study procedures but, in the opinion of the study team, can reasonably be explained by the known characteristics of the participant's clinical state or therapies.

- Definitely Not Related: The adverse event was definitely produced by the participant's clinical state or therapies and not by the study procedures.

- Uncertain Relationship: The adverse event does not meet any of the criteria outlined above and the study team cannot ascertain enough information to classify relatedness of the event.

For the purposes of this study, an adverse event is considered related to study procedures if there is a "reasonable possibility" of a causal relationship between a study procedure and the adverse event or the relationship cannot be determined; this includes events that are classified as definitely related, possibly related, or of uncertain relationship.

(iv) Adverse events of special interest (AESIs)

An adverse event of special interest (AESI) is defined as a pre-specified event of scientific or medical concern that has the potential of being related to study procedures and is important to understand regardless of investigator classifications. In this trial, AESIs listed below will be recorded as an AE and will require a written narrative regardless of a site's study team classification of seriousness and relatedness. For this study, the following events are AESIs:

- **AESI #1**: New or worsening hypoxemia within 30 minutes of a study-specified NBBAL. For AESI consideration, new or worsening hypoxemia is defined by the event meeting both of the following criteria: (i) a decrease in SpO2 by at least 10 percentage points in the time window between the beginning of the procedure and 30 minutes after the procedure is completed; and (ii) a persistent absolute increase in FiO2 by at least 10% for at least 6 hours after the procedure compared to before the procedure.

(v) Unanticipated Problem (UP)

An unanticipated problem is a finding discovered during the conduct of the study that suggests participation in the study may have more risk than was anticipated at the time of study initiation. UPs have the potential to change the risk: benefit balance of the study compared to what was known at the time of study launch.

14.2.3 Reporting Adverse Events

AEs that meet criteria for reporting should be entered into the system's electronic data collection system within 3 calendar days of the study team becoming aware of the AE. Reporting an AE will include a clinical narrative explaining the context of the AE and rationale for the investigator's classification of the event as serious and related, an AESI, or an UP. The study team should also alert the Consortium Coordinating Center for reported events. The coordinating center will report the AE to the sIRB and NHLBI within 4 calendar days of receiving the report of an AE (thus, within 7 days of site awareness of the event). The OSMB will be altered about AEs from NHLBI. Reported events will be followed until

resolution. Figure 3 is a summary flow diagram to assist study teams with deciding which adverse events to report in this study.

Figure 3. Flow diagram to assist with decisions about AE recording and reporting.
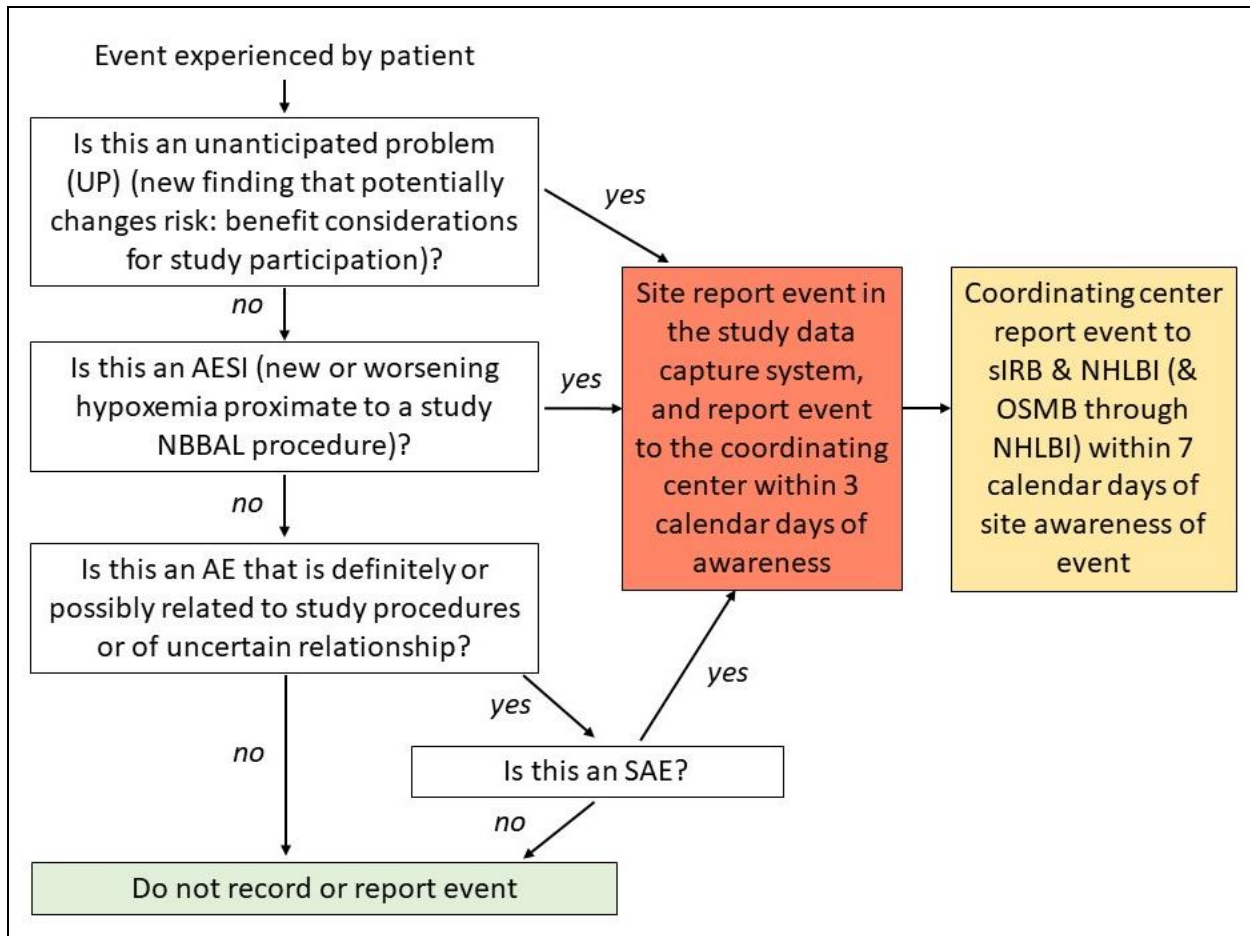
14.3 Observational Study Monitoring Board (OSMB)

The OSMB will be comprised of experts in APS syndromes and fields relevant for this study. The OSMB is appointed by NHLBI. The principal role of the OSMB is to evaluate the safety and integrity of the study. Full details of the OSMB structure and function will be provided in an OSMB charter, which will be reviewed with the OSBM at its first meeting with the study team.

Prior to initiation of study enrollment, the OSMB will review the study protocol and informed consent documents. OSMB meetings will be scheduled regularly in accordance with the OSMB charter. Additionally, the NIH, OSMB, and investigators may call ad hoc OSMB meetings.

The OSMB will regularly monitor several aspects of the study, including the safety, enrollment rates, protocol compliance, cohort demographics and geographic distribution, and data quality and completeness.

Recommendations to end, modify, or continue aspects of the study will be communicated by the OSMB, through the OSMB executive secretary, to the Consortium Coordinating Center.

14.4 Institutional Review Boards (IRBs)

This is a multi-center cohort study for which a single IRB will be used for the ethical review of the proposed research per NIH policy (https://grants.nih.gov/grants/guide/notice-files/NOT-OD-16-094.html). Vanderbilt University Medical Center will serve as the single IRB of record. Local context will be reviewed by local IRBs for each participating site.

This study utilizes a 2-part consent document. Part 1 of the informed consent document is the master consent document and contains information that applies to all study sites. Part 2 of the informed consent document is a site-specific document which outlines local, site-specific information.

Processes of obtaining informed consent should be reviewed during local context review to ensure study procedures are consistent with local laws and standards.

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16. Protocol Appendices

16.1 Appendix A (Aim 1): Determining and interrogating the role of microbiota (pathogen and microbiome) in the clinical and biological heterogeneity of ARDS, pneumonia, and sepsis, and their long-term outcomes.

16.1.1 Aim 1 Background and Rationale

Background: Efforts to date to understand clinical heterogeneity in ARDS, pneumonia, and sepsis have focused exclusively on variation in host-derived features.[1-5] These studies have overlooked two crucially important *microbial* sources of variation among critically ill patients: the *pathogen* and the *microbiome*.

Pathogens: All pneumonia and sepsis, and most ARDS, is initially provoked by infection with a pathogen: bacterial, fungal, or viral. Pathogens differ profoundly in their mechanisms of virulence and their effects on the host response and are frequently not identified using existing clinical microbiology practices[6,7]. Cutting-edge molecular techniques (metagenomics and metatranscriptomics) hold tremendous potential to (1) identify previously undetected pathogens and (2) advance our understanding of the pathogen's role in the biological and clinical heterogeneity of these syndromes.

Microbiome: The microbiome – the communities of microbiota living on and in the human body – represents a major source of biological variation that propels heterogeneity in diverse diseases and therapeutic responses (e.g., cancer[8,9] and cardiovascular disease[10,11]). Critically ill patients exhibit tremendous variation in the *density*, *diversity*, *composition*, and *function* of communities within the gut and lungs.[9,12-17] Via its production of systemically active metabolites, the gut microbiome is a major determinant of the serum metabolome in health and disease,[18,19] and gut microbiota play a crucial role in the maturation and calibration of systemic and alveolar immunity.[20-24]

Rationale: The pathogen and the microbiome are important yet incompletely understood sources of biologic heterogeneity in APS conditions, and both represent a highly promising treatment target. Yet we lack an actionable understanding of their role in ARDS, pneumonia, and sepsis. By determining and interrogating the role of the pathogen and microbiome in the *development*, *trajectory*, and *recovery* of these conditions, this Aim will both 1) facilitate the development of microbially-targeted therapeutics and 2) enhance our understanding of the role of varying pathogens and microbial environments on the variability in host response observed in critically ill patients with ARDS, pneumonia and sepsis.

16.1.2 Aim 1 Study Objectives

Primary Objective (Aim 1):

Establish and understand the relationship between patients' microbiota (pathogen and microbiome) and their long-term physical, cognitive, and mental health outcomes. Specifically, we will evaluate the associations between:

Predictor 1: metagenomics- and clinically-identified etiologic pathogen
Predictor 2: final in-hospital gut microbiome diversity

and 3-month measurements of:

Outcome 1: Short Physical Performance Battery Protocol (*physical performance*)
Outcome 2: CNS-Vital Signs assessment (*neurocognitive performance*)
Outcome 3: Handgrip strength (*musculoskeletal function*)

These analyses for the primary objective will be adjusted for baseline comorbidity and health status.

Exploratory Objectives (Aim 1):

1. Determine how patient-associated microbiota (pathogen and microbiome) contribute to the heterogeneity of previously defined phenotypes of ARDS, pneumonia, and sepsis.
2. Determine how patient-associated microbiota (pathogen and microbiome) inform the heterogeneity of ARDS, sepsis, and pneumonia using unsupervised analyses (development of novel phenotypes).
3. Evaluate the extent to which recovery of microbiome at 3 months (vs. final in-hospital time point) is associated with 6- and 12-month physical, cognitive and mental health outcomes, adjusting for baseline comorbidity and health status.

16.1.3 Aim 1 Study Population
The study population will be the complete cohort of 4000 hospitalized patients as described in the study protocol.

16.1.4 Aim 1 Study Design
Prospective observational cohort study.

16.1.5 Aim 1 Study Procedures
We will characterize the *pathogen* (and its corresponding host response) using metagenomic and metatranscriptomic next-generation sequencing using previously described methods.[25,26] We will characterize the *microbiome* using the following approach: we will assess *bacterial density* in rectal swabs and oral and nasal swabs using droplet digital PCR (Bio-Rad) using universal 16S primers. *Bacterial communities* of all specimens will be characterized using 16S rRNA amplicon sequencing (Illumina MiSeq).

Additionally, plasma protein biomarkers needed to identify the previously defined LCA-derived "hyperinflammatory" and "hypoinflammatory" phenotypes will be measured in all 4000 patients, using

previously described multiplex assays. The "hyperinflammatory" and "hypoinflammatory" phenotypes will then be identified using previously published parsimonious biomarker models.[27] As a secondary analysis, latent class models will be fit to the entire cohort using previously described approaches[28] to confirm concordance with the parsimonious classifier model and test for variance according to clinical syndrome (i.e., ARDS, pneumonia, sepsis).

16.1.6 Aim 1 Data, Images, and Biospecimens Used from the APS Consortium

The primary measurements performed in this Aim will be made using: plasma from all patients (day 0: mNGS), endotracheal aspirates from all mechanically ventilated patients (day of intubation: mNGS), rectal swabs (day 0, 2, 6; 3-month, 6-month, 12-month: 16S rRNA gene amplicon sequencing), and oral and nasal swabs from all mechanically ventilated patients (day of intubation: 16S rRNA gene amplicon sequencing). As indicated in Sub-Aims, these measurements will be compared with plasma biomarker-adjudicated phenotypes as well as clinical and functional outcomes.

16.1.7 Aim 1 Pre-processing and Statistical Analysis

Methods for analysis to be used in this Aim continue to be developed at a rapid pace, and the approach should be optimized based on knowledge at the time of analysis. To that end, the SAP will be fixed prior to analyzing the digital data. The general approach to rigor and reproducibility described in the protocol will be followed, with the aim-specific details incorporated into the SAP. To provide an example of the kinds of analyses we may pursue for this aim, we outline one possible approach here.

To analyze the pathogen data, samples will be processed using the open-source CZ-ID pipeline,[25] which performs reference-based alignment at both the nucleotide and amino acid level against sequences in the National Center for Biotechnology Information (NCBI) NT and NR databases, respectively, followed by assembly of reads matching each detected taxon. The resulting matrix of taxon counts in the patient samples and water controls will be used for downstream analyses, as detailed below.[25] We will first fit a negative binomial background model of taxon-specific counts, normalized to the total number of ERCC spike-in reverse-transcribed cDNA counts in the water control samples. We will use this model to filter out taxa whose abundance does not significantly exceed the background model. Background-corrected taxonomic count matrices will be used for downstream analyses. From here, analyses will diverge for plasma and respiratory samples. In plasma, a generally sterile compartment, we expect to identify only a few taxa above background, even in cases of bacteremia. We will employ a previously developed rules-based model[25] that ranks species within each sample by abundance and identifies those above the maximum drop-off in abundance, i.e. the dominant taxa. These taxa will then be intersected with a literature-curated list of established causal agents of bacteremia, resulting in a per-sample determination of mNGS-identified bacteremia and likely pathogen.

We will use the R package metagenomeSeq to perform differential abundance testing at the individual genus level. We will identify probable pathogens using a validated rules-based model[25] that identifies respiratory viruses and established bacterial and fungal respiratory pathogens present at disproportionate abundance. We will then compare the distributions of detected pathogens between the phenotypes, as

described for plasma. For both plasma and respiratory sample data, metagenomics-identified pathogens will be compared with clinical microbiologic data and identified clinical pathogens.

For microbiome analysis, pre-processing steps will use *mothur*, *R*, and *vegan*, or comparable packages. Informed by published studies and preliminary data, for each specimen, we will derive key community features for analysis, such as: 1) bacterial density (quantified via ddPCR); 2) bacterial diversity (calculated using the Shannon Diversity index); and 3) community composition (using PERMANOVA analysis of beta-diversity and relative abundance of key bacterial taxa).

Comparative analyses for Aim 1a will compare features between previously derived LCA-defined phenotypes to test for enrichment of bacteremia, specific pathogens, or categories of pathogens (e.g., gram-positive or gram-negative bacteria) in a particular phenotype. In respiratory samples, we expect a more complex microbial community, including both airway commensals and potential pathogens so phenotypes may be compared using more global assessments of the microbiome, such as Bray-Curtis dissimilarity at the genus level as well as the Shannon diversity index.

For Aim 1b, we will incorporate data derived from whole blood host gene expression (Aim 2) in addition to the data described in this Aim. We will apply unsupervised analysis to the high-dimensional data (including whole-blood host gene expression, ETA host gene expression, ETA and plasma microbial composition) to characterize and interpret biological variation in the cohort. For host gene expression, we may perform principal component analysis (PCA) of gene counts following variance stabilizing transformation and selection of the top 1000 (or top 5000) most variable genes. Principal components representing >5% of total variance then enter the permutation-based jackstraw method to assign statistical significance to the association of individual genes with each principal component (or combinations thereof). The resulting gene loadings and p-values can then serve as input to functional/pathway enrichment methods, such as gene set enrichment analysis and IPA, in a manner analogous to traditional differential expression analysis, to facilitate biological interpretation. Canonical correlation analysis can then be used to characterize co-variation of biological features between the assayed compartments, namely blood and the lower airway.

To harness all the high-dimensional data types for generating novel phenotypes, one approach we may use is to apply variance stabilizing transformation to count-based features, or log transformation for other continuous measurements, such as abundance of pathogens detected in mNGS. Then, reduced representations of high-dimensional data can be generated using linear combinations of the most variable original features derived from PCA. Principal components representing >5% of total data type variance might then be included in the reduced representation. Reduced datasets are then concatenated and can be clustered using the K-means algorithm. The optimal number of clusters can be determined using metrics of clustering quality, such as within cluster sums of squares, silhouette score, and gap statistics.

Once novel clusters have been established, we can compare the new clusters and LCA classes based on the maximum overlap and assess the similarity between novel cluster labels and LCA labels using the adjusted Rand index. The prognostic value of the new cluster labels and LCA labels can then be assessed for various outcomes, including mortality. Of particular interest when comparing methods is samples

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allocated to different classes by different methods. Data types and features driving class discordance can be highly informative. Once novel clusters are established and validated, associations of clusters with various pathogen and microbiome features will be assessed similarly to Aim 1a.

16.1.8 Aim 1 Adequacy of sample size

The statistical approaches that will be used in the ultimate analysis will be specified *a priori* in a Statistical Analysis Plan. Here, we provide an assessment of the adequacy of the sample size based on reasonable assumptions, recognizing that the final methods will be highly optimized using rapidly developing methodology.

Given (1) the pathophysiological centrality of the pathogen and microbiome in APS conditions, (2) the inadequacy of current clinical practices to identify both pathogenic and non-pathogenic host-associated microbiota, and (3) the dynamic nature of the microbiome in response to critical illness and ICU exposures (e.g., antibiotics), we plan on generating microbe-related measurements at the following timepoints:

- plasma mNGS on 1,500 APS participants at day 0
- ETA mNGS on all mechanically ventilated APS participants on the day of intubation
- rectal swab microbiota on all APS participants with rectal swabs collected at day 0, 2, 6 and months 3, 6, and 12
- oral and nasal swab microbiota on 300 mechanically ventilated APS participants on the day of intubation

Based on preliminary and published data, these measurements will (1) meaningfully increase our ability to identify etiological pathogens in most APS patients, (2) provide rich and dynamic characterization of changes in lower gastrointestinal microbiota across our cohort both during hospitalization and in the months following discharge, and (3) provide sufficient time-matched measurements to compare pharyngeal microbiota with lower respiratory tract microbiota and determine if noninvasive respiratory sampling can improve our ability to predict and prevent ventilator-associated pneumonia.

Specifically, for Aim 1a (determine how patient-associated microbiota contribute to the heterogeneity of previously derived LCA-defined phenotypes of ARDS, sepsis, and pneumonia), we anticipate that roughly 25-30% of the cohort will be in the "hyper-inflammatory" phenotype and 70-75% will be in the "hypo-inflammatory" phenotype. With a total sample size of 1500 participants, we should therefore have approximately 450 patients in the hyperinflammatory phenotype, with 50-60% of those being mechanically ventilated.(Sinha LRM 2023) In preliminary analyses of blood and plasma mNGS data from 156 sepsis patients, we identified 44% higher prevalence of mNGS-defined bacteremia in hyper-inflammatory compared to hypo-inflammatory patients (74% vs 30%; $p<0.001$), reflecting the large magnitude of effect sizes that might be observed. As noted in the rationale for cohort sample size, for this ratio of group sizes, a sample size of just 400, and a modest effect (odds ratio of 0.5), the half width of the confidence interval is about 0.25. If the group size is 1500, the half width of the odds ratio is 0.13, assuming a 50% effect rate. Analyses involving all 4,000 persons would result in a half width of 0.08, demonstrating an ability to estimate even small effects with precision. This suggests that the sample is

sufficient to detect meaningful differences in pathogens and microbiota between hyper and hypo classes both within and across the two LCA-defined phenotypes.

Aim 1b (Determine how patient-associated microbiota inform the heterogeneity of ARDS, sepsis, and pneumonia using unsupervised analyses) will follow the approaches to cluster analysis as laid out in the main protocol. We anticipate that at least 500 features can be evaluated for the purposes of unsupervised (or supervised) analyses. The number of features can increase with employment of data reduction techniques.

16.1.9 Aim 1 Expected Output

Via these measurement, analyses, and integration with host-directed measurements acquired on the same 4,000 patients, we anticipate the following outputs:

- Discovery of specific <u>microbe-host interactions</u> (pathogen-host, gut-systemic, respiratory-respiratory) that contribute to immune calibration and clinical heterogeneity, confirming the significance of the microbiome as an "upstream" driver of dysregulated immunity and providing specific targets for coordinated microbe/host modulation.
- Earlier and more informative <u>recognition of pathogens</u>, improving our ability to provide tailored antimicrobial treatment with fewer off-target effects.
- Identification and interrogation of <u>the gut as a reservoir of secondary pathogens</u>, facilitating development of <u>real-time tools for characterizing the microbiome</u>, improving our ability to provide selective, narrow gut and pharyngeal decontamination.
- We anticipate that the hyper-inflammatory phenotype will be characterized by increased expression of IFN-stimulated genes and activation of innate immune and integrated stress responses in the lower respiratory tract, and increased expression of genes related to neutrophil function/degranulation in the blood, in comparison to the hypo-inflammatory phenotype (Aim 1a). We also anticipate that the pathogens and microbiota characterizing each phenotype will differ (Aim 1a). These results would have important implications for identification of future targeted therapies for testing in each phenotype.
- We anticipate that unsupervised analysis of mNGS data will identify novel phenotypes that partially overlap but will not be fully concordant with prevously derived LCA-defined phenotypes (Aim 1b). We anticipate that these analyses will identify host response patterns associated with particular pathogens or pathogen types, indicating the importance of incorporation of pathogen data into critical illness phenotypes.

16.1.10 Aim 1 References

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16.2 Appendix B (Aim 2): Determine host response phenotypes that most strongly associate with organ failure and 3, 6 and 12-month health status, and test the incremental utility of markers of vascular dysfunction, plasma DAMPs, and leukocyte expression for APS short- and long-term outcomes.

16.2.1 Aim 2 Background and Rationale

APS syndromes overlap physiologically and display similar sequelae, yet pathways from mechanism to outcomes are unclear. Dysregulation in both vascular and damage-associated molecular pattern (DAMP) signaling may explain pharmacologically targetable clinical heterogeneity in APS critical illness. Vascular injury is a central pathogenic mechanism in APS that may explain progression from limited pneumonia to multi-organ involvement. Dysfunctional endothelium is leaky, attracts leukocytes and platelets, forms neutrophil-endothelial traps (NETs), and sheds glycocalyx.[1–3] Rarely, vessel injury manifests as overt or threatened disseminated intravascular coagulopathy with fibrinolysis.[4] However, each of these pathologies requires unique treatment strategies, so deconvoluting vascular injury using proven markers has value. A more precise recognition of which features of endothelial dysfunction drive organ injury and persistent health impairments will clarify the best therapeutic strategy for APS. Each marker (ANGPT2, sTM, sICAM, Syndecan-1) is selected based on our preliminary data in our large sepsis population and in published literature.[1,2,5]

Dysregulated damage-associated molecular pattern (DAMP) signaling is another central mechanism in APS that may explain heterogeneity in pauci- versus multi-organ failure.[6,7] The DAMP axis may interact with vascular injury and host inflammation, and drugs to neutralize specific features of DAMP signaling (including markers such as cell free nucleic acids, alarmins, and soluble RAGE), are increasingly available. Thus, understanding the contribution of aberrant or excessive DAMP signaling, and which specific features are most prognostic for specific organ failure, APS state, and clinical outcomes, is important.

Survivors of APS experience varied impairments in their physical, psychological, cognitive, and socioeconomic health that persist for months to years following discharge.[8–10] These multiple impairments are often collectively termed post-intensive care syndrome (PICS)[11,12] and experts recommend serial sequential assessments post-discharge for its diagnosis.[13] Improved prediction of impairments might allow testing of targeted interventions yet our ability to predict these impairments is limited. No externally validated models exist. Better predictive tools are needed for long term outcomes, and simple ADL metrics may have value both for predicting these impairments and should be accounted for in analyses testing associations with new markers. Furthermore, the links between acute biologic events and post-ICU health are poorly understood. A longitudinal cohort with repeated batteries and molecular sampling is needed to understand the relationships between host premorbid status, acute illness, and long-term impairments. We hypothesize that integrating both pre-ICU health and molecular features of illness response will result in more accurate prediction of long-term health. If we can validate a predictive tool for specific health states, then we could aid patients and families in decision-making during acute illness, better anticipate the workforce needed for long-term recovery, and perform more efficient trials of interventions to improve post-APS outcomes via prognostic enrichment. Specific markers for excessive

vascular injury, DAMP signaling, circulating markers for sarcopenia (GDF-15), and markers for neuronal injury (neurofilament light chain, tau protein) will be tested as candidate markers to predict long term health status. Post-APS health will be assessed using patient-reported long term outcome recovery questions and WHODAS, EQ-5D utility score and clinical frailty score, adjusted for pre-APS comorbidity and retrospective baseline status, at 3, 6, and 12-month follow-up.

16.2.2 Aim 2 Study Objectives

Primary Objective (Aim 2):

To utilize day 0 plasma measurements of vascular injury (permeability, vascular activation, coagulopathy/fibrinolysis, and glycocalyx degradation), altered damaged associated molecular pattern (DAMP) signaling, and indicators of sarcopenia and/or neuronal injury to test association with 60-day mortality (primary objective), organ failure free days (secondary objective), and individual organ-specific outcomes (ventilator free days, vasopressor-free days, dialysis-free days; secondary).

Exploratory Objectives (Aim 2):

1. To determine reproducible multi-marker plasma phenotypes of immune activation, vascular injury, DAMP activation, and sarcopenia/neuronal injury and test for association with 60-day survival (primary), organ failure free days (secondary), and individual organ-specific outcomes (VFD, vasopressor-free days, dialysis-free days)
2. To test whether the association between multi-marker plasma phenotypes and 60-day mortality is modified by classically defined syndromes (ARDS, pneumonia, and sepsis).
3. To test whether day 0 plasma measurements of vascular injury (permeability, vascular activation, coagulopathy/fibrinolysis, and glycocalyx degradation), altered damaged associated molecular pattern (DAMP) signaling, and indicators of sarcopenia and/or neuronal injury associate with for patient-reported health states (cognitive, emotional, functional, or respiratory) at 3, 6, and 12 months.
4. To test whether multi-marker plasma phenotypes early during the APS illness have predictive utility for patient-reported health states at 3, 6, and 12 months post-APS, specifically in cognitive, emotional, physical function, and respiratory domains.
5. To test whether a change in multi-marker plasma phenotypes between acute illness and 90 days associates with improved patient-reported health states at 3, 6, and 12 months.

16.2.3 Aim 2 Study Population
All participants enrolled in the APS Consortium will be eligible to participate in this aim. Our analytic plan, as detailed below, calls for a sample size of 1500 individuals at day 0 to test associations of both individual markers and patterns of co-expression of multiple plasma analytes while retaining adequate power to test for effect modification by APS state (ARDS, pneumonia, and sepsis). The 1500 subset for this profiling will be drawn from the first 2200 participants enrolled to allow for balance of demographic features, geographic areas, and APS states.

16.2.4 Aim 2 Study Design

Prospective observational cohort study with scheduled blood sampling, and longitudinal follow-up at 3, 6, and 12 months after critical illness.

16.2.5 Aim 2 Study Procedures

We will quantify the following proteins in plasma: Syndecan-1, angiopoietin-2, soluble ICAM, soluble thrombomodulin, growth differentiation factor 15, soluble RAGE, S100A12, neurofilament light chain, and tau protein using multiplex electrochemiluminescence, conventional enzyme-linked immunosorbent assay (ELISA), or other automated multiplex immunoassay platforms (Simoa and/or ELLA). We will also quantify cell free DNA and mitochrondrial DNA in plasma using Qiagen DNeasy miniprep kits and then quantitative polymerase chain reaction (qPCR) to quantify ND-1 and COX-IV genes to represent mtDNA and nuclear DNA, respectively.

16.2.6 Aim 2 Data, Images, and Biospecimens Used from the APS Consortium

Clinical data including syndromic phenotype (ARDS, pneumonia, sepsis), presence/type of infection, risk factors for ARDS, demographic information, comorbidities, severity of illness, physiologic data, and outcomes (survival, duration of organ support) will be utilized from each participant's inpatient admission. Long term outcomes including measures of functional status, quality of life, emotional health, cognitive performance, and respiratory symptoms at 3, 6, and 12 months will also be tested outcomes, and will be adjusted for metrics of pre-illness health. Images from the inpatient stay or the longitudinal 12-month visit will be utilized, and from longitudinal muscle ultrasound, will be used.

Biological samples will include the plasma drawn at 0, 2, and 6 days, as well as 90 days. Depending on findings, additional timepoints including d180 and d360 may be assayed for leading candidate markers. In addition, whole blood gene expression from the PAXgene RNA-stabilizing tubes will be utilized for the clustering aim.

16.2.7 Aim 2 Statistical Analysis

It is recognized that methods for analysis continue to be developed. A detailed SAP will be generated before analysis begins, in which we will specify the method considered optimal at the time or, if an optimal method cannot be easily determined, competing methods will be compared. The following outlines a general approach that might be considered for this aim.

Unadjusted tests for association between individual markers and outcomes (mortality, organ failure, or post-ICU health state) might be conducted as a t-test or Wilcoxon rank sum test as appropriate to the data distribution. Marker concentrations can be transformed for normality as needed and then used in multivariable regression analysis controlling for factors that might act as confounders (including but not exclusive to age, sex, pulmonary versus non-pulmonary source of infection, presence of sepsis, and risk factors or comorbidities). A directed acyclic graph (i.e., DAG) will be derived by the study team to guide the analysis.

Longitudinal data might use a 'joint longitudinal and time-to-event' model, with a longitudinal model appropriate for the outcome distribution (e.g., logistic, linear, negative binomial), and a competing risk accelerated failure time model for dropout (e.g., death, moribund, lost). The shared parameter specification can be selected for each outcome based on model fit.

Multiple methods can be enacted for clustering, and consistency across methodologies will be assessed. For example, we may employ agglomerative hierarchical clustering, Wald's clustering, and uniform manifold approximation and projection (UMAP), and other potentially informative clustering approaches to visually inspect data.[18–20] Within the UMAP space, we can visualize the overall gradient, or contribution, of specific biologic features using a feature-weighted kernel density weighting as we have previously published.[18] Latent class modeling can also be accomplished,[16] and the number of classes tested by Vuong-Lo-Mendell-Rubin test. Longitudinal data can be incorporated into longitudinal clustering by growth mixture modeling or a 2-step approach with growth curve modeling and K-means.[21] Once we have established discernable and reproducible clusters, we can then use regression modeling to test the association between cluster and organ failure, mortality, and disability scores (CNS Vital Signs, WHODAS II, and HADS).

As discussed in the protocol, the SAP will be written and filed centrally a priori. If there are competing, plausible approaches, theses will be compared. Adjustments to pre-specified plan will be documented with rationale. The SAP will follow the principles outlined in the protocol.

16.2.8 Aim 2 Adequacy of sample size
The statistical approaches that will be used in the ultimate analysis will be specified *a priori* in a Statistical Analysis Plan. Here, we provide an assessment of the adequacy of the sample size based on reasonable assumptions, recognizing that the final methods will be highly optimized using rapidly developing methodology.

With continuous traits such as plasma protein or nucleic acid quantification, we will have ample power to test for associations with mortality (estimated 30%), specific organ failure (estimated 10 – 40%), or specific post-ICU health impairment (estimated 30%) with the targeted deep phenotyping sample size of 1000-1500 participants at inpatient timepoints and approximately 600 participants at day 90 (3 months). This sample size will also provide adequate power to test for statistical interaction with APS state or other clinical or biological states such as sex, or race/ethnicity. This sample size will also facilitate the ability to preserve cohort participants in the remaining n=2500 of the APS cohort as a validation set for findings identified in the first n=1500 (as a secondary validation procedure to the cross-validation described in Section 12).

For clustering analyses, prior published experience in critical illness phenotypes has successfully identified between 2 and 4 biologic clusters using sample sizes ranging from n=265 to n=1200.[14–17] With 1500 we expect adequate power for clustering with up to 150 features (more if dimension reduction is employed) as described in the rationale for the cohort sample size.

16.2.9 Aim 2 Expected Output

With this careful characterization of vascular, DAMP, and immune plasma protein and whole blood gene expression profiling across 1500 participants from the APS cohort, we anticipate:

- the generation of multivariable models including both clinically-derived and lab-derived variables that display predictive value for ARDS, AKI, 28-day mortality, and overall change in SOFA score
- the identification of plasma or gene expression features that will enhance prediction of post-APS cognitive or functional health impairments
- that better classification of the specific vascular health or DAMP signaling dysregulation across APS states, and a careful mapping of their trajectories, will suggest new therapeutic strategies
- that specific vascular, DAMP, or whole blood gene expression features will be informative to integrated subphenotype classification, and that such features can be leveraged to test for heterogeneous treatment effect in future trials

16.2.10 Aim 2 References

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16.3 Appendix C (Aim 3): Test the hypothesis that sampling the airspaces of mechanically ventilated patients with ARDS, pneumonia and sepsis will uncover novel APS phenotypes and endotypes and inform our understanding of central mechanisms that drive short- and long-term outcomes, including pulmonary dysfunction.

16.3.1 Aim 3 Background and Rationale

ARDS and pneumonia are diseases of the lungs. Similarly, up to 50% of severe sepsis cases result from lower respiratory tract infections. Compared to the bloodstream, the luminal compartment of the lungs has been largely understudied. Moreover, despite the fact that studies of airspace biology in ARDS have yielded novel mechanistic insights,[1] phenotypes identified in ARDS patients are predominantly driven by markers of systemic inflammation and coagulation rather than respiratory variables, and airspace biomarkers were not included in the models.[2] There are few published studies that have sought to determine whether the previously derived hyper- and hypoinflammatory phenotypes are mirrored in airspace biology and available evidence suggests that airspace phenotypes are discordant with systemic phenotypes.[3,4] Taken together, these factors have limited our ability to identify APS phenotypes and represent a major barrier in the field.

A new method for serially sampling the airspace in mechanically ventilated patients using fluid that condenses on the heat moisture exchanger (HME) filter has recently been described. This method is completely non-invasive, inexpensive, requires minimal investigator time and can be done serially and safely over the course of mechanical ventilation.[5,6] We have prospectively enrolled over 400 mechanically ventilated patients at Vanderbilt in HME filter fluid studies and we have implemented this method in NHLBI-funded studies at multiple sites including University of Colorado, University of Washington, Stanford and UCSF. Moreover, we have demonstrated that protein biomarkers can reliably be measured in HME filter fluid collected from mechanically ventilated patients with ARDS and other causes of acute respiratory failure.[5,6]

Non-bronchoscopic bronchoalveolar lavage (NBBAL) is a well-recognized approach that enables sampling of the distal airways and airspaces of individuals that are on mechanical ventilation. The technique involves instillation of saline into the distal airspaces, followed by gentle aspiration. Accordingly, cells, bacteria, lipids and proteins from the distal airspaces can be sampled. NBBAL is minimally invasive and has been demonstrated to be safe in critically ill participants.[7]

The overarching goal of this scientific aim is to test the hypothesis that sampling the airspaces of mechanically ventilated patients with ARDS, pneumonia and sepsis using HME filter fluid and NBBAL will uncover novel APS phenotypes and endotypes and inform our understanding of central mechanisms that drive short- and long-term clinical outcomes, including pulmonary dysfunction.

16.3.2 Aim 3 Study Objectives

Primary objectives (Aim 3):

1. Identify acute phenotypes of APS in mechanically ventilated patients using bulk RNA sequencing of mononuclear cells isolated from NBBAL and identify their association with short-term and long-term clinical outcomes.
2. Identify acute phenotypes of APS in mechanically ventilated patients using a panel of protein biomarkers measured in exhaled breath condensate obtained from HME filters on day 1 of mechanical ventilation along with clinical respiratory variables and identify their association with short-term and long-term clinical outcomes.

Secondary objectives (Aim 3):

1. Use longitudinal collection of HME filter fluid to determine whether phenotypes identified at the onset of acute respiratory failure are stable over time and whether new respiratory phenotypes emerge.
2. Determine which biologic processes in the airspaces are reflected in the bloodstream and which processes are lung-specific by comparing bulk RNA sequencing of mononuclear cells from blood and lungs and proteins measured in HME fluid and plasma.
3. Determine whether phenotypes derived from lung compartment biomarkers improve the ability to predict clinical outcomes versus currently accepted phenotypes defined from blood markers alone.

16.3.3 Aim 3 Study Population
All mechanically ventilated patients enrolled in APS will be eligible for inclusion if mechanical ventilation is initiated during the period of in-hospital biospecimen collection. We estimate that 1500 of the 4000 patients enrolled in the APS study will be mechanically ventilated during the first four study days.

16.3.4 Aim 3 Study Design
Prospective observational cohort study

16.3.5 Aim 3 Study Procedures

HME filter fluid studies:

After passing a safety screen to rule out severe hypoxemia (FiO2 > 80%) or high PEEP (>15 cm H2O), HME fluid will be collected on days 0, 2, 4, 6 and 14 if patient is mechanically ventilated by placing a fresh Airlife HME filter in the ventilator circuit for 4 hours. Patients who are intubated prior to enrollment will have the first (day 0) sample collected on the day of enrollment. The Airlife HME filter is optimal for these studies, with a small dead space volume and a reliable HME fluid yield of ~1-2 ml after a 4-hour dwell time.[5] At study sites where heated ventilator circuits are routinely used, the heated circuit will be removed or turned off for the 4-hour HME dwell time. Once removed, HME filters will be centrifuged to collect exhaled breath condensate which will be frozen in small aliquots.

A panel of protein biomarkers representing key facets of lung pathology during acute respiratory failure will be measured in duplicate in HME samples including IL-6, IL-8, TNFR1, MCP1, RAGE, SP-D, Angiopoietin-2, ICAM-1, procollagen peptide-3, protein C, PAI-1 and total protein.

NBBAL studies:

All patients that are initiated on mechanical ventilation will be considered for NBBAL. Ideally, NBBAL will be performed within 24 hours of initiation of mechanical ventilation. However, we recognize that this might not be feasible for all participants. Therefore, NBBAL may be performed as late as 96-hours after initiation of mechanical ventilation. Safety criteria for NBBAL include FiO2 $\leq$ 75%, PEEP < 15, INR $\leq$ 3.0, and platelets > 50,000 / ul. All must be met at the time of the procedure. Prior to performing NBBAL, the patient is placed on 100% FiO2 for 10 minutes. BAL is performed using a flexible 16 gauge catheter that is gently advanced through the endotracheal tube to "wedge" the distal tip of the catheter. Serial aliquots of saline are instilled into the catheter and then aspirated. The maximum instillation volume is 120 ml.

Aspirated BAL fluid will be pooled and placed in a sterile collection container and kept on ice until processing. BAL fluid will be run through a 70 micron strainer to remove mucus and debris. Fluid will be centrifuged to pellet cells. Cell-free fluid is gently aspirated and aliquoted for banking. Cells will be washed once, and then preserved for RNA sequencing studies.

16.3.6 Aim 3 Data, Images, and Biospecimens Used from the APS Consortium

To identify acute lung-specific APS phenotypes and endotypes using airspace biospecimens (Objective 1) we will utilize clinical data, chest radiograph images and HME fluid that are collected for the APS study. To determine relationships between acute lung-specific APS phenotypes and short- and long-term pulmonary dysfunction (Objective 2) we will utilize clinical data including duration of mechanical ventilation and mortality from APS and long-term outcome data including pulmonary function testing (spirometry, DLCO) and CT fibrosis scoring that will be obtained in approximately 500 survivors at 12 months. In addition, we will utilize plasma biomarker levels and LCA phenotyping results that are planned for the entire APS cohort for comparison to lung-specific phenotypes derived from respiratory variables and biomarkers measured in HME fluid (Objective 3).

16.3.7 Aim 3 Statistical Analysis

As with all aims, the final statistical analysis plan will be fixed and filed prior to conducting the analysis. Here, we describe one potential approach for this aim, which will either be confirmed and fleshed out or updated with new methods available at the time of analysis. The primary analysis will include all enrollees to determine if there are latent respiratory phenotypes that are common to all critically ill patients; mechanically ventilated patients enrolled in the APS study will include patients intubated for pneumonia, sepsis, ARDS, and other causes of acute respiratory failure. Secondary analyses will focus within subgroups of patients who have been clinically phenotyped as having sepsis (both pulmonary and non-pulmonary), ARDS, and pneumonia. Some patients will be classified into more than one subgroup.

To approach this aim, a *de novo* latent class analysis (LCA) using previously described methods might be proposed.[2] For the primary analysis, clinical and respiratory variables along with HME biomarkers measured on the first day of mechanical ventilation would be considered. These variables include protein biomarkers (IL-6, IL-8, TNFR1, MCP1, RAGE, SP-D, Angiopoietin-2, ICAM-1, procollagen peptide-3, protein C, PAI-1 and total protein) along with age, sex, etiology of acute respiratory failure, PaO2/FiO2, PEEP, static compliance of the respiratory system, ventilatory ratio,[10] and RALE score.[11] Respiratory and HME biomarker data available at a later timepoints for patients who are still intubated may be included in the analysis to determine whether phenotypes identified at the onset of ARF are stable over time and whether new respiratory phenotypes emerge in those who remain in ARF. Once we have determined whether latent respiratory phenotypes are present, we will investigate phenotype generalizability and stability, and test associations between respiratory phenotypes and pre-illness factors such as air pollutant exposure and socioeconomic factors.

For long-term outcomes, we will focus first on two key hypotheses. First, we hypothesize that acute lung epithelial injury as measured by HME fluid RAGE and SP-D will be associated with development of fibrotic lung changes (increased lung fibrosis scoring on CT, decreased FVC). Second, we hypothesize that the severity of lung endothelial injury as measured by HME fluid Ang-2 and ICAM-1 will be associated with long-term destruction of the lung vascular bed as measured by DLCO. The dependent variables of interest are continuous variables and will be DLCO, FVC, lung fibrosis score. Independent variables include age, sex, HME biomarker data from both day 1 and latest day available, ventilator settings, RALE score, duration of mechanical ventilation, underlying cause of ARF (pneumonia, ARDS, sepsis, other). The initial proposal is for use of linear regression models; dependent variables may be log-transformed to improve fit. Analyses done are expected to include the entire cohort, as well as in subgroups divided by clinical syndrome (pneumonia, ARDS, sepsis). To determine whether long term respiratory outcomes differ by any *de novo* respiratory phenotypes, we can include the phenotype variable and its interaction terms with the main independent variables of interest.

We plan to check for concordance and discordance between novel respiratory phenotypes identified in this Aim and previously derived phenotypes based on clinical and biomarker data and the LCA approach. We will then assess reasons for concordance and discordance. If the same number of clusters are identified with each analysis, standard metrics including Cohen's Kappa will be used to assess the degree of concordance. Identifying a different number of clusters for systemic vs. respiratory phenotypes, or the same number with lack of concordance, may indicate that respiratory phenotypes differ from systemic inflammatory phenotypes. When different numbers of clusters are identified between aims, correlation between cluster variables will be assessed, as well as evaluating whether some clusters function as sub-clusters of those identified in other aims.

Finally, a key question is whether the biologic mechanisms that form the basis for NBBAL endotypes are reflected in the blood. This can be achieved by exploring targeted gene expression profiles and weighted gene cluster network analysis (WGCNA) in bulk RNA seq between peripheral blood PBMCs and NBBAL.

16.3.8 Aim 3 Adequacy of sample size

The statistical approaches that will be used in the ultimate analysis will be specified *a priori* in a Statistical Analysis Plan. Here, we provide an assessment of the adequacy of the sample size based on reasonable assumptions, recognizing that the final methods will be highly optimized using rapidly developing methodology.

Among the estimated 1500 ventilated patients with HME fluid collected, we estimate that 1050 will have sepsis (both pulmonary and non-pulmonary), 600 will have ARDS, and 600 will have pneumonia. All subgroups are larger than n=500, which is sufficient for the proposed analyses as shown in the rationale for the cohort sample size, and is true even if feature quality is suboptimal.[8] For example, Hsieh et al,[9] showed that with n=600 (the smallest subgroup) a multivariable logistic regression to detect an association between a covariate of interest (COI) and the response variable, has 90% power to detect an odds ratio of 1.55 to 1.60, for each standardized unit increment in the COI when the COI is moderately correlated with other covariates in the model (multiple correlation of 0.2 to 0.5).

For analysis of long-term respiratory outcomes, approximately 500 hospital survivors will have follow-up testing (PFT, CT) collected and among these we anticipate that the majority will have been mechanically ventilated during the acute hospitalization. Enrollment of mechanically ventilated patients in the in person LTO cohort will be monitored over time and sampling strategy can be adjusted to further enrich for mechanically ventilated patients if needed. Assuming just 200 mechanically ventilated patients, the sample size is sufficient for complex regression models with interactions and regression splines; Cohen's F^2 will be 0.08 for 90% power and 5% type I error rate when the base model has 10 degrees of freedom and the new model adds 4 degrees of freedom (e.g., adding a three-level categorical variable and its interaction with two-level categorical variable). Thus, power is sufficient to detect a medium effect in terms of improvement in model fit by including new respiratory phenotypes and their interactions, represented by relative increase in R^2 values.

Finally, of the 1500 subjects that we expect will be on mechanical ventilation in the study, we anticipate that 700 will have NBBAL. We can assess the association between NBBAL and blood expression of 500 candidate genes and up to 50 WGCNA networks. We estimate an FDR adjusted significance threshold of 0.0047 assuming 10% of genes are truly associated in blood and BAL, 80% power and a 5% FDR. Using this adjusted significance threshold, 700 participants will provide 80% power to detect correlations of 0.14 between blood and NBBAL expression measures.

16.3.9 Aim 3 Expected Output

We anticipate that we will find lung-specific phenotypes, and that sepsis, ARDS and pneumonia will share respiratory phenotypes that will differ from systemic hyper- and hypo-inflammatory phenotypes. In addition, we expect to definitively answer the longstanding question as to how well the biological processes in the airspace can be reflected in the plasma. This study will allow direct comparison between plasma and airspace biomarkers from contemporaneous samples in a large and clinically diverse cohort allowing us to directly address that question.

With regard to long-term outcomes, we anticipate that we will identify key biological signatures in the airspace that will correlate with different long-term outcomes. Specifically, lung epithelial injury biomarkers will correlate with long term restrictive/fibrotic lung dysfunction, and lung endothelial injury markers will correlate with reduced DLCO. It may be that our selected biomarkers do not correlate with these outcomes, in which case we will explore associations with biomarkers of other mechanistic pathways that will be measured for the respiratory LCA.

Lastly, we expect that results from this analysis will shed light on relationships between the alveolar environment and systemic disease. As an example, it has been suggested that COVID-19 pneumonia is driven by self-sustaining inflammatory circuits. However, it is unclear how this mechanism contributes to systemic inflammation and extra-pulmonary organ dysfunction. Similarly, it is unclear how severe pulmonary infections set the stage for impaired systemic immune function. Head-to-head assessment of gene expression pathways and network modules between lung and blood will help address these questions and will define central biologic mechanisms that contribute to disease pathogenesis in the lung versus the periphery.

16.3.10 Aim 3 References

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16.4 Appendix D (Aim 4): Determine trajectories of established phenotypes over time, and utilize longitudinal data to identify novel phenotypes of APS during the acute phase and during recovery.

16.4.1 Aim 4 Background and Rationale

Molecularly-defined phenotypes with different predicted survival and differential response to multiple randomized therapies have been validated in multiple critical illness populations with APS.[1,2,3] However, the vast majority of existing work has been performed using plasma protein and clinical phenotyping from only the earliest timepoint, and not considering the dynamic nature of class-defining markers over time. We hypothesize that the trajectory of molecular phenotypes have important prognostic information about patients' condition, and that the resolution of 'hyperinflammatory' phenotype will associate with improved survival. Furthermore, by considering new plasma proteomic features, transcriptomic features, and microbial data over time, we hypothesize that novel phenotypes with distinct biological dysregulation can be identified, which in turn may yield novel precision treatment paradigms for APS.

16.4.2 Aim 4 Study Objectives

Primary Objective (Aim 4):

To utilize longitudinal molecular (plasma and HME), transcriptomic, and microbial data over the first seven days of critical illness to identify novel longitudinal, trajectories-based phenotypes, to determine the prognostic value for short-term clinical outcomes (primary: in-hospital mortality; secondary: organ-failure free days).

Exploratory Objectives (Aim 4):

1. To utilize longitudinal molecular, transcriptomic, and microbial data over the course of critical illness to identify novel longitudinal, trajectories-based phenotypes, to determine the prognostic value for and long-term patient outcomes assessed at 3, 6 and 12 months via physical, cognitive, and mental health outcome measures.
2. To determine the biologic trajectories of novel molecular and transcriptional phenotypes of APS over time and whether phenotype resolution is associated with recovery (Recovery = survival; 1-item recovery question; WHODAS ≤ baseline WHODAS), and compare their natural course to previously described phenotypes.

16.4.3 Aim 4 Study Population
All participants enrolled in the APS Consortium will be eligible to participate in Substudy D.

16.4.4 Aim 4 Study Design
Prospective molecular cohort study with scheduled blood and microbiome sampling during the index hospitalization and with longitudinal follow-up at 3, 6, and 12 months after critical illness.

16.4.5 Aim 4 Study Procedures

Clinical and molecular data generated in the previously described in Aims 1, 2, and 3 will be collated and standardized.

16.4.6 Aim 4 Data, Images, and Biospecimens Used from the APS Consortium

Clinical data including syndromic phenotype (ARDS, pneumonia, sepsis), presence/type of infection, risk factors for ARDS, demographic information, comorbidities, severity of illness, physiologic data, and outcomes (survival, duration of organ support) will be utilized from each participant's inpatient admission. Clinical laboratory values and treatments will also be collected. Long term outcomes including measures of functional status, quality of life, emotional health, cognitive performance, and respiratory symptoms at 3, 6, and 12 months will also be tested outcomes, and will be adjusted for metrics of pre-illness health. Images from the inpatient stay or the longitudinal 12 month visit will be utilized, and from longitudinal muscle ultrasound, will be used.

Biological samples will include the results from all assays performed on the plasma, host whole blood RNA expression, and pathogen and microbiome assessment from samples obtained on days 0, 2, 4, and 6, along with respiratory sample profiling (HME filter fluid proteins) obtained at intubation and day 6.

16.4.7 Aim 4 Statistical Analysis

It is recognized that methods for analysis continue to be developed and evaluated. A detailed SAP will be generated before analysis begins, in which we will specify the method considered optimal at the time or, if an optimal method cannot be easily determined, competing methods will be compared. The following sections outlines a general approach that might be expected undertaken for this aim.

16.4.7.1 Evaluate existing phenotypes

Initially, we propose that for each cohort, at each time point, we will take two complementary modelling approaches to identify classes and/or phenotypes: 1) latent class analysis (LCA); 2) parsimonious classifier model (or these will be substituted or augmented if comparable of better preforming models are identified). To date, LCA has been the gold-standard algorithm to identify the molecular phenotypes at cross-sectional timepoints. However, LCA uses variables standardized and scaled to the distribution of each cohort / timepoint. Therefore, at later timepoints, as disease states resolve and/or therapies take effect, physiology will likely normalize, leading to a divergence in the statistical solution and biological signature captured by LCA. It is conceivable, that while LCA at later time points may identify two classes, their biological characteristics may differ from those identified at baseline, as overall distributions in the population changes. Thus, we currently propose to evaluate the temporal kinetics of the biological signature of the previously derived "molecular phenotypes", defined as Hypoinflammatory and Hyperinflammatory phenotypes identified using LCA at enrollment. At each time point, we will fit a parsimonious logistic regression classifier model which, unlike LCA which uses z-score standardized variables, uses the original scales of protein biomarkers and clinical variables;[7] therefore, it is more likely to capture the biological signatures that define the phenotypes.

The variables used to perform LCA will be similar to those used in our prior studies and include clinical variables and protein biomarkers. Non-normally distributed data will be log-transformed and continuous variables will be scaled using z-scoring using values specific to the study day. Outcome data and severity scores (e.g., APACHE and SOFA scores) will be excluded from the modelling. We will build a minimum of five models, comprising of 1 to 5 classes respectively. We will use fit statistics and the Vuong-Lo-Mendall-Rubin test to determine the best fitting model in each cohort at each timepoint. Once identified, we will use the highest probability generated by the best fitting model to assign class membership. We will use latent transition modelling to evaluate the temporal stability of the classes at sequential time points. To establish overlap with the baseline molecular phenotypes, we will use the parsimonious classifier models comprising of IL-8, protein C, and serum bicarbonate. We plan to use a probability of $\geq$ 0.5 to assign molecular phenotypes. We will perform mixed effect modeling assessing for overall group differences in parsimonious model probabilities over time between survivorship groups in each phenotype independently. We will perform unadjusted (main) and confounder-adjusted analysis of probabilities trajectories, to ascertain cluster association with outcomes.

For gene-based existing phenotypes, we will use repeated RNA-sequencing data to classify patients to the various phenotypes (SRS, MARS, etc.). We will use the probabilities of belonging to the phenotypes on each day to model for trajectories and use outcome data to evaluate the prognostic value of the changing probabilities and switching of phenotypes, adjusting the models for treatment such as corticosteroids.

16.4.7.2 Discovery of novel phenotypes

For discovery of novel phenotypes that are based on trajectories of patient characteristics, we will use two approaches: 1) trajectories-based latent class analysis; and 2) group-based trajectories modelling (or these will be substituted or augmented if comparable of better preforming models are identified). Given the computational demands of trajectories-based LCA, we may only perform this modelling on clinical data and protein biomarkers and not on transcriptomics data; if methods for transcriptomic data become feasible they will be considered. The time-dependent LCA will be performed using the procedures and features described in the section above, with the addition of repeated measures to identify novel phenotypes. The group-based trajectories modeling (GBTM) will be performed on the repeated measures of the most important variables identified in the time-dependent latent class analyses, and we will seek overlap between this single variable derived cluster and its corresponding latent class. GBTM, like LCA, is a finite mixture modelling approach, and the rationale for its use to interrogate trajectories of a single variable is that it may be more clinically implementable than a multivariate trajectories LCA. The GBTM trajectory monitoring algorithm computes unique equations of the variable of interest as a function of time, which is used to define a subgroup. Individual patients are then classified into the trajectory subgroups using the equation and how closely their measurements match the function defined by the equation. Depending on the variable of interest in the model, we will adjust the GBTM for the effects of treatments such as corticosteroids.

Both for novel and existing phenotypes, we will leverage the breadth of physiological and biological data to better understand the underlying biology of the phenotypes. We will use differential gene-expression analysis and pathway analyses of RNA-sequencing data to study within group and between group

differences in longitudinal gene-expression patterns. Within groups we will use time as variable stratified by survivorship to further study distinct temporal pathways associated with recovery and death.

Longitudinal models will be fit using mixed-effects models that will include a random effect for individual participants. Similar to Aim 2, we will examine the results of joint longitudinal and time-to-event models to account for various dropout mechanisms.

16.4.8 Aim 4 Adequacy of sample size

While the statistical approaches that will be used in the ultimate analysis will be specified *a priori* in a Statistical Analysis Plan, we provide an assessment of the adequacy of the sample size, recognizing that the final methods will be highly optimized using rapidly developing methodology. For the LCA, one of many potential clustering methods, a sample size of 300-500 observations is generally considered sufficient, and we have shown in the cohort sample size rationale that a sample size of 1500 is sufficient for a cluster analysis with 150 variables or more. In our prior work, we identified well separated classes and demonstrated that the proposed variables in our LCA are high quality. The sample sizes proposed here (n=1500-4000 depending on analysis) for the deeply phenotyped subset, and n=1500 for the mechanically ventilated subset, should be sufficient to interpret fit statistics from the LCA overall, and for each component syndrome of APS. These estimates also include later time points where we anticipate at least 1000-1800 patients having molecular data assayed at Day 6 (depending on the variable), with the sample size expected to be slightly larger in sepsis and pneumonia than for ARDS.[2-7]

16.4.9 Aim 4 Expected Output

The completion of these described study objectives will generate the following expected output:

- Evidence to accept or refute a state change from hyperinflammatory subphenotype to a less inflamed phenotype as a prognostic indicator, which can then be tested as a theragnostic marker in future trials
- Novel biologic subphenotypes that incorporate longitudinal data and more diverse data elements than currently exist, which may yield better tools for prediction of long-term health states
- Novel biologic subphenotypes that may demonstrate unique biology that is both identifiable and pharmacologically targetable

16.4.10 Aim 4 References

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16.5 Appendix E (Aim 5): Determine and interrogate the role of patient comorbidities, exposures, and biophysical constitution in the clinical and biological heterogeneity of ARDS, pneumonia, and sepsis.

16.5.1 Aim 5 Background and Rationale

Background: Efforts to date to understand clinical heterogeneity in ARDS, pneumonia, and sepsis have focused on biological measurements (e.g., plasma biomarkers and vital signs) obtained at the time of acute illness. Yet chronic and constitutional elements of patient biology are important in APS trajectories, both in acute and long-term outcomes. *Rationale*: By performing granular measurements of patients' comorbidities, exposures, and biophysical constitution, we will determine their relative influence and significance in the heterogeneity of APS conditions. This knowledge will equip us to design and trial tailored therapies, informed by each patient's underlying clinical substrate.

16.5.2 Aim 5 Study Objectives

Primary Objective (Aim 5):

We will assess the association between patients' comorbidities, environmental exposures, and biophysical constitution with membership in both previously described and newly identified phenotypes of ARDS, pneumonia, and sepsis. This approach will allow us to identify the contribution of pre-acute illness factors to the pathophysiology of ARDS, pneumonia, and sepsis. There is no intent to develop novel phenotypes under this aim. We will assess the association between exposure history and clinical syndromes, existing molecular phenotypes (e.g., hypo/hyperinflammatory, SRS classes, MARS classes), and phenotypes newly developed under the other aims of this protocol.

The comorbidities of interest are cancer, diabetes mellitus, and cirrhosis; current state and history of these conditions will be considered. The Functional Comorbidity index will also be assessed for contribution to phenotype assignment.

The environmental exposures of interest are cigarette smoking, alcohol use measured using the AUDIT and via blood phosphatidylethanol (PeTH) concentration, air quality index based on 9-digit zip code of residence, and drug use based on urine toxicology screen.

Measures of biophysical constitution are biological sex, age, and body composition (BMI, CT scan imaging), as well as disability assessed using the WHODAS II, cognition assessed using the IQCODE, and frailty assessed using the Clinical Frailty Score.

Exploratory Objectives (Aim 5):

We will assess whether patients' comorbidities, environmental exposures, and biophysical constitution contribute to short-term and long-term outcomes of ARDS, pneumonia, and sepsis, and whether these factors modify the association between phenotype membership and outcomes. Short-term outcomes

include mortality, hospital length of stay, and ICU length of stay. Long-term outcomes include 3-, 6-, and 12-month assessments of physical, respiratory, cognitive, and mental health status.

16.5.3 Aim 5 Study Population
The study population will be the complete cohort of 4000 hospitalized patients as described in the study protocol.

16.5.4 Aim 5 Study Design
Prospective observational cohort study.

16.5.5 Aim 5 Study Procedures
As detailed in the protocol, for each patient we will use surveys to assess the following:

Biophysical constitution: Comorbidities (Functional Comorbidity Index), disability (WHODAS II), cognition (IQCODE), frailty (Clinical Frailty Score), and obesity (BMI). We will also obtain DICOM images from clinically acquired chest and abdominal CT scans to derive indices of body composition.

Psychosocial factors: Social vulnerability (social vulnerability index), social isolation (NHATS 6-item)

Exposures: Air pollution (AQI), tobacco, alcohol (AUDIT). We will also obtain phosphatidylethanol measurements from plasma specimens to estimate cumulative alcohol consumption.

16.5.6 Aim 5 Data, Images, and Biospecimens Used from the APS Consortium
In addition to the listed surveys, we will collect DICOM images from clinically acquired chest and abdominal CT scans to derive indices of body composition and will measure phosphatidylethanol in plasma to estimate alcohol consumption.

16.5.7 Aim 5 Statistical Analysis
As noted for other aims, analyses will either compare outcomes or characteristics between classes, or will identify *de novo* classes. Following the principles laid out in the protocol, each analysis will be pre-specified in a statistical analysis plan. When an optimal approach is not clear, different methods will be compared; comparison of different approaches will be a strong contribution to the methods literature. We also note that discordance between methods can identify informative features and help to further interrogate individual approaches for biological meaning. we have offered multiple approaches for identifying *de novo* classes, for example in Aims 1, 3 and 6. We will follow one of these approaches (or another approach that is considered optimal at the time), replacing the data used in that aim with patient comorbidity, exposure, and biophysical constitution related features.

16.5.8 Aim 5 Adequacy of sample size
Aim 5 includes all 4000 participants. As described in the rationale for the cohort size, this is sufficient for both supervised (e.g., outcome prediction) and unsupervised (e.g. clustering) considering 500 features of

more. Also, when n=4000 it is possible to estimate odds ratios of about 0.8 or 1.2 with the half width of the confidence intervals being 0.1 or smaller. This aim has robust statistical power for understanding the role of comorbidities and biophysical constitution on both APS phenotypes and outcomes from ARDS, pneumonia and sepsis.

16.5.9 Aim 5 Expected Output

These analyses will provide us with the following new insights into APS conditions:

- How patient comorbidities differ across previously described subphenotypes and newly identified subphenotypes
- How patient exposures (tobacco, alcohol, air pollution) differ across previously-described subphenotypes and newly identified subphenotypes; whether patient exposures represent a "treatable trait" that module patient risk for clinically unfavorable heterogeneity
- How biophysical constitution (as assessed using body composition and integrative functional assesssments) contributes to acute and long-term trajectories of APS conditions
- How these contributing factors interact with common ICU interventions (corticosteroids, neuromuscular blockade, early mobilization and antibiotics) as related to acute and long-term outcomes

16.6 Appendix F (Aim 6): Develop approaches for translating phenotypes to the bedside to enable follow-on precision clinical trials.

16.6.1 Aim 6 Background and Rationale

ARDS, sepsis, and pneumonia cause severe morbidity and mortality globally. While supportive care has improved outcomes over the last decades, persistently high morbidity and mortality indicate that new treatments are needed. Unfortunately, the history of trials in ARDS, sepsis, and pneumonia has been disappointing. At most 5% of Phase 3 trials in critical care demonstrate efficacy,[1–3] even in settings where pre-clinical data has been promising.[4,5] This finding is likely due to improperly specified target populations, inadequate predictive enrichment, and inappropriate trial endpoints.[6–9]

Robust statistical/machine-learning techniques hold promise for the definition of relevant subgroups of patients within complex syndromes. Such subgroups may include modifiable biological pathways (e.g., fibrosis and repair), an influential polymorphism (e.g., IL-6 promoter),[10] a comorbidity (e.g., diabetes mellitus), or an important social context (e.g., economic deprivation). Identification of such subgroups holds promise as the foundation of a subsequent generation of targeted therapeutics.

The focus in this appendix is on phenotypes as actionable target populations—i.e., homogenous, treatment-responsive subgroups of patients identifiable at the time a treatment decision must be made. We do so recognizing that such phenotype identification has improved clinical care in, e.g., pulmonary hypertension[11] and asthma.[12] Multiple analytic aspects are important to accomplishing the ultimate goal in this appendix of designing high-quality trials in the right target populations within ARDS, pneumonia, and sepsis, with higher probability of success.

Logistically, the use of phenotypes as actionable target populations includes two critical components.

The first component is the ability to identify phenotype membership at the time a treatment decision must be made. Usual phenotyping methods are based on high-dimensional data in the derivation and validation cohorts and cannot be straightforwardly applied in clinical practice. In order to be used in clinical practice, simpler, parsimonious definitions are required. These parsimonious models utilize a restricted set of variables—all of which can be obtained clinically—to identify phenotype membership in real time. Treatment decisions can then be made based upon the phenotype assignments of the parsimonious models. Substantial progress in machine learning, combined with the richness of the APS Consortium cohort dataset, makes possible the development and validation of such classification rules for application in future trials.

The second component is to identify the most promising future trial candidates in ARDS, pneumonia, and sepsis, whether repurposed agents (i.e., those already in clinical use among patients enrolled in the APS Phenotypes Consortium cohort) or hypothetical novel agents (i.e., the expected effect of modulating a protein biomarker or other biological signal among Consortium patients). Fortunately, statistical methods for causal inference and observational data methods allow for the performance of simulated clinical trials in a way that has been demonstrated to closely approximate inferences drawn from prospective randomized trials.[13] These methods of "target trial emulation" provide the opportunity to make key observations relevant to the design and conduct of future clinical trials.[14]

16.6.2 Aim 6 Study Objectives

Primary Objective (Aim 6):

Aim 6a. Perform target trial emulations of observed or potential novel therapies, with prespecified assessment of possible treatment effect heterogeneity. As an illustrative example of one such emulation, we will estimate the effect of a potential DAMP-blocking agent administered to lower DAMP to the below the median level versus usual care alone on all-cause mortality at 60 days. The baseline historic syndrome (ARDS, pneumonia, or sepsis) will be the primary subgroups for treatment effect heterogeneity.

Aim 6b. Derive and validate parsimonious classification rules using clinically available baseline data to assign patients to APS-identified phenotypes.

Exploratory Objectives (Aim 6):

Aim 6a:

- Assess treatment effect heterogeneity within target trial emulations based on (a) the hyper- vs. hypo-inflammatory phenotypes identified by Calfee and colleagues and (b) novel phenotypes identified within the APS Consortium
- Assess secondary endpoints for the target trial emulation, including mortality at 30- and 90-days, ICU-free days, organ-support-free days, WHO ordinal outcome scale, and the 12-month outcomes measured among the long-term outcome cohort within the APS Consortium.

Aim 6b:

- Compare the accuracy of (a) classification rules restricted to currently available clinical values with (b) classification rules that incorporate results that could feasibly be reduced to clinical application (e.g., cytokine results that could be performed at the point of care).

16.6.3 Aim 6 Study Population
All enrolled APS Phenotypes Consortium cohort patients

16.6.4 Aim 6 Study Design

No additional laboratory procedures will be performed for this Aim.

Objective 1 will be approached using statistical learning classification models based on clinically available data and/or parsimonious biological data, we will derive and validate classification models, with an intraclass correlation coefficient (ICC) ≥ 0.75.

Objective 2 will be approached using treatment normalization, causal inference, and design of simulated trials using structured design deliberations and target trial emulation techniques. Using these methods, we

will define and perform 5 high-priority trial simulations. Results of those simulated trials will be reported back to the Steering Committee and NIH.

16.6.5 Aim 6 Study Procedures

No additional non-statistical procedures will be performed for this aim beyond those in the main protocol and the Appendices that generate biomarker data.

16.6.6 Aim 6 Data, Images, and Biospecimens Used from the APS Consortium

All data, with the exception of biomarkers or other biological assays (e.g., microbiome) that are not expected to be reducible to a clinically relevant turnaround time (i.e., biomarkers or other biological assays that would take >24h to return results). These data centrally include (1) medications and other therapies (e.g., respiratory support modes) for simulated trials of repurposed medications and (2) protein biomarkers and other biological assays (e.g., transcriptomic results given recent progress in turn-around times for peripheral blood RNA sequencing) for simulated trials of potential novel agents that modulate a given biological signal. The biomarkers and other bioassays proposed in the APS Phenotypes Consortium cohort Aims have been chosen on the basis of their expected causal roles in ARDS, pneumonia and sepsis pathophysiology. It is therefore anticipated that the output of simulated trials of potential novel agents will be highly relevant to the development and design of future clinical trials.

16.6.7 Aim 6 Adequacy of sample size

The statistical approaches that will be used in the ultimate analysis will be specified *a priori* in a Statistical Analysis Plan. Here, we provide an assessment of the adequacy of the sample size based on reasonable assumptions, recognizing that the final methods will be highly optimized using rapidly developing methodology.

For Objective 1, historically, formal sample size calculations have not been performed for classification problems such as these, with general reliance on heuristics (e.g., 5–7 observations per variable in the classification problem). Nevertheless, Walter's method[15] makes possible a power estimate based on comparisons against a null hypothesis for ICC. In this case, an overall sample size of 460 provides approximately 90% power to detect an ICC difference of 0.05 against a null hypothesis of 0.70, 80% with a null of 0.60, and 70% with a null of 0.50. (Note that this sample size refers to the cohort size for all phenotypes for a given phenotyping problem, rather than the size of any individual phenotype within the overall cohort.)

For Objective 2, the overall sample size of 4,000 is 2.5–10 times larger than other prospective phenotyping efforts in this disease area. Recognizing the complexities of the data and associated analyses, power estimates by simulation would be heavily assumption-laden. We focus here on power associated with trial emulation. A conservative assessment of power can be determined by considering inference about the weighted average causal effect obtained using matching weights.[16]

This approach mimics 1:1 propensity score matching and emphasizes trials that enroll participants whose propensity scores are close to 0.5.[16] This is consistent with the goal of trial emulation, as patients with propensities close to 0.5 represent patients for whom greater equipoise exists. Using the analogue to 1:1 matching, we can estimate the minimum detectable treatment effects, based on the sample size of the

smaller of the two comparison groups (Table 14). The table rows apply either to the full cohort or to a subgroup of interest when evaluating treatment effect heterogeneity.

16.6.7 Aim 6 Statistical Analysis

As noted for other aims, following the principles laid out in the protocol, each analysis will be pre-specified in an SAP. When an optimal approach is not clear, different methods may be compared; comparison of different approaches will be a strong contribution to the methods literature. Below, we outline the general direction that we plan to undertake to accomplish this aim.

For Objective 1, we plan to first pursue a dimension reduction exercise (e.g., penalized regression) to identify the variables most likely to identify class membership. The next step will be use of a machine learning technique (e.g., classification and regression trees) to derive the classification rule. Validation can be performed by assessment of the ICC, comparing the underlying phenotype assignment with the classification generated by the classification rule.

For Objective 2, a rigorous, multi-stage method will be employed to minimize bias and maximize the probability of accurate inference. The key features of the multi-stage method are treatment normalization, phenotype identification, phenotype assignment (e.g., using the methods of Objective 1), definition and prioritization of simulated trials, and the actual performance of the simulated trials. The phases of analysis are displayed in Table 14.

Table 14. Aim 6: Flow of analysis for Objective 2.

Step	Task	Methods	Output
A1	Account for the role of treatment in characterization of phenotypes	Normalize disease trajectories via dynamic treatment regime and inverse probability weighting	A normalized pseudo-cohort for phenotyping
A2	Identify disease phenotypes using longitudinal data	Hidden Markov Models and machine-learning techniques	3–5 distinct disease phenotypes
A3	Predict disease phenotypes for individual patients	Elastic net multinomial regression	Patient-level probabilities of phenotype membership
A4	Define and prioritize trial emulations	Structured Steering Committee review of phenotype attributes and potential treatments	Prioritized list of ≤5 trial emulations
A5	Identify efficacy signals for treatments among phenotypes	Target trial emulations with exploration of treatment effect heterogeneity by phenotype	Results of trial emulations, by phenotype

Treatment normalization is a crucial initial step, given the risk that variations in treatment may shape the measured features for phenotyping, independent of the patient's acute biology and premorbid status. Treatments may change over time, and their effect may similarly vary over time. Thus, for example, glucocorticoids decrease inflammatory biomarkers,[17-19] while low-tidal volume ventilation decreases

inflammatory markers, including interleukin-6.[20] Failure to control for the dynamic effect of treatment would thus introduce severe bias, motivating techniques to control for treatment over time. Treatment normalization generates a reweighted cohort on which the relevant phenotyping (and phenotype assignment; steps A2–A3) are performed. After the results of these initial steps, a structured deliberation at the Steering Committee level (step A4) before performance of simulated trials will minimize the risk of alpha inflation (i.e., false positive inferences from simulated trials). The performance of simulated trials (step A5) will follow valid techniques for causal inference, including appropriate cohort definition and simulated treatment plus adjustment for propensity to receive the treatment. For repurposed medications, the treatment definition is simple and familiar—the treatment actually received by the patient in the cohort. In addition to repurposed medications, the simulated trials are able to include assessment of potential novel treatments. For simulated trials of potential novel treatments, levels of measured biomarkers are used as surrogates for treatments that could modulate a biomarker level by a certain level (e.g., assess a hypothetical agent that would lower plasma levels of sRAGE by 50%). Continuous-variable approaches to treatment and treatment adjustment are used to support these analyses for hypothetical novel treatments.[21,22] Note that where a given phenotype has a valid classification rule (as developed in Objective 1), that classification rule will be used for the simulated trials to improve generalizability.

16.6.8 Aim 6 Adequacy of sample size

The statistical approaches that will be used in the ultimate analysis will be specified *a priori* in a Statistical Analysis Plan. Here, we provide an assessment of the adequacy of the sample size based on the approach described above, recognizing that the final methods will be highly optimized using rapidly developing methodology.

As described in the rationale for the cohort sample size, formal sample size calculations have traditionally not been performed for classification problems such as these, with general reliance on heuristics (e.g., 5–7 observations per variable in the classification problem). We have shown that with the overall sample size of 4,000, cluster analyses with as many as 500 features are feasible. As well as the approach described in the sample size justification, Walter's method[15] makes possible a power estimate based on comparisons against a null hypothesis for ICC – the ICC is used as a measure of concordance between actual and predicted class membership with a high ICC reflecting good concordance. Sample sizes calculated in this way are consistent with sample sizes based on the correlation coefficient or detectable difference approach; an overall sample size of 460 provides approximately 90% power to detect an ICC difference of 0.05 against a null hypothesis of 0.70, 80% with a null of 0.60, and 70% with a null of 0.50.

For objective 2, we focus on power associated with trial emulation. A conservative assessment of power can be determined by considering inference about the weighted average causal effect obtained using matching weights.[16] This approach mimics 1:1 propensity score matching and emphasizes trials that enroll participants whose propensity scores are close to 0.5.[16] This is consistent with the goal of trial emulation, as patients with propensities close to 0.5 represent patients for whom greater equipoise exists. Using the analogue to 1:1 matching, we can estimate the minimum detectable treatment effects, based on the sample size of the smaller of the two comparison groups (Table 15). The table rows apply either to the full cohort or to a subgroup of interest when evaluating treatment effect heterogeneity, and show that small to modest differences can be observed even in the smaller subgroups.

Table 15. Aim 6: Minimum detectable average treatment effect (90% power, 2-sided α = 0.05)

Evaluable patients in smaller group (N)	Detectable mean difference in continuous outcome (% SD)	Detectable % reduction in hazard ratio		
		10% of pts with events	20% of pts with events	40% of pts with events
200	32.5%	64.1%	51.5%	40.1%
400	22.9%	51.5%	40.1%	30.4%
800	16.2%	40.1%	30.4%	22.6%
1200	13.2%	34.2%	25.6%	18.9%

16.6.9 Aim 6 Expected Output

The expected output from Objective 1 is a classification rule for each major phenotyping task for which ≥ 460 overall patients are available. These classification rules can be immediately applied to future patient populations for future trials.

The expected output from Objective 2 is a suite of simulated trials of repurposed and/or hypothetical novel therapies. These trials would be used to motivate and to design next-generation trials in ARDS, pneumonia, and sepsis.

16.6.10 Aim 6 References

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16.7 Appendix G (Aim 7): Determine whether APS phenotypes identify differences in multiple organ dysfunction syndrome (MODS).

16.7.1 Aim 7 Background and Rationale

Multiple organ dysfunction syndrome (MODS) is a life-threatening disruption of organismal homeostasis. MODS is the hallmark of sepsis, is extremely common in ARDS, and is frequently present in pneumonia treated with respiratory support. In health, organs maintain their own homeostasis and communicate with other organs to maintain organismal homeostasis, a phenomenon termed crosstalk. The physiology of MODS is highly complex, in terms of the range of initial organs affected, disruptions in crosstalk pathways, and the pattern of organ dysfunction. In its terminal stages, MODS appears to be a final common pathway of a fatal insult; prior to those terminal stages, however, MODS may be a diverse syndrome. By convention, MODS in critically ill populations has been graded in terms of severity, using one of several scoring systems, e.g., the Sequential Organ Failure Assessment (SOFA).[1] Prior work in sepsis-related organ dysfunction has suggested various phenotypes that differ in clinical presentation and possible treatment effect heterogeneity in prior sepsis trials.[2] While these clinical phenotyping efforts provide preliminary evidence supporting the existence of phenotyping, deep phenotyping of MODS has not yet occurred. This Appendix seeks to determine whether deep phenotyping will identify distinct phenotypes within MODS.

16.7.2 Aim 7 Study Objectives

Primary Objective (Aim 7):

To determine whether the pattern of multiple organ dysfunction syndrome (MODS) differs by APS-identified phenotypes, using dimension reduction techniques to summarize the baseline Sequential Organ Failure Assessment (SOFA) scores, the most common clinical measure of MODS.

Exploratory Objectives (Aim 7):

1. Explore associations among constituent biomarkers (i.e., those used to define the APS phenotypes) and SOFA sub-scores at baseline.
2. Explore evolution of MODS over time, including its interaction with longitudinal phenotypes.
3. Explore the implications of alternative definitions of organ dysfunction (e.g., incorporation of lactate levels into cardiovascular sub-score, alternative methods of central nervous system dysfunction, relevance of specific biomarkers to organ failure definition)

16.7.3 Aim 7 Study Population

All APS Phenotypes Consortium Cohort Patients (N=4000)

16.7.4 Aim 7 Study Design

Broadly, dimension reduction techniques will be used to compare the distribution of organ dysfunction among the phenotypes identified within the APS Cohort.

16.7.5 Aim 7 Study Procedures

No additional laboratory procedures will be performed for this Aim. Procedures for this Aim are statistical and are outlined below. SOFA subscores will be calculated from clinically available results.

16.7.6 Aim 7 Data, Images, and Biospecimens Used from the APS Consortium

All available data results will be available for the underlying phenotyping. For the definition of MODS, daily SOFA scores will be utilized. (Based on previous analyses, last observation carried forward (LOCF) imputation will be employed for SOFA subscore constituents that are not obtained clinically.)

16.7.7 Aim 7 Statistical Analysis

The analysis for Aim 7 will follow principles of reproducibility and validation laid out on the protocol. There are two aspects of the proposed analysis: first is to use a dimension reduction technique such as PCA on the components of the SOFA score. This is expected to generate interpretable principal components reflecting combinations of organ dysfunction. Additional variables reflecting organ-specific functionality may be included in the analysis to fully capture dysfunction. Then, the principal components can be compared among *de novo* phenotypes to determine whether MODS differs among both previously derived and novel phenotypes. Comparison among phenotypes can utilize multiple group rank-based statistics (e.g., Wilcoxon rank sum) or regression modeling.

16.7.8 Aim 7 Adequacy of sample size

As indicated in the master protocol, there is no clear method for establishing precise sample sizes for unsupervised analyses such as clustering. We have shown in the rationale for the cohort sample size that group sizes of 2000 or more sufficient for identifying clusters when using 500 features or more. For comparison among phenotypes, we have shown that a small effect such as an odds ratio of 0.8 can be estimated with a half width of the confidence interval ranging from about 0.1 to 0.2 under the full range of assumptions.

16.7.9 Aim 7 Expected Output

It is expected that this Appendix will identify relevant differences in the distribution of organ failure among the APS phenotypes. It is anticipated that this will further illuminate the possibility of phenotypes that span the historic syndromes of ARDS, pneumonia, and sepsis.

16.7.10 Aim 7 References

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