Study Title: A Multi-Center Observational Study: The RECOVER Post Acute Sequelae of SARS-CoV-2 (PASC) Pediatric Cohort Study

Short plain language title: Understanding the long-term impact of COVID on children and families

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Statement of Compliance

This study will be conducted in accordance with the Code of Federal Regulations on the Protection of Human Subjects (45 CFR Part 46), any other applicable US government research regulations, and institutional research policies and procedures. The Principal Investigator will assure that no deviation from, or changes to the protocol will take place without prior agreement from the sponsor and documented approval from the Institutional Review Board (IRB), except where necessary to eliminate an immediate hazard(s) to the study participants. All personnel involved in the conduct of this study have completed Human Subjects Protection Training.

List of Abbreviations

ABCD study	Adolescent Brain Cognitive Development study
AE	Adverse Event/Adverse Experience
ATO	Authority to Operate
CBCL	Child Behavior Checklist
CDC	Centers for Disease Control
CFR	Code of Federal Regulations
CI	Confidence Interval
CLIA	Clinical Laboratory Improvement Amendments
COVID-19	Coronavirus Disease 2019
CRF	Case Report Form
DRC	Data Resource Core
EDTA	Ethylenediaminetetraacetic acid
EHR	Electronic Health Record
FISMA	Federal Information Security Modernization Act of 2002
HIPAA	Health Insurance Portability and Accountability Act of 1996
ICF	Informed Consent Form
IRB	Institutional Review Board
IRR	Incident Rate Ratio
MIS-C	Multisystem Inflammatory Syndrome In Children
MOP	Manual of Procedures
MRI	Magnetic Resonance Imaging
N	Number (typically refers to participants)
NHLBI	National Heart, Lung, and Blood Institute
NIH	National Institutes of Health
NYULH	New York University Langone Health
OSMB	Observational Study Monitoring Board
PASC	Post-Acute Sequelae of SARS-CoV-2
PBMC	Peripheral Blood Mononuclear Cells
PHI	Private Health Information
PI	Principal Investigator
PII	Personal Identifiable Information
Post-vax MC	Post-COVID vaccine myocarditis
RECOVER	Researching COVID to Enhance Recovery
REDCap	Research Electronic Data Capture
SST	Serum Separator Tube
US	United States
UUID	Universal Unique identifier
WGS	Whole Genome Sequencing
WHO	World Health Organization

1 Summary of Protocol Revisions

March 1, 2022: Version 2.2 to 2.3

Location and description of revision	Rationale for revision
Section 8.2: Added description of IRB-approved eligibility screener for recruitment	To standardize screening procedures at enrolling sites
Section 8.3: Added description of randomization of uninfected control participants for Tier 2 longitudinal follow-up	To maintain harmonization with the updated statistical analysis plan
Section 10.3: Changed description of intermediate visits after newly identified infection	To clarify study procedures and harmonize with the updated acute Tier 2 visit schedule described in revised section 11.4
Section 11.4 and Appendix B: Reduced number of acute Tier 2 remote visits	To reduce participant burden and increase feasibility of the acute Tier 2 procedures
Section 11: Added text to indicate that EHR and mobile health platform may be used to collect health status and symptom data during longitudinal follow up in acute and post-acute Tier 2	Clarification
Section 12.3: Changed the event reporting requirements	To align with NYU IRB reporting requirements
Remove Appendix F	To eliminate redundancy with other sections of the protocol

January 31, 2022: Version 2.1 to 2.2

Location and description of revision	Rationale for revision
Section 9 clarifications	Clarified entry criteria for primary caregiver, other biological parent, and children/young adults with history of MIS-C.
Section 10 new study visit procedure	Added description of intermediate visit procedures in case of a newly discovered SARS-CoV-2 infection or re-infection after study entry.
Section 11 minor changes in study procedures and biospecimen collection	1.Simplified post-acute Tier 2 visit schedule for pediatric main cohort to harmonize with pediatric statistical analysis plan revision. Visits will be timed from date of study entry instead of date of COVID infection. 2. Added description of Tasso M-20 blood collection device for home Tier 1 blood spot collection. 3. Added Tier 2 and Tier 3 optional blood collection for MIS-C cohort
Sections 9 and 15 clarification of study recruitment procedures	Clarification of use of supporting materials to enhance understanding of the study as part of the informed consent process.

December 24, 2021: Version 2.0 to 2.1

Location and description of revision	Rationale for revision
Title Page	Addition of clinicaltrials.gov registration number; updating contact information
Section 6 and 15	Clarifications of procedures and risks for return of new information from incidental findings and genetic testing

Location and description of revision	Rationale for revision
Section 11	Clarifications of timing of study visits for post-acute Tier 2 main cohort, MIS-C and post-vaccine myocarditis sub-cohorts. Reformatting and minor edits to the Tier 3 panel of neurocognitive testing.
Appendix F	Updated to maintain consistency with the revised protocol sections

December 10, 2021: Version 1.0 to 2.0:

Location and description of revision	Rationale for revision
Section 1 added	To provide summary of protocol revisions
Section 4 Figure 1 Overview of Study Protocol Enrollment changed	More detailed overview of the study protocol enrollment to be harmonized with the pediatric statistical analysis plan
Section 5 Background updated	Added relevant citations published since last version
Section 6 Risks updated	Clarify risk mitigation by age of participant
Section 8 Study Design updated and re-organized	Clarify description of cohort types, recruitment strategies and recruitment pools
Section 9 Study enrollment updated	Clarify study entry criteria
Section 10 Study Schedule updated	Clarify scheduling of study visits
Section 11 Study Procedures re-organized	Clarify study procedures by age of participant and sub-cohort
Section 11 Study Procedures revised	To reduce participant burden
Section 14 Statistical considerations updated; Data management plan updated	Clarify plan for subgroup analysis by age group; data management plan harmonized with RECOVER DRC manual of operations
Section 15 Ethics/Protection of human subjects updated; Data security section updated	Clarify informed consent procedures; added additional information on data security procedures to harmonize with RECOVER DRC manual of operations
Appendix figures revised	Updated schedule of assessment figures and tables

2 Protocol Summary

Title	A Multi-Center Observational Study: The RECOVER Post-Acute Sequelae of SARS-CoV-2 (PASC) Pediatric Cohort Study
Short Title	Understanding the long-term impact of COVID on children and families
Brief Summary	This is a combined retrospective and prospective, longitudinal, observational meta-cohort of individuals ages newborn-25 years who will enter the cohort with and without SARS-CoV-2 infection at varying stages before and after infection. Individuals with and without SARS-CoV-2 infection and with or without PASC symptoms will be followed to identify risk factors and occurrence of PASC. This study recruit participants inpatient, outpatient, and community-based settings in the United States. Study data including age, demographics, social determinants of health, medical history, vaccination history, details of acute SARS-CoV-2 infection, overall health and physical function, and PASC symptoms will be reported by participants or collected from the electronic health record using a case report form at specified intervals. Biologic specimens will be collected at specified intervals, with some tests performed in local clinical laboratories and others performed by centralized research centers or banked in the Biospecimen Repository. Advanced clinical and radiologic examinations will be performed at local study sites with cross-site standardization.
Objectives	1. Characterize the incidence and prevalence of sequelae of SARS-CoV-2 infection occurring >30 days after study entry. 2. Characterize the spectrum of clinical symptoms, subclinical organ dysfunction, natural history, and distinct phenotypes identified as sequelae of SARS-CoV-2 infection occurring >30 days after study entry. 3. Define the biological mechanisms underlying pathogenesis of the sequelae of SARS-CoV-2 infection occurring >30 days after study entry.
Methodology	Ambidirectional longitudinal meta-cohort study (combined retrospective and prospective) with nested case-control studies.
Endpoints	Primary Endpoint: Presence of candidate PASC symptoms over time. Secondary Endpoints: Clinical and biological recovery trajectories from SARS-CoV-2 infection; clinical and subclinical organ injury; incident post-SARS-CoV-2 clinical disease.
Study Duration	Four years
Participant Duration	Up to four years after study entry

Population	Infected: Individuals ages newborn-25 years meeting WHO criteria for suspected, probable or confirmed SARS-CoV-2 infection on or after March 1, 2020, including participants with history of MIS-C, and infants born to a mother meeting the same infected criteria during pregnancy. Uninfected: Individuals ages newborn-25 years who have never met any of the WHO criteria for suspected, probable or confirmed SARS-CoV-2 infection, including infants born to a mother meeting the same uninfected criteria during pregnancy. Individuals age 3-25 years with history of post-COVID vaccine myocarditis. The primary caregiver of the child (or children) enrolled in the RECOVER pediatric cohort may provide consent to participate in prospective data and biological sample collection as specified in the protocol procedures as a member of a caregiver/child dyad. If the identified primary caregiver is a biological parent of the child, the other biological parent may provide consent to participate in prospective biospecimen sample collection.
Study Sites	Phase 2 PASC Consortium Pediatric Cohort Sites (Please see Appendix E)
Number of participants	Up to 20,000 dyads (children and young adults with and without history of SARS-CoV-2 infection and their primary caregiver), including 800 children with MIS-C and 200 children and young adults with history of post-COVID vaccine associated myocarditis. Up to 20,000 caregivers, and 20,000 other biological parents are anticipated to enroll.
Statistical Analysis	A flexible study design is proposed to allow modifications to PASC case definition, tiered phenotyping assessments, comparator groups, and/or statistical plan to optimize public health and scientific impact of study findings. Modifications in study design may be based on analyses of structured cohort data, unstructured cohort EHR data, other cohort EHR data, or other new knowledge of PASC acquired after study initiation.

3 Key Roles

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4 Overview of Study Protocol Enrollment for RECOVER Meta-cohort

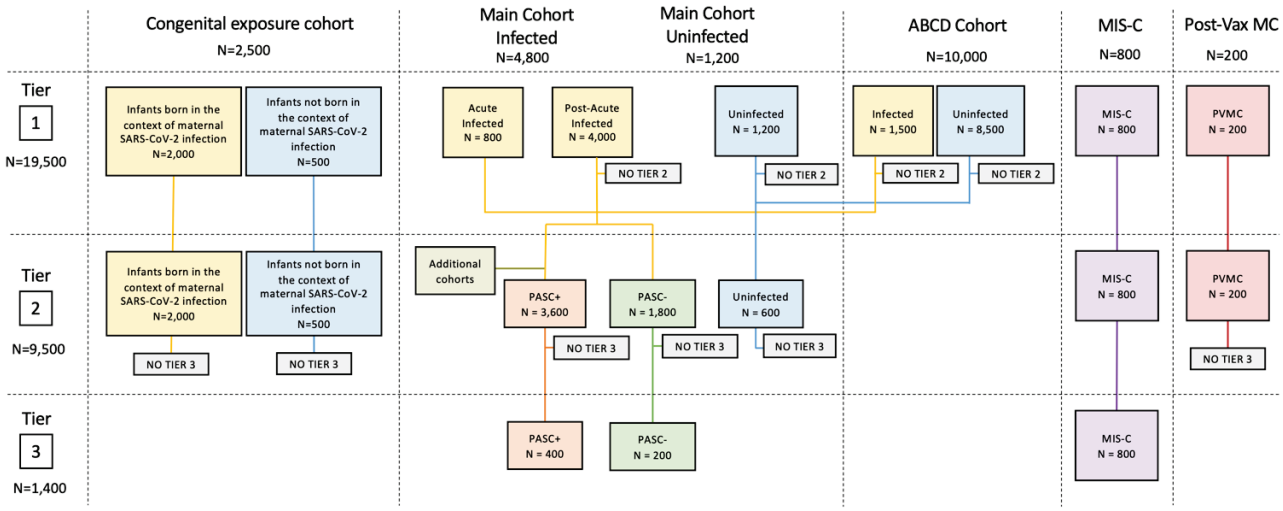


Figure 1. Overview of Study protocol enrollment for RECOVER meta-cohort. Up to 20,000 children and young adults ages newborn-25 years will be enrolled as a meta-cohort, including 2500 infants born to mothers with and without SARS-CoV-2 infection during pregnancy (congenitally exposed infant cohort), 6,000 children and young adults age newborn-25 years from the main PASC cohort with and without SARS-CoV-2 infection, 10,000 adolescents from the Adolescent Brain Cognitive Development (ABCD) study, 800 children and young adults ages 3-25 years with Multisystem Inflammatory Syndrome in Children (MIS-C), and 200 children and young adults age 3-25 years with post-COVID vaccine myocarditis. Each enrolled subject will undergo a tailored tiered testing approach based on history of SARS-CoV-2 infection, presence of PASC symptoms, age range, and other considerations specific to each sub-cohort of the RECOVER meta-cohort. Subjects will be selected for participation in Tiers 2 and 3 based on their history of COVID exposure and PASC symptoms as determined in Tier1 for post-acute participant and uninfected controls, and at the week 8 visit for acute Tier 2 participants. Subjects will be selected for participation in Tiers 2 and Tiers 3 to achieve the enrollment targets as summarized in Figure 1 and to achieve pre-specified targets for age distribution and diversity.

5 Introduction, Background Information and Scientific Rationale

5.1 Background Information and Relevant Literature

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel coronavirus strain that was first detected at the end of 2019. This strain primarily spreads through aerosols in expiratory gases in infected individuals with and without symptoms, resulting in the highly contagious coronavirus disease 2019 (COVID-19) and a global pandemic. As of June 2021, approximately 177 million people were infected with COVID-19, with at least 3.8 million deaths globally (1). COVID-19 positive cases are identified with a SARS-CoV-2 polymerase chain reaction test or an antigen test using saliva, nasopharyngeal or bronchial samples (2). Fever, chills, cough, shortness of breath, fatigue, muscle aches, loss of taste and/or smell, nausea, diarrhea, and other symptoms are typical of the acute phase of the disease (3,4). The spectrum of symptoms in acute SARS-CoV-2 infection in children is similar to adults, but typically less severe, and with minimal or no reported symptoms in almost half of all cases (5). Chest pain or burning, labored breathing, disorientation, and delirium are less common emergent symptoms in both adults and children associated with increased risk of hospitalization and death. Increased age, (6) male sex, obesity, and co-morbidities such as diabetes, cardiac disease, or cancer (3,7) are associated with increased risk of severe COVID-19 disease and death in adults. Additionally, non-White people have experienced much higher rates of infection, severe disease, and death when compared with non-Hispanic Whites (8). Case fatality rates in children with acute COVID-19 are lower than adults. In hospitalized children with COVID-19, age < 2 years, indigenous ethnicity, and presence of ≥2 pre-existing conditions were associated with increased risk of death (9).

Multi-organ injury and dysfunction in acute COVID-19 have been reported in both adults and children (7,9-11). One distinct manifestation of PASC in children became recognized in April 2020, with the first reports from Europe of what is now called Multisystem Inflammatory Syndrome in Children, or MIS-C (13,14). MIS-C was initially defined as a multisystem hyperinflammatory syndrome in children and adolescents temporarily related to COVID-19, with features overlapping with Kawasaki disease and toxic shock syndrome. The case definition was revised by CDC as disease of children <21 years of age characterized by fever, laboratory evidence of inflammation, and clinically severe illness with multisystem organ involvement requiring hospitalization within 4 weeks of a laboratory confirmed SARS-CoV-2 infection (15). MIS-C is considered a subset of PASC because it occurs 4-6 weeks after the acute SARS-CoV-2 infection. More than 5,000 children and adolescents in the US have been diagnosed with MIS-C. Acute COVID-19 in children may also manifest as severe disease with multi-organ involvement but with clinical and laboratory patterns that are distinct from those observed in children with MIS-C (16).

After recovery from acute COVID-19, 30-70% of adults infected report a diverse array of persistent mild to severe symptoms lasting >4 weeks. The persistent symptoms after acute have been termed Long COVID or post COVID-19 condition. The term post-acute sequelae of SARS-CoV-2 (PASC) has been proposed to encompass a broader array of post-COVID-19 disease including evidence of organ dysfunction or emergence of new diseases that may occur independently of symptoms. There is no established definition for any of these terms; the WHO has proposed a working clinical case definition of post COVID-19 condition by a Delphi consensus based on a global consensus of a diverse stakeholders.(12) The WHO working case definition is based on an established history of COVID-19, and presence of symptoms in 12 identified domains that persist over time without alternative explanation.

The underlying pathophysiology of persistent symptoms after SARS-CoV-2 infection is unknown but has been proposed to be attributable to viral persistence, reactivation of other viruses such as Epstein-Barr virus, vascular endothelial damage, small fiber autonomic nerve damage, neuroinflammation in the central nervous system, immune dysregulation including activation of auto-immunity, and organ damage caused by hyper-inflammatory response during the acute phase of the disease (8). Myocarditis has been reported after SARS-CoV-2 infection in young adults and rarely in children after administration of mRNA-based COVID-19 vaccines (13,14); this observation suggests that immunological response to the SARS-CoV-2 spike protein may be contributing to cardiac manifestations of PASC in children with and without evidence of MIS-C. Other contributing causes include complications from critical illness related to prolonged intubation, malnutrition, and prolonged bed rest, and effects of pandemic-related stressors, including disruption of school, disruption of family dynamics by COVID-19 in multiple family members, household financial stress, and disruptions of health care access.

Commonly reported persistent symptoms after SARS-CoV-2 infection include fatigue, post-exertional malaise, dyspnea, cough, chest pain, palpitations, cognitive dysfunction and neuropsychiatric symptoms, gastrointestinal symptoms, extreme thirst, hair loss, and persistent loss of taste or smell (8,15-19). Persistent respiratory symptoms may occur independently of demonstrable abnormalities in lung structure or function (8,15,19). The severity of the acute COVID-19 manifestation has consistently been found to be directly proportional to the severity of post-COVID manifestation, but severe post-acute symptoms have been reported in subjects with mild or asymptomatic acute COVID-19 (15).

The clinical manifestations of non-MIS-C PASC in children are less well characterized when compared with adults with wide-ranging estimates of incidence across available studies. The WHO working definition may not be applicable in children. A study from Italy examined health data in 129 children under the age of 18 obtained via a questionnaire between September 2020 and 1 January 2021. 53% of the group experienced COVID-19 symptoms more than 120 days after their diagnosis. Symptoms included chest tightness and pain, nasal congestion, tiredness, difficulty concentrating and muscle pain (20). Among 55 children hospitalized for COVID-19 in Sweden, 22% were found to have persistent symptoms four months after hospitalization, with fatigue being the most common symptoms (21). An analysis of private health claims data indicated that pain and difficulty breathing were the two most commonly documented medical problems after acute SARS-CoV-2 infection in children ≤18 years of age (33). In 518 hospitalized children age ≤18 years in Russia, 24% reported persistent symptoms after median 256 days after hospital discharge (22). Fatigue, sleep disturbance and sensory disturbance were the most commonly reported symptoms. Increased age and history of allergic

disease were associated with increased risk of persistent symptoms. In a community-based sample of school aged children from the United Kingdom, 4.4% of the children with symptomatic laboratory-confirmed SARS-CoV-2 infection had symptoms lasting ≥28 days. The most frequently reported persistent symptoms were fatigue, headache and anosmia (23). A seroprevalence study of Swiss school children reported overall prevalence of prolonged symptoms <5%, and no difference between children with and without antibodies to SARS-CoV-2 (24). An analysis of electronic health records in 11,950 children/adolescents in Germany, demonstrated increased incidence rate ratio of overall health problems after laboratory COVID-19 diagnosis when compared with uninfected controls (IRR=1.30, 95%-CI=[1.25-1.35]). Malaise/fatigue/exhaustion was the symptom complex with the highest IRR versus controls (2.28, 95%-CI=[[1.71-3.06]) (25).

The goal of this study is to identify, evaluate, and characterize the clinical course of PASC symptoms and gain insight into underlying mechanisms in children, adolescents and young adults ages newborn to 25 years with previous SARS-CoV-2 infections with and without a history of PASC, and in infants born in the context of maternal SARS-CoV-2 infection, and the risk and resiliency factors associated with the severity of the clinical course of PASC. This ambidirectional (combined retrospective and prospective) longitudinal observational cohort study will focus on the long-term effects of SARS-CoV-2 infection, while explicitly considering sex and racial and ethnic disparities in risks and outcomes. Data acquired from this study will provide accurate and quantifiable measures for PASC symptoms in selected case and control populations to allow for comparisons among groups and provide insights into mechanisms related to PASC progression and recovery. Additionally, it is hoped that such findings will identify predictive and prognostic factors for PASC that will inform study design and enrich participant selection for future clinical trials of the prevention and/or treatment of PASC.

5.2 Rationale

PASC stands to pose a profound public health crisis in future years, with anticipated increased risk of morbidity, mortality, and disability in both adults and children. As of August 2021, there were over 37 million diagnosed cases of COVID-19 in the United States; as of the end of October 2021, nearly 25% of new cases were in the pediatric age group (26). The true US prevalence of SARS-CoV-2 infection is likely much greater when accounting for asymptomatic disease, limited access to testing and underreporting, particularly in children (27,28). Given the large number of cases of COVID-19 in the United States, even a low incidence of PASC might affect millions with immense impact on health care resource utilization, and public health measures of morbidity, mortality and disability. The long-term public health impact of PASC may be greater in the pediatric population when compared with adults despite less severe acute disease in children. The incidence, prevalence, phenotypes, risks, and etiology of PASC in US children are currently unknown, limiting opportunities for prevention and treatment (27). Therefore, prospective longitudinal studies in the pediatric population are urgently needed. The proposed study will enroll child or young adult/caregiver dyads to characterize indirects of effects of the SARS-CoV-2 pandemic on study endpoints, and if possible DNA samples from the child or young adult and both biological parents for future studies of genetic risks of long-term effects of SARS-CoV-2 infection. This pediatric study will enhance knowledge of children's recovery from SARS-CoV-2 infections and define and categorize the clinical spectrum and risk factors for PASC and elucidate potential mechanisms to inform future preventive and treatment studies.

6 Potential Risks & Benefits

6.1 Known Potential Risks

All study procedures are considered minimal risk, with exception of Tier 3 exercise testing and sputum induction, which are considered to be a minor increase over minimal risk. All study procedures will be performed when clinically appropriate based on age and investigator judgment. Participation in the study is associated with a small risk of breach of confidentiality. Study procedures to reduce risk of breach are described below.

Tier 1 Procedures Risk

Tier 1 procedures ages newborn-5 years

- Questionnaires: Questionnaires for this age group are tailored to the caregiver as the primary respondent with little or no additional input from the child. Questionnaires may include questions that make people feel uncomfortable, anxious, angry, or sad. Questionnaires can also cause fatigue. To minimize risk, study personnel will explain that all questions are optional, and that a question can be skipped if the participant prefers not to answer. The platform for the questionnaires will allow participants to take a break and come back to where they left off. All questionnaires will be approved by the IRB prior to use.
- Saliva collection is an optional biospecimen collection for children age 3-5 years. Saliva collection has no known risks. Collection is expected to be messy in this age group. Caregivers will receive written and oral instructions for saliva collection and will determine whether optional collection is feasible for their child.
- Blood collection (age 2-5 years). Blood collection at home is associated with risk of pain, lightheadedness and fainting. Specialized lancet devices designed to create a small drop of blood from the shoulder region, or other areas of the body with reduced density of pain receptors will be used for blood collection.

Tier 1 procedures ages 6-17 years

- Questionnaires: Questionnaires for this age group are tailored to the caregiver as the primary respondent with optional additional input from the child (with exception of PROMIS global health self-report in children ages 12-17 years). Questionnaires may include questions that make people feel uncomfortable, anxious, angry, or sad. Questionnaires can also cause fatigue. To minimize risk, study personnel will explain that all questions are optional, and that a question can be skipped if the participant prefers not to answer. The platform for the questionnaires will allow participants to take a break and come back to where they left off. All questionnaires will be approved by the IRB prior to use.
- Saliva collection has no known risks. Collection might be messy. Caregivers will receive written and oral instructions for collection.
- Blood collection at home is associated with risk of pain, lightheadedness and fainting. Specialized lancet devices designed to create a small drop of blood from the shoulder region, or other areas of the body with reduced density of pain receptors will be used for blood collection.

Tier 1 procedures ages 18-25 years

- Questionnaires: Questionnaires for this age group are tailored to the young adult as the primary respondent with optional input from the caregiver. Questionnaires may include questions that make people feel uncomfortable, anxious, angry, or sad. Questionnaires can also cause fatigue. To minimize risk, study personnel will explain that all questions are optional, and that a question can be skipped if the participant prefers not to answer. The platform for the questionnaires will allow participants to take a break and come back to where they left off. All questionnaires will be approved by the IRB prior to use.
- Saliva collection has no known risks. Collection might be messy. Young adults will receive written and oral instructions for collection.
- Blood collection at home is associated with risk of pain, lightheadedness and fainting. Specialized lancet devices designed to create a small drop of blood from the shoulder region, or other areas of the body with reduced density of pain receptors will be used for blood collection.

Tier 1 procedures primary caregivers

- Questionnaires: Questionnaires for the primary caregiver are tailored to the caregiver adult as the primary respondent. If the primary caregiver is below the age of majority, the caregivers parent or legal guardian of the caregiver will be the primary respondent, with input from the minor caregiver. Questionnaires may include questions that make people feel uncomfortable, anxious, angry, or sad. Questionnaires can also cause fatigue. To minimize risk, study personnel will explain that all questions are optional, and that a question can be skipped if the participant prefers not to answer. The platform for the questionnaires will allow participants to take a break and come back to where they left off.
- Saliva collection has no known risks. Collection might be messy. Caregivers and their parent or legal guardian if appropriate will receive written and oral instructions for collection.

- Blood collection at home is associated with risk of pain, lightheadedness and fainting. Specialized lancet devices designed to create a small drop of blood from the shoulder region, or other areas of the body with reduced density of pain receptors will be used for blood collection.

Tier 2 Procedures Risks

Procedures in Tier 2 will only be performed when clinically appropriate based on age and investigator judgment.

Tier 2 ages newborn-5 years

- Questionnaires: Questionnaires for this age group are tailored to the caregiver as the primary respondent with little or no additional input from the child. Questionnaires may include questions that make persons feel uncomfortable, anxious, angry, or sad. Questionnaires can also cause fatigue. To minimize risk, study personnel will explain that all questions are optional, and that a question can be skipped if the participant prefers not to answer. The platform for the questionnaires will allow participants to take a break and come back to where they left off. All questionnaires will be approved by the IRB prior to use.
- Child anthropometry and vital signs including oximetry: Child blood pressure, heart rate, oxygen saturation, oral, axillary or skin temperature, height (length), weight, head circumference, waist circumference and skin fold thickness will be measured. These procedures have no known risk, but could make some participants feel uncomfortable. All examination and vital sign procedures will be performed in a private examination room, in the company of the caregiver, with trained research personnel and chaperones if needed or requested.
- Home pulse oximetry. There are no known risks associated with use of an FDA-approved pulse oximeter sized to age of the child. The use of an oximeter is optional for children <5 years of age per judgment of the site investigator and caregiver.
- Child electrocardiogram: The electrocardiogram procedure is optional in this age group per judgment of the site investigator and caregiver. A 12-lead or 15-lead electrocardiogram will be performed based on site practice with FDA-approved equipment. Self-sticking pediatric size electrodes will be placed on the extremities and chest for the electrocardiogram recording and will be removed after the recording. The electrocardiogram will be completed in approximately 5 minutes. Electrocardiography is considered a minimal risk procedure. The adhesive on the gel electrodes may cause transient minor skin irritation.
- Phlebotomy for blood collection is considered a minimal risk procedure (ages 3-5 years, maximal single phlebotomy volume 15 ml). Children <3 years of age will not undergo phlebotomy for Tier 2. Blood collection may be associated with minor temporary discomfort. There is a risk of bruising and a very small amount of bleeding associated with the blood collection. There is also a very small risk of infection at the site of blood collection. Standard of care phlebotomy procedures will be used to mitigate these risks.

Tier 2 ages 6-17 years (child) and ages 18-25 years (young adult)

- Questionnaires: Questionnaires for the 6-17 years (child) age group are tailored to the caregiver as the primary respondent with optional additional input from the child. Questionnaires for the 18-25 years (young adult) group are tailored to the young adult as the primary respondent with optional input from the caregiver. Questionnaires may include questions that make children or young adults feel uncomfortable, anxious, angry, or sad. Questionnaires can also cause fatigue. To minimize risk, study personnel will explain that all questions are optional, and that a question can be skipped if the participant prefers not to answer. The platform for the questionnaires will allow participants to take a break and come back to where they left off. All questionnaires will be approved by the IRB prior to use.
- Home pulse oximetry. There are no known risks associated with use of an FDA-approved pulse oximeter sized to age of the child. The use of an oximeter is optional for children <7 years of age per judgment of the site investigator and caregiver.
- Child and young adult anthropometry and vital signs including oximetry: Child blood pressure, heart rate, oxygen saturation, oral, axillary or skin temperature, height, weight, head circumference, waist circumference and skin fold thickness will be measured. These procedures are considered minimal risk, but could make some participants feel uncomfortable. All examination and vital sign procedures

will be performed in a private examination room, in the company of the caregiver, with trained research personnel and chaperones if needed or requested.

- Child and young adult electrocardiogram: A 12-lead or 15-lead electrocardiogram will be performed based on site practice with FDA-approved equipment. Self-sticking pediatric size electrodes will be placed on the extremities and chest for the electrocardiogram recording and will be removed after the recording. The electrocardiogram will be completed in approximately 5 minutes. Electrocardiography is considered a minimal risk procedure. The adhesive on the gel electrodes may cause transient minor skin irritation.
- Child and young adult spirometry testing: The spirometry procedure is optional in children <7 years of age per judgment of the site investigator and caregiver. An FDA-approved hand-held spirometry system will be used to measure lung function. Subjects will be asked to blow into a single-use tube to capture exhaled gases. Three exhalations will be assessed. Spirometry is considered a minimal risk procedure. Blowing into a tube may cause transient fatigue or lightheadedness.
- Phlebotomy for blood collection is considered a minimal risk procedure (maximal single phlebotomy volume for ages 6-9 years 25 ml, maximal single phlebotomy volume for ages 10-25 years 38 ml). Blood collection may be associated with minor temporary discomfort. There is a risk of bruising and a very small amount of bleeding associated with the blood collection. There is also a very small risk of infection at the site of blood collection. Standard of care phlebotomy procedures will be used to mitigate these risks.
- Ziopatch is a small wearable device is applied against the left chest using a simple adhesive and fits under normal clothing (the device is approximately 5 inches x 2 inches (including adhesive strips) with a central button that is one-half-inch raised, and it weighs 24.5 grams). This device has no known risks, but the adhesive strips may cause skin irritation. Participants can remove the device if they experience irritation. This procedure is optional for children <7 years of age per judgment of the site investigator and caregiver. The device is designed to record up to 14 days, but the duration of the monitoring may be shortened, or temporarily interrupted to coincide with days off from school and participant preference as per judgment of the site investigator and caregiver.

Tier 2 primary caregiver

- Questionnaires: Questionnaires for the primary caregiver are tailored to the caregiver adult as the primary respondent. If the primary caregiver is below the age of majority, the caregivers parent or legal guardian of the caregiver will be the primary respondent, with input from the minor caregiver. Questionnaires may include questions that make people feel uncomfortable, anxious, angry, or sad. Questionnaires can also cause fatigue. To minimize risk, study personnel will explain that all questions are optional, and that a question can be skipped if the participant prefers not to answer. The platform for the questionnaires will allow participants to take a break and come back to where they left off.

Tier 3 Procedures Risk

Procedures in Tier 3 will only be performed when clinically appropriate based on age and investigator judgment.

Tier 3 ages 3-25 years

- Child cardiac structure and function (transthoracic echocardiogram, cardiac MRI)
    - Echocardiogram (age 3-25 years). An echocardiogram will be performed according to standard clinical protocols and is optional for age <7 years per judgement of the site investigator and caregiver. This is considered a minimal risk procedure. There is no known risk to cardiac ultrasound imaging. Gel electrodes for electrocardiogram monitoring during the test may cause skin irritation. The hand-held probe may cause a sensation of pressure, and rarely pain on the chest wall. Echocardiograms will be performed by trained technicians with pediatric experience.
    - Cardiac MRI (age 5-25 years). A cardiac MRI will be performed according to standard clinical protocols in children who do not require sedation and is optional for age <9 years per judgment of site investigator and caregiver. This is considered a minimal risk procedure. Participants may experience anxiety or claustrophobia in the MRI scanner. These symptoms will be mitigated by the use of entertainment/distraction whenever possible. Children with

metallic implants will not be permitted to participate. Children who require sedation for the MRI will not be allowed to participate. No intravenous contrast agent will be administered for research purposes. To minimize risk, the MRI will be conducted by trained technicians under supervision of pediatric cardiologists or licensed radiologists at each study site.

- Pulmonary function tests including diffusing capacity (age 5-25 years). Pulmonary function testing will be completed according to clinical protocols and is optional for age <9 years per judgment of the site investigator and caregiver. Pulmonary function tests are considered minimal risk procedures, but can be associated with transient fatigue and lightheadedness.
- Child sputum induction (ages 10-25 years). A standard of care clinical protocol for sputum induction will be used and is optional for children <12 years per judgment of the site investigator and caregiver. Sputum induction is considered a minor increase over minimal risk procedure due to small risk of bronchospasm, and small risks of use of levalbuterol to reduce the risk of bronchospasm. Participants will inhale nebulized hypertonic saline solution (3%) for 5-15 minutes to liquify airway secretions, promote coughing and allow expectoration of respiratory secretions. Coughing may be an uncomfortable feeling. If a participant is uncomfortable from coughing, the mist will be stopped. The mist may also induce a transient sore throat. Hypertonic saline can induce bronchospasm, with estimated risk of symptomatic bronchospasm in non-asthmatic children of 0.1% (29,30). To minimize risk of bronchospasm, children with asthma will be excluded and all children will receive pre-treatment with a bronchodilator (levalbuterol). Levalbuterol is an analog of endogenous catecholamines with established safety profile in studies of >600 children (31). Oxygen saturation will be monitored throughout the procedure. Handheld spirometry will be used to monitor subjects at 5-minute intervals during the inhalation procedure. The inhalation will be stopped if the $FEV_{1.0}$ decreases by more than 20% from pre-testing baseline or if the participant has symptoms of wheezing or shortness of breath, or if oxygen saturation decreases to <94%. The laboratory will be supervised by a licensed pediatric pulmonologist and emergency bronchodilator medications will be available in the room used for sputum induction.
- Symptom-limited cardiopulmonary exercise testing (ages 10-25 years). Participants will perform symptom-limited graded exercise on a treadmill or stationary bicycle ergometer according to clinical protocols in children and is optional for children <12 years per judgment of local site investigator and caregiver. Cardiopulmonary exercise testing is considered to be a minor increase over minimal risk procedure. Pediatric size gel electrodes will be placed to measure the electrocardiogram before, during and after exercise. Exercise testing mimics exertion experienced during normal activities of living and is associated with a very small risk of arrhythmia or low blood pressure during or after exercise (32). A trained exercise technician in an experienced center that performs pediatric cardiopulmonary exercise testing will be present throughout the exercise test to monitor the participant; cardiac monitoring of the rhythm and serial blood pressure measurements will be performed throughout the course of the exercise test and during recovery. Participants with history of exercise intolerance will be carefully monitored and testing procedures may be modified if symptoms emerge. Children and young adults with known heart disease, including history of pericarditis, myocarditis, or MIS-C with cardiac involvement within the prior 6 months, abnormal resting electrocardiogram, or evidence of active myocardial inflammation on cardiac MRI findings will not undergo exercise testing.
- Abdominal ultrasound (age 3-25 years). An abdominal ultrasound examination will be performed by a trained technician according to clinical protocols and is optional for age <7 years per judgment of the site PI and caregiver). Abdominal ultrasound is considered a minimal risk procedure. The liver, pancreas, kidneys, and bladder will be imaged. There is no known risk to abdominal ultrasound. Local pressure of the handheld probe may cause discomfort or pain.
- Brain MRI (age 3-25 years): A 3T or lower field strength brain MRI will be performed according to standard clinical protocols in children who do not require sedation and is optional for age <9 years per judgment of the site investigator and caregiver. Brain MRI is considered a minimal risk procedure. Children with metallic implants will not be permitted to participate. Children who require sedation for the MRI will not be allowed to participate. No intravenous contrast agent will be administered. To minimize risk, the MRI will be conducted by trained technicians under supervision of licensed pediatricians and/or radiologists at each study site.
- Awake EEG (age 3-25 years): An electroencepholagram (EEG) will be performed according to clinical protocols with FDA approved equipment and is optional for age <9 years per judgement of the site investigator and caregiver. An EEG is considered a minimal risk procedure. A trained technician will

apply scalp electrodes and record the EEG for up to 60 minutes. There is no known risk to EEG. The scalp electrodes may cause skin irritation. The test can be terminated early if necessary to relieve irritation.

- Neurocognitive testing (age 3-25 years). Neurocognitive testing will be performed under the supervision of a child psychologist according to clinical protocols with use of standard of care validated instruments for assessment of selected age-appropriate domains of neurocognitive function. Neurocognitive testing is considered a minimal risk procedure. These tests may require 4 hours total for completion. Neurocognitive testing may ask questions that make persons feel uncomfortable, anxious, angry, or sad. Questionnaires can also cause fatigue. To minimize risk, study personnel will explain that all questions are optional, and that a question can be skipped if the participant prefers not to answer. The platform for the questionnaires will allow participants to take a break and come back to where they left off.
- Blood collection for clinical laboratory and biorepository (maximal single phlebotomy volume for ages 3-5 years 15 ml, maximal single phlebotomy volume for ages 6-9 years 25 ml, maximal single phlebotomy volume for ages 10-25 years 38 ml). Phlebotomy for blood collection is considered a minimal risk procedure. Clinical standard of care phlebotomy procedures will be used. Phlebotomy is associated with minor temporary discomfort. There is a risk of bruising and a very small amount of bleeding associated with the blood collection. There is also a very small risk of infection at the site.
- Microbiome biospecimen collection (age 3-25 years). There is no known risk to specimen collection. Collection of urine and stool may be embarrassing and messy and can be performed at home and is optional for age <7 years per judgment of site investigator and caregiver. Nasal swabs can cause local discomfort or pain.

Risks of use of mobile health technology

Commercial products or devices made by third party companies, including wearable fitness trackers, wearable sleep monitors, mobile apps for use on smartphone and tablets, websites and web apps, and types of computer software that permit screen sharing, record keystrokes, gain access to device files and/or use location tracking technology may be used to collect study data. These devices will be used in accord with the Terms of Service and/or the End User License Agreements (EULA) provided by the product or device vendor. Use of such products and devices is associated with loss of privacy and risk of breach of confidentiality. These products and devices will only be used to collect study data in ages 6-25 years with IRB approval and if the participant has agreed to all applicable Terms of Service and EULAs.

Risks of Incidental findings

Biospecimen test results determined in CLIA-certified clinical laboratories and imaging and other clinical testing results determined by licensed medical professionals that are analytically valid will be recorded in the participant medical record and will be reviewed by the Principal Investigator or other designated licensed medical professional at each site. If the Principal Investigator or licensed designee determine that the result is clinically significant or medically actionable, the participant will be contacted by telephone or in-person to explain the test findings within one week of the return of the test results. The participant will also be advised to follow up with their primary care physician. Any additional testing ordered by the primary care physician will be paid by the participant or their insurance company. Disclosure of such incidental findings can cause or increase anxiety, and may result in increased clinical care costs to the participant.

Test results determined in research laboratories that cannot be validated in CLIA-certified clinical laboratories will not be recorded in the medial record and will not be returned to the participant.

Risks of Genetic Testing

A known risk of WGS is the incidental discovery of potentially pathogenic genetic variants during the course of the planned research. The WGS studies for the RECOVER study will be performed in a CLIA-certified laboratory with results interpreted by a board-certified clinical molecular geneticist or molecular genetic pathologist or equivalent. Analytically valid replicated results from the CLIA-certified laboratory that are defined as clinically actionable according to the standards and guidelines defined by the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMB-AMP) guidelines may be disclosed to the participants if all of the following criteria are met (33-35):

- The genetic finding has important health implications for the participant and the associated risks are established and substantial.
- The genetic finding is actionable, that is, there are established therapeutic or preventive interventions or other available actions that have the potential to change the clinical course of the disease.
- The test is analytically valid and the disclosure plan complies with all applicable laws.
- During the informed consent process or subsequently, the study participant has opted to receive his/her individual genetic results.

All disclosure of clinically actionable genetic results will be guided by the RECOVER WGS Core Laboratory in collaboration with the RECOVER Clinical Science Core. The consent form will inform participants of the potential for return of actionable results from WGS and the potential risks associated with the disclosure of the genetic information. For participants who elect to be informed of their clinically actionable genetic results, the validated, replicated result will be shared with the site PI or designated study personnel, who will use their local process and policies for re-identification of the participant and referral if needed for evaluation and counseling, which may include involvement of their local genetics team and/or the participant's cardiologist or other healthcare providers. Participants who reach the legal age of majority during the study will be re-consented and given the opportunity to opt-in or opt-out of return of genetic information.

There is a risk when performing genetic testing on participants and their parents that the genetic testing may detect cases of non-paternity (i.e., where the father of the child someone other than who it was thought to be). Non-paternity will be kept in the strictest confidence and will not be shared with the participant, parent(s), or family members.

Disclosure of individual genetic test results can cause or increase anxiety, damage family relationships, and/or compromise future insurability and employability. Some people involved in genetic studies feel anxious about the possibility of carrying (or their child carrying) an altered gene that may place them at risk or that might be passed on to subsequent generations. Genetic counseling will be provided by their clinical health care providers. Even without disclosure of individual results, genetic findings may be used to support harmful stereotypes, stigmatize, or discriminate against members of a socially defined group such as race or ethnicity; which could further impact employability as well as marital, adoption, and child-custody opportunities.

6.2 Known Potential Benefits

For most participants and caregivers in this observational study, there will be no direct benefit. It is possible that results of certified clinical laboratory testing available in the medical record will provide primary care physicians of the participants with clinically useful and medically actionable information. Although an individual participant may not benefit from participation, the results of the study will make important contributions to the understanding of healthcare providers, caregivers, parents, and patients about the long-term outcomes after SARS-CoV-2 infection.

Currently, there is no known direct benefit from the participation of the participant in the RECOVER biorepository. However, future biospecimen analyses may help physicians provide better answers to families' questions regarding causes, risk, and recurrence risks. It may also provide clues to future interventions and/or treatments.

At the end of the study, participants and/or parents will be sent a description of the aggregate study results in lay language. In this study, families will be given the contact information for the study PI and/or coordinator, in the event the patient wishes to discuss the results or has questions. If desired, a separate notification will also be provided to the treating/referring physician (cardiologist, etc.) describing overall study results.

7 Objectives and Purpose

The objective of this proposed study is to enhance knowledge of children's recovery from SARS-CoV-2 infections in order to support development of novel diagnostic and therapeutic interventions. Overarching scientific objectives are as follows:

1. Characterize the incidence and prevalence of sequelae of SARS-CoV-2 infection occurring >30 days after study entry across the early life spectrum from age newborn-25 years.
2. Characterize the spectrum of clinical symptoms, subclinical organ dysfunction, natural history, and distinct phenotypes identified as sequelae of SARS-CoV-2 infection occurring >30 days after study entry infection across the early life spectrum from age newborn-25 years.
3. Define the biological mechanisms underlying pathogenesis of the sequelae of SARS-CoV-2 infection occurring >30 days after study entry across the early life spectrum from age newborn-25 years.

7.1 Specific Aims

The following aims are proposed to support achievement of the scientific objectives of the study:

Aim 1. Characterize the incidence, prevalence, and long-term sequelae, including clinical and biological features, severity, and distinct sub-phenotypes following SARS-CoV-2 infection across the early life spectrum from ages newborn-25 years.

1a. Define PASC phenotypes, sub-phenotypes, and severity based on clinical and biological features across the early life spectrum from ages newborn-25 years.

1b. Estimate the incidence of PASC phenotypes (new onset or exacerbation) among children with SARS-CoV-2 infection or born in the context of maternal SARS-CoV-2 infection during pregnancy compared with uninfected controls followed over the same time interval.

1c. Estimate the incidence and prevalence of clinical and subclinical organ injury/disease after SARS-CoV-2 infection.

Aim 2. Characterize the clinical course and recovery of acute and post-acute sequelae over time and determine associated risk factors for PASC among SARS-CoV-2 infected individuals compared to SARS-CoV-2 infected individuals without PASC and compared to uninfected individuals across the early life spectrum from ages newborn-25 years.

2a. Characterize the patterns of outcomes of acute and post-acute sequelae of SARS-CoV-2 infection over time.

2b. Determine whether pre-infection and peri-infection risk and resiliency factors (e.g., social determinants of health, family dynamics, demographic, behavioral, and biological factors, preexisting clinical and subclinical co-morbidities and acute infection severity and treatment) are associated with sequelae of SARS-CoV-2 infection and their resolution over time.

2c. Compare the prognostic significance of subclinical organ injury/disease for incident clinical disease over time among SARS-CoV-2 infected versus uninfected individuals.

Aim 3. Define the pathophysiology of and mechanisms associated with the development of acute and post-acute sequelae, including the direct and indirect effects, of SARS-CoV-2 infection on symptom onset and potential modifiers across the early life spectrum from ages newborn-25 years.

3a. Evaluate the direct and indirect effects of SARS-CoV-2 infection on the development of acute and post-acute sequelae, including potential mediation by severe disease (e.g., ICU stay and/or intubation), treatment (e.g., steroids), and pandemic-related stressors.

3b. Determine whether SARS-CoV-2 infection modifies the trajectory of prior organ dysfunction, and/or the risk of developing new organ injury compared with pre-pandemic status, and identify possible pathophysiological mechanisms.

3c. Compare the long-term outcomes of children with MIS-C, children with post-COVID-19 vaccine myocarditis, and SARS-CoV-2 infected children without history of MIS-C, and elucidate the mechanisms and pathobiology behind these events.

3d. Characterize the impact of clinical manifestations of PASC on measures of child development in caregiver-child dyads comparing:
   i. Children with SARS-CoV-2 infection with PASC to those with SARS-CoV-2 infection without PASC, and those without SARS-CoV-2 infection.
   ii. Children with and without caregiver history of SARS-CoV-2 infection including infants born to mothers with SARS-CoV-2 infection during pregnancy.

8 Study Design and Endpoints

8.1 Overall Study Design

This is a combined retrospective and prospective, longitudinal, observational meta-cohort of individuals who will enter the cohort with and without SARS-CoV-2 infection and at varying stages before and after infection (see Study Overview Figure 1). Individuals with and without SARS-CoV-2 infection and with or without PASC symptoms will be followed to identify risk factors and occurrence of PASC. This study will be conducted in the United States and participants will be recruited through inpatient, outpatient, and community-based settings. No recruitment materials will be used without first being approved by the IRB.

Each enrolled subject will undergo a tailored tiered testing approach based on history of SARS-CoV-2 infection, presence of PASC symptoms, age range, and other considerations specific to each sub-cohort of the RECOVER meta-cohort. Subjects will be selected for participation in Tiers 2 and 3 based on their history of COVID exposure and PASC symptoms as determined in Tier1 for post-acute participant and uninfected controls, and at the week 8 visit for acute Tier 2 participants. Subjects will be selected for participation in Tiers 2 and Tiers 3 to achieve the enrollment targets as summarized in Figure 1 and to achieve pre-specified targets for age distribution and diversity.

8.2 Recruitment procedures

Recruitment into the pediatric protocol will occur through direct contact with potential participants in clinical settings, electronic health record query, social media platforms, websites, outreach to community-based organizations, and conventional mass media (radio and print publications). Flyers, post-cards, business cards, narrated slides shows, animations, and videos may be used across different media platforms to provide information about study participation. All materials used in recruitment activities will be IRB-approved, and any recruitment information sent by email will use a secure encrypted email platform.

If the potential subject has provided prior consent to be contacted for research at their study site institution, the site study team may create a query in the local electronic health record system to identify potential subjects based on study entry criteria (based on age, diagnostic codes, SARS-CoV-2 testing results, or other information in the electronic health record found to be related to the PASC phenotype). A secure email will be sent to potential subjects to solicit interest in the study, with instructions for contact of the study team if interested to participate. Once contact has been established with a potential subject, an IRB-approved eligibility script and screener may be used by the enrolling site. The study Principal Investigator or designated study staff members may provide additional IRB-approved information to the potential subject as described below and may schedule a study visit. Other PHI or medical information will not be available to the study team. The electronic health record query may be repeated at 3-month intervals for the duration of the 4-year study. All query responses will be deleted at the end of the study.

If a potential subject requests information regarding opting out of further recruitment for all research, subjects will be directed to site Principal Investigator or designated study team member.

Once potential subjects have been identified, the study team may need to notify the treating physician that they have patients eligible to participate. If notification to the treating physician is necessary, one or more of the following methods will be used to notify the treating physician:

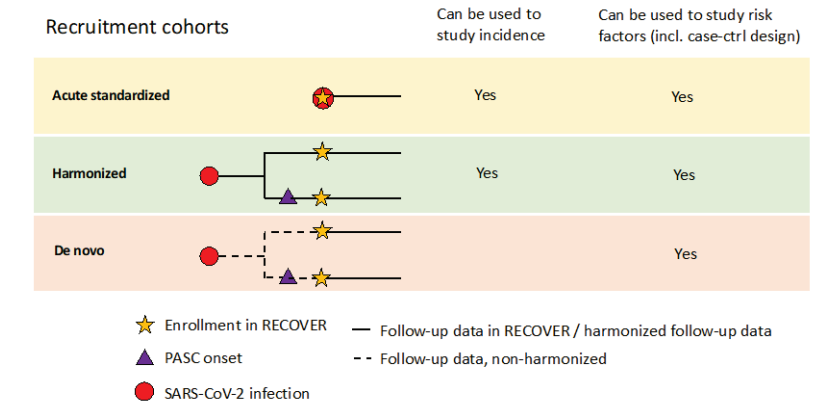
1. The treating physician may be given a list, advertisement, letters or oral script to use when contacting potential subjects
2. The treating physician and site Principal Investigator may send a letter to all potential subjects (letter must have both names)
3. If the treating physician agrees, the study team may directly contact potential subjects on behalf of the treating physician by letter, phone, email, or an electronic medical record patient portal.

Once contact is made, approved recruitment language will be used to communicate the reason they are being contacted and potential subjects will be asked if they are interested in participating in this specific study. An IRB-approved eligibility script and screener may be used by the enrolling site. Should the potential subjects agree, the study team will provide the subjects with information regarding the next steps for participation.

8.3 Cohort and Case-Noncase Study Recruitment Pools

The SARS-CoV-2 Pediatric Recovery Cohort PASC Study will enroll individuals ages newborn-25 years with and without SARS-CoV-2 infection, defined as probable, suspected or confirmed according to the WHO criteria, and at varying stages before and after infection in a meta-cohort design.

Figure 2. Schematic of Meta-Cohort Recruitment Cohorts



Key points
1. Given that we need the harmonized cohort to study the incidence of PASC pre-delta, we think a comparable sample size to the acute standardized cohort is preferable. We think analyses should be stratified by recruitment cohort to examine differences by variant (i.e., acute infected will include delta and subsequent variants; others will include largely pre-delta variant data)
2. Relative to analyses in the acute standardized cohort, analyses can be conducted more quickly in the harmonized and de novo cohorts because a proportion will have already developed PASC by RECOVER enrollment.
3. In order to study PASC incidence using the harmonized cohort, the harmonized cohort should not be enriched for PASC.
4. The de novo cohort may be enriched for cases to maximize power, but individuals with PASC who are included may ultimately represent those with the most severe symptoms.

8.3.1 Overview of cohort types contributing to the RECOVER meta-cohort

Extant, clinical and de novo (newly enrolled in RECOVER) cohorts:

- SARS-CoV-2 infected children and young adults with and without current or prior PASC-like symptoms, including infected individuals with history of MIS-C, and infants born in the context of maternal SARS-CoV-2 infection during pregnancy (congenitally exposed).
- SARS-CoV-2 uninfected children and infants born to uninfected mothers
- Enrollment procedures and RECOVER data collection procedures will be harmonized with the extant research protocol for participants enrolled in other studies; separate RECOVER consent will be signed.

Acute cohort:

- Newly SARS-CoV-2 infected individuals (≤30 days since onset of symptoms or positive laboratory testing)
- Contemporaneous SARS-CoV-2 uninfected individuals selected from the same population as newly SARS-CoV-2 infected individuals
- Eligibility for the post-acute phase of Tier 2 in the acute cohort will be determined at the 8-week acute Tier 2 visit as described in Section 11.

Post-acute cohort:

- Post-acute infected individuals (>30 days after initial symptoms or positive laboratory testing) in the extant, clinical and de novo cohorts, including infected individuals with history of MIS-C, and infants born in the context of maternal SARS-CoV-2 infection during pregnancy (congenitally exposed), will be enrolled after initial SARS-CoV-2 infection.
- If available, relevant retrospective data prior to enrollment will be extracted from the electronic health record or existing research and clinical cohort data.
- Uninfected individuals will be derived from a similar population with respect to age, sex, race and ethnicity, geographic origin, sociodemographics, and time of enrollment as the infected individuals.
- Individuals with Tier 1 positive antibody test to the nucleocapsid antigen will be classified as post-acute infected with or without history of prior laboratory testing or symptoms of SARS-CoV-2 infection. An index date for these individuals may be the time of a past known family or school exposure, past symptoms, or at study entry if no history of exposure or symptoms is known.

Post-COVID-19 vaccine myocarditis cohort

- Recipient of mRNA COVID-19 vaccination within last 4 weeks with evidence of myocarditis per enrollment criteria listed below.
- Children or young adults with or without history of SARS-CoV-2 infection are eligible
- Children or young adults with or without history of MIS-C are eligible (if any prior MIS-C-related cardiac abnormalities are known to have resolved pre-vaccination)
- It is anticipated that most individuals in this cohort will be enrolled in extant research cohorts. Enrollment procedures and RECOVER data collection procedures will be harmonized with the extant research protocol for participants enrolled in other studies; separate RECOVER consent will be signed.
- If available, relevant retrospective data prior to enrollment will be extracted from the electronic health record or existing research and clinical cohort data.

8.3.2 Categories of recruitment pools in the RECOVER meta-cohort

Based on these types of cohorts available within the RECOVER meta-cohort, the RECOVER study population will include 9 categories of participants:

1. Acute infected participants in the main cohort (ages newborn-25 years, symptomatic and asymptomatic),
2. Post-acute infected participants in the main cohort (ages newborn-25 years, symptomatic and asymptomatic),
3. Uninfected participants matched to acute and post-acute infected participants in the main cohort,
4. Infected participants recruited from the Adolescent Brain Cognitive Development (ABCD) study,
5. Uninfected participants recruited from the ABCD study,
6. Infants and toddlers (≤3 years of age) born in the context of maternal SARS-CoV-2 infection during pregnancy (congenitally exposed)
7. Uninfected control infants and toddlers (≤3 years of age) not born in the context of maternal SARS-CoV-2 infection during pregnancy
8. Children and young adults with history of MIS-C, and
9. Children and young adults with history of post-COVID vaccine myocarditis.

Additional procedures participant enrollment within these categories is described below. Primary caregivers of participants will also be enrolled in the study for limited data collection.

Acute and post-acute infected participants in the main cohort. Participants with SARS-CoV-2 infection will be recruited at or within 30 days after infection (acute) or more than 30 days after infection (post-acute).

Symptomatic acute infected participants will meet the WHO criteria for suspected, probable, or confirmed infection and may include both vaccinated and unvaccinated participants. For participants with confirmed SARS-CoV-2 infection, the index date is the infection date, i.e., the date at which a participant meets test result inclusion criteria; for participants with probable or suspected infection, infection date is an approximate date at which infection occurred (by self-report or documented by chart review). For participants with asymptomatic infection and no known exposure history to determine data of infection, no index date will be assigned. A proportion of the symptomatic acute participants with and without PASC symptoms will be randomly selected to be followed longitudinally beyond the initial 8-week acute phase for the duration of the study.

Symptomatic post-acute infected participants in the main cohort will be recruited based on meeting WHO criteria for a suspected, probable, or confirmed case of SARS-CoV-2 infection. Participants with history of MIS-C who have not been previously enrolled in another research cohort may be enrolled in the pediatric main cohort. Participants with history of MIS-C may be identified in RECOVER PASC consortium EHR cohorts, active surveillance of the EHR at RECOVER PASC consortium pediatric cohort sites, existing patient advocacy social networks for MIS-C, referral from other health care providers at the RECOVER PASC consortium pediatric cohort health care systems, and self-referral. Recruitment from PASC clinics or other referral sources based on the presence or absence of post-acute symptoms (rather WHO criteria) will be minimized. Self-referral patients will be accepted but the combination of participants recruited based on the presence of PASC symptoms and self-referral patients with reported PASC symptoms cannot account for more than 50% of enrolled patients within any single racial/ethnic group. Participants recruited based on the presence of post-acute symptoms and self-referral patients with post-acute symptoms will be excluded from prevalence/incidence calculations due to selection bias.

Asymptomatic acute and post-acute infected participants may be identified by positive PCR or antigen screening test in the medical history or by positive nucleocapsid antibody test in the medical history or from results of Tier 1 nucleocapsid antibody testing, or symptomatic infected participants will meet WHO criteria for suspected, probable or confirmed infection that are not symptom-based. For asymptomatic infected participants, the index date is the historical date of exposure if known, or the date of study entry. A proportion of the asymptomatic acute infected participants will be randomly selected to be followed longitudinally beyond the initial 8-week acute phase for the duration of the study, as determined by the presence of suspected PASC symptoms.

Matched acute and post-acute uninfected participants in the main cohort. Uninfected acute subjects must meet the following conditions at the time of RECOVER screening/enrollment:

1. Never met the WHO criteria for a suspected, probable, or confirmed case of SARS-CoV-2 infection,
2. No known history of SARS-CoV-2 test from a respiratory specimen (PCR or antigen testing) and
3. No known history of positive SARS-CoV-2 nucleocapsid protein antibody (if vaccinated) and spike protein antibody test (if not vaccinated).

Uninfected participants will be recruited contemporaneously with acute participants and will be frequency matched on sex, age, race/ethnicity, and vaccination status. For uninfected participants, the *index date* is defined as the date of RECOVER enrollment. A subset of uninfected participants will be followed longitudinally for the duration of the study. Uninfected control subjects will be recruited from screening test records (negative tests due to household or school contacts with no symptoms at the time) and from well-child ambulatory clinics, vaccination clinics, or other non-COVID related clinical encounters contemporaneous with an identified acute or post-acute case from the same clinic setting. Acute and post-acute uninfected participants must have no known history of positive screening SARS-CoV-2 test and no known history of a positive antibody test (spike and nucleocapsid protein) from medical records or from Tier 1 testing. The uninfected subject may be the sibling or classmate of another participant in RECOVER. When possible, at recruitment, the uninfected and infected participants may also be matched on important covariates such as sex, age, race / ethnicity, hospitalization, and vaccination status. The date of a negative SARS-CoV-2 test (if available) or the date of a well-child visit from the same recruitment pool contemporaneous with an infected case will be considered the index date. A proportion of post-acute uninfected participants will be selected

based on the presence or absence of PASC symptoms to be followed longitudinally for the duration of the study (n=600, Figure 1). Uninfected control subjects selected for longitudinal follow-up in Tier 2 will be randomly assigned to Tier 2 procedures matching the acute infected main cohort (n=200) or Tier 2 procedures matching the post-acute infected main cohort (n=400).

Infected and uninfected children recruited from the Adolescent Brain Cognitive Development (ABCD) study (ages 12-17 years). The Adolescent Brain Cognitive Development (ABCD) Study is an NIH-funded study of brain development and child health. Participants belonging to this extant cohort include both adolescents who were infected with SARS-CoV-2 prior to RECOVER enrollment and those who were not. Most ABCD cohort participants will contribute Tier 1 data only and will not be followed longitudinally; however, a small proportion of ABCD Study participants may contribute longitudinal Tier 2 data to RECOVER depending in part on the presence of suspected PASC symptoms and geographic proximity to other pediatric cohort sites. Infected post-acute cases and non-infected controls will be identified according to the same criteria as the main cohort. Index date determination for these participants will follow the index determination for post-acute infected and uninfected participants.

Children (≤3 years of age) born in and out of the context of maternal SARS-CoV-2 infection during pregnancy. Children ≤3 years of age born to a childbearing parent with history of SARS-COV-2 infection during pregnancy will be enrolled in the study from existing research cohorts. Children ≤3 years of age born to a childbearing parent without history of SARS-COV-2 infection during pregnancy will also be enrolled from the same existing research cohorts. These participants will all be followed longitudinally until the age of 48 months. It is anticipated that these infants will mostly be recruited between the ages of 9 and 18 months. Caregiver dyads are not being enrolled for this cohort. The mothers of these children may participate in the RECOVER adult cohort.

Children (ages newborn-5 years) from the pediatric cohorts who were infected with COVID after birth (excluding those with only in utero exposure by being born to a childbearing parent with history of SARS-COV-2 infection during pregnancy) and uninfected controls will be enrolled in the study. These participants will receive similar assessments to the infants born in the context of maternal SARS-CoV-2 infection during pregnancy until the age of 48 months. After age of 48 months, these participants will continue participation in the main pediatric cohort Tier 2 and Tier 3 procedures as appropriate for age.

Children and young adults with history of MIS-C in extant cohorts. MIS-C is considered a subset of PASC. Participants with history of MIS-C according to the CDC case definition (36) will be recruited from RECOVER sites participating in existing research cohort studies. These participants will all be followed longitudinally until 2 years after study enrollment (up to 5 years after index infection). The existing research cohort data will be harmonized with RECOVER prospective data collection.

Children and young adults with history of post-vaccine myocarditis. Participants with post-vaccine myocarditis will have received an mRNA COVID-19 vaccine within 4 weeks prior to RECOVER enrollment. Participants with and without history of SARS-CoV-2 infection or MIS-C are eligible for inclusion into this cohort. Participants with known auto-immune or immune dysregulation diseases will be excluded. These participants will have evidence of or probable confirmed myocarditis as described in the protocol. These participants will all be followed longitudinally until 2 years after their vaccination date.

Primary caregivers of participants. A primary caregiver is the adult responsible for the care of the enrolled child and resides in the same household as the child. For children ages newborn-18 years, the caregiver will be the primary respondent for child health questionnaires. Caregivers will answer questions about their own SARS-CoV-2 exposure and experience with COVID-19, if any, as well as provide information about household social determinants of health and limited biological specimens.

Other biological parents of participants. If the primary caregiver is a biological parent of the child or young adult who is willing to participate in the study, the other biological parent may be enrolled to provide a home sample of saliva for DNA analysis.

Recruitment targets by demographics, symptoms, and calendar time of infection. Recruitment will occur across all pediatric sites in the RECOVER consortium. Within each site, recruitment of infected individuals will be monitored to ensure balance across strata as follows:

- 50% of participants will be symptomatic and 50% will be asymptomatic during the 30 days after SARS-CoV-2 infection.
- 50% of participants will be female,
- 53% of participants will be non-Hispanic Whites, 16% non-Hispanic Blacks, 27% Hispanic/Latinx, and 4% Asian Americans, Native Hawaiians, Pacific Islanders, American Indians, and Native Alaskan.

Uninfected participants will be recruited from the same communities and sources as infected participants. Recruitment will be stratified to match the SARS-CoV-2 infected group as follows:

- 50% of participants will be female,
- 53% of participants will be non-Hispanic Whites, 16% non-Hispanic Blacks, 27% Hispanic/Latinx, and 4% Asian Americans, Native Hawaiians, Pacific Islanders, American Indians, and Native Alaskan.

All participants (acute, post-acute and uninfected controls) will contribute to aims according to their SARS-CoV-2 infection/PASC history and clinical course during prospective follow-up. Patients without SARS-CoV-2 infection may include individuals with or without hospitalization in the prior three months. A proportion of individuals with prior or acute SARS-CoV-2 infection will be selected to be followed prospectively from enrollment, based on presence or absence of suspected PASC symptoms, with collection of relevant retrospective data when available. Selection for prospective follow-up will be determined by the Tier 2 targets for symptomatic vs. asymptomatic infection, PASC positive vs. PASC negative symptom status, and race and ethnicity as described above.

9 Study Enrollment and Withdrawal

9.1 Inclusion Criteria

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

9.1.1 Infected Cohort:

Patients will be eligible for inclusion according to the following criteria:

i. Ages newborn-25 years
ii. Infected individuals will have suspected, probable, or confirmed SARS-CoV-2 infection as defined by WHO criteria within 24 months of enrollment or have been born to a mother meeting these criteria during pregnancy (congenitally exposed)
iii. Children/young adults with or without history of MIS-C are eligible
iv. Children/young adults with or without history of SARS-CoV-2 vaccination are eligible
v. Children/young adults with evidence of past SARS-CoV-2 infection based on serum antibody profile are eligible (with or without history of acute symptoms)
vi. Children/young adults with recurrent SARS-CoV-2 infections and those with post-vaccination (breakthrough) infections are eligible to participate
vii. Participants are eligible without exclusion related to sex, race/ethnicity, geography, nationality, severity of disease, or underlying health conditions

9.1.2 Children/Young Adults with Suspected SARS-Cov-2 Infection

a) Children/young adults who meet these clinical criteria:

At least one of these clinical criteria:

i. Acute onset of fever and cough OR
ii. Acute onset of ANY THREE OR MORE of the following signs or symptoms: fever, cough, general weakness /fatigue, headache, myalgia, sore throat, coryza, dyspnea, anorexia/nausea/vomiting, diarrhea, altered mental status.

AND at least one of these epidemiological criteria:

i. Residing or working in an area with a high risk of transmission of virus: closed residential, school or camp settings anytime within the 14 days before symptom onset; OR
ii. Residing or travel to an area with community transmission anytime within the 14 days before symptom onset; OR
iii. Any known household contact or any member of the household working in any health care setting, including within health facilities or within the community; anytime within the 14 days before symptom onset.

b) A patient with history of **severe acute respiratory illness (SARI):**
SARI: acute respiratory infection with history of fever or measured fever of ≥ 38 C°; and cough; with onset within the last 10 days; and requires hospitalization

c) An asymptomatic person not meeting epidemiologic criteria with a positive SARS-CoV-2 Antigen-RDT.

9.1.3 Children/Young Adults with Probable SARS-Cov-2 Infection

a) A patient who meets clinical criteria above AND is a contact of a probable or confirmed case or linked to a COVID-19 cluster; OR
b) A suspect case with chest imaging showing findings suggestive of COVID-19 disease; OR
c) A person with recent onset of anosmia (loss of smell) or ageusia (loss of taste) in the absence of any other identified cause

9.1.4 Children/Young Adults with Confirmed SARS-Cov-2 Infection

a) A person with a positive Nucleic Acid Amplification Test (NAAT); OR
b) A person with a positive SARS-CoV-2Antigen-RDT AND meeting either the probable case definition or suspect criteria A OR B; OR
c) An asymptomatic person with a positive SARS-CoV-2 Antigen-RDT who is a contact of a probable or confirmed case

9.1.5 Children/Young Adults with Asymptomatic SARS-CoV-2 Infection

a) A person without history of acute COVID-19 symptoms who has one or more of the epidemiological exposures for suspected infection and who also meets criteria b or c for suspected or probable infection, or who meets any of the criteria for confirmed infection
b) A person without history of acute COVID-19 symptoms who has positive nucleocapsid antibody test result in medical history or Tier 1 testing with or without NAAT or RDT testing or known contact to a probable or confirmed case.

9.1.6 Non-Infected Cohort

A person who meets the following criteria will qualify for enrollment as a non-infected control subject:
a) Does not meet WHO criteria for a suspected, probable, or confirmed case of SARS-CoV-2 infection AND
b) Does not have serological evidence of past asymptomatic SARS-CoV-2 infection in medical history or Tier 1 testing, AND
c) Lives in the same communities or recruited from the same sources as those in the SARS-CoV-2 infected cohort, AND
d) Either not hospitalized for any reason in prior 3 months, or hospitalized (with or without ICU stay) within the prior 3 months
e) Uninfected individuals may participate independent of their vaccination status
f) Uninfected individuals who develop SARS-CoV-2 infection during the study period will be reassigned to the SARS-Cov-2 infected group and will be considered to have been enrolled prior to SARS-CoV-2 infection.

9.1.7 *Children (≤3 years of age) born in and out of the context of maternal SARS-CoV-2 infection during pregnancy.*

- Children ≤3 years of age born to a childbearing parent with history of suspected, probable, or confirmed SARS-COV-2 infection during pregnancy (according the same criteria listed for the infected child cohort) will be enrolled in the study from existing research cohorts at the maternal fetal medicine sites in the RECOVER. network.
- Children ≤3 years of age born to a childbearing parent without history of SARS-COV-2 infection during pregnancy (according to the same criteria listed for the non-infected child cohort) will also be enrolled from the same existing research cohorts at maternal fetal medicine sites in the RECOVER network.

9.1.8 Children with MIS-C

Children/young adults with SARS-CoV-2 infection who have history of MIS-C meeting the CDC definition:

- An individual aged <21 years presenting with fever*, laboratory evidence of inflammation**, and evidence of clinically severe illness requiring hospitalization, with multisystem (≥2) organ involvement (cardiac, renal, respiratory, hematologic, gastrointestinal, dermatologic or neurological); AND
- No alternative plausible diagnoses; AND
- Positive for current or recent SARS-CoV-2 infection by RT-PCR, serology, or antigen test; or exposure to a suspected or confirmed COVID-19 case within the 4 weeks prior to the onset of symptoms.

*Fever ≥38.0°C for ≥24 hours, or report of subjective fever lasting ≥24 hours
**Including, but not limited to, one or more of the following: an elevated C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), fibrinogen, procalcitonin, d-dimer, ferritin, lactic acid dehydrogenase (LDH), or interleukin 6 (IL-6), elevated neutrophils, reduced lymphocytes and low albumin

Young adults with past history of MIS-C with current ages 22-25 years are eligible to participate.

9.1.9 Children/Young Adults with Post-Vaccine Myocarditis

a) Age 3-25 years
b) Recipient of mRNA COVID-19 vaccination within last 4 weeks
c) Children or young adults with or without history of SARS-CoV-2 infection are eligible
d) Children or young adults with or without history of MIS-C are eligible (if any prior MIS-C-related cardiac abnormalities are known to have resolved pre-vaccination)
e) No other known auto-immune or other immune dysregulation disease
f) Participants are eligible without exclusion related to sex, race/ethnicity, geography, nationality, severity of disease, or underlying health conditions
g) Clinical evidence of probable or confirmed myocarditis based on the following criteria:

Children and young adults ages 3-25 years with presence of ≥1 new or worsening of the following clinical symptoms:

- chest pain, pressure, or discomfort
- dyspnea, shortness of breath, or pain with breathing
- palpitations
- syncope

OR, children aged 3-12 years might instead have ≥2 of the following symptoms:

- irritability
- vomiting
- poor feeding
- tachypnea
- lethargy

AND
≥1 new finding of:

- troponin level above upper limit of normal (any type of troponin)

- abnormal electrocardiogram (ECG or EKG) or rhythm monitoring findings consistent with myocarditis
- abnormal cardiac function or wall motion abnormalities on echocardiogram
- cardiac MRI findings consistent with myocarditis
- histopathologic confirmation of myocarditis (Definite myocarditis)

AND

- No other identifiable cause of the symptoms and finding

Entry criteria are adapted from the CDC definition based on the assumptions that COVID-19 vaccines will be available in the future to children <5 years of age.

9.1.10 Primary Caregiver Entry Criteria

a) A primary caregiver is defined as an individual, such as a family member (biological or nonbiological) or legal guardian, who is responsible for the care of the enrolled child and resides in the same household as the child. When possible, the primary caregiver identified at study entry will remain in the same role throughout the study.
b) The designated. primary caregiver is the family member (biological or nonbiological) or legal guardian who spends the most time with the child or young adult, has substantial responsibility for taking care of her/him on a daily basis, and is most knowledgeable about her/him.
c) If two or more persons share equally in the caregiver responsibilities for the child or young adult, the person selected by the family to fill out study forms both about themselves and the child will be designated the primary caregiver.
d) If a biological family member primary caregiver has not reached the legal age of majority in their jurisdiction, the parent/legal guardian for the minor designated primary caregiver will provide consent for participation, with assent provided by the minor caregiver.
e) A nonbiological primary caregiver or legal guardian serving as the designated primary caregiver must be above the legal age of majority in their jurisdiction.
f) The designated primary caregiver cannot be a babysitter or other childcare provider who receives money to care for the child.

9.1.11 Biological Parent Entry Criteria

a) If the designated primary caregiver who is participating in the study is a biological parent of the enrolled child or young adult, the other biological parent may be enrolled to provide a home sample of saliva for DNA analysis.

9.2 Exclusion Criteria

An individual who meets any of the following criteria will be excluded from participation in this study:

a) Any child, young adult, designated caregiver, or other biological parent who in the opinion of the site investigator may be at increased risk of adverse events during participation in the study, or who may not be able to complete study procedures due to co-morbid disease or disability.
b) Any young adult above the age of majority who lacks capacity to provide consent
c) Nonviable neonates and neonates of uncertain viability as determined by the treating physician
d) Any child, young, adult, or designated caregiver with co-morbid illness with expected survival <2 years
e) Any child who is being given up for adoption or is a ward of the state
f) Any young adult, designated caregiver or other biological parent who is incarcerated, or who lacks capacity to provide consent
g) Currently enrolled in the study Understanding the Long-term Impact of COVID-19 in Adults

9.3 Vulnerable Subjects

This observational study is conducted in subjects ages newborn-25 years, their primary caregivers, and optionally the other biological parent if the designated primary caregiver is a biological parent. Some of the participants may be women who are pregnant or breastfeeding. Some of the caregivers may be under the age of majority in their local jurisdiction. Study participation in caregivers is limited to questionnaires and collection

of blood and saliva biospecimens. For children and young adults enrolled in the study, the study procedures are considered to be minimal risk (Tiers 1 and 2), or a minor increase over minimal risk (some of the procedures in Tier 3).

For Tier 3 procedures, symptom-limited exercise testing and sputum induction are considered to include a minor increase over minimal risk. This means that the increase in the probability and magnitude of harm is only slightly more than minimal risk, and that any potential harms associated with the procedure will be transient and reversible in consideration of the nature of the harm, and there is no or an extremely small probability that subjects will experience significant pain, discomfort, stress or harm. Exercise is a normal activity in children, and the level of exercise will be stopped as directed by the child symptoms. Monitoring of electrocardiogram, heart rate, blood pressure, and symptoms by a trained professional will reduce risk of harm. Sputum induction is considered as a minor increase over minimal risk due to a small risk of bronchospasm (0.1%) and possible side effects with levalbuterol pre-treatment. To minimize risk, participants with history of bronchospasm will be excluded. Oxygen saturation and spirometry will be monitored during the procedure. Trained technicians under supervision of a licensed medical professional will be present with access to emergency bronchodilator medicines if needed.

Inclusion of exercise testing and sputum induction for collection of lung microbiome biospecimens is vitally important to the understanding of the long-term effects of SARS-CoV-2 infection in children. There is clear and significant evidence that these procedures will provide important information to further the understanding of the etiologies, prevention, diagnosis, pathophysiology, or alleviation or treatment of PASC in children.

Inclusion of pregnant subjects is vitally important to the understanding of the long-term effects of SARS-CoV-2 infection in pregnant women and their child. The proposed study procedures do not require radiation exposure or other procedures that pose risk to pregnant or breastfeeding research subjects. If a participant younger than the age of majority is pregnant, or becomes pregnant, during the course of the study, the consent process may be modified if the participant is allowed under state and local regulation to provide consent for themselves. Otherwise, consent from two parents will be obtained and subject assent will be sought.

There is possible direct medical benefit to participants in the study related to return of medical information to the participant and their primary care physician, and potential future benefit related to discoveries about PASC from study data and biospecimens.

9.4 Strategies for Recruitment and Retention

9.4.1 Study Recruitment Strategy and Sampling

Recruitment of children and young adults with and without SARS-CoV-2 infection or born to a mother with SARS-CoV-2 infection during pregnancy may be stratified to ensure adequate representation by age, sex, race/ethnicity.

Children and young adults with acute SARS-CoV-2 infection will be recruited from extant research and clinical cohorts not selected for the study of PASC, including:

- Acute case surveillance testing in PASC consortium pediatric cohort health care systems
- Community engagement cohorts for surveillance of acute cases
- Acute case surveillance in extant populations enrolled in aim 1
- State and local public health resources
- PASC consortium EHR cohorts
- Participant self-referral

Recruitment for acute SARS-CoV-2 infection may use CDC data to target regions with higher case rates.

Children and young adults with post-acute SARS-CoV-2 infection including children born to a mother with SARS-CoV-2 infection (>4 weeks after infection) will be recruited from:

- Post-acute case surveillance in PASC consortium pediatric cohort health care systems, including surveillance of infants born to a mother with SARS-CoV-2 infection
- Community engagement surveillance for post-acute cases
- Post-acute cases identified in extant populations
- COVID vaccine clinics
- Existing research and clinical cohorts of post-acute children (including MIS-C research cohorts) and infants born to a mother with SARS-CoV-2 infection
- PASC consortium EHR cohorts
- Existing patient advocacy groups and social networks of PASC patients
- Referral from other health care providers at the PASC consortium pediatric cohort health care systems
- Participant self-referral

Children and young adults with SARS-CoV-2 infection will be sampled and recruited from an unbiased denominator of SARS-CoV-2 infected children, including a diversity of sites of care (e.g., not only patients seeking medical care at a post-COVID clinic or only patients cared for in academic medical centers) and severity of illness (i.e., not only from hospitalized patients).

For children and young adults without SARS-CoV-2 infection, participants will be sampled and recruited from similar communities, demographics, and sites and dates of care as those being recruited into the SARS-CoV-2 positive cohort. Screening testing after household or school contacts will be used when possible to identify uninfected control subjects. Uninfected control subjects may also be recruited from well-child ambulatory clinic visits or COVID vaccine clinics that are contemporaneous with acute and post-acute cases (within 6 months of acute index date). Recruitment may be stratified to match the SARS-CoV-2 positive group in terms of racial/ethnic diversity and severity of acute SARS-CoV-2 illness based on history of hospitalization and history of treatment in an intensive care unit.

Participants with history of MIS-C will be recruited from existing research cohorts, PASC consortium EHR cohorts, active surveillance of the EHR at PASC consortium pediatric cohort sites, existing patient advocacy social networks for MIS-C, referral from other health care providers at the PASC consortium pediatric cohort health care systems, and self-referral.

Participants with post-vaccine myocarditis will be recruited from PASC consortium EHR cohorts, clinical surveillance in pediatric vaccine clinics at PASC consortium pediatric cohort sites, active surveillance of the EHR at PASC consortium pediatric cohort sites, existing patient advocacy social networks for post-vaccine myocarditis, referral from other health care providers at the PASC consortium pediatric cohort health care systems, and self-referral.

Caregivers will be recruited at the time of child enrollment when feasible. Caregiver participation is optional; eligible child and young adult participants can be enrolled with or without caregiver participation. The other biological parent of the child/young adult will be recruited at the time of caregiver recruitment, if the primary caregiver is a biological parent. Participation by the other biological parent is optional.

9.4.2 Retention Strategy

Subject retention will be promoted by promoting self-efficacy and self-monitoring behaviors with a home pulse oximetry device provided by the study site, by promoting family engagement with educational materials related to PASC symptom management, by creation of a patient web portal to provide access to study personnel for questions, and by using feedback received from subjects to enhance participant experience. Additional efforts will be made to minimize attrition: 1) reminder calls for assessments, rescheduled if missed; 2) maintaining contact information (e.g., updating at each contact, obtaining alternate contacts, re-connecting in primary care), and ongoing contact with all subjects during the study by email (with encrypted messaging if PHI included) and by text (with subject consent); 3) compensation for subject participation in study procedures.

Contacts with participants may include: reminders for completion of study surveys, reminders for study appointments, a post-visit thank-you card or call, a quarterly newsletter, a birthday or greeting card, and a holiday or end-of-year card. We aim to design both culturally and religiously appropriate contact documents. Therefore, these contacts will be initiated by each site and will be conducted in the language of choice of the participant. In addition, because some religions (e.g., Jehovah's Witnesses) may not celebrate birthdays or holidays, specialized cards will be designed to accommodate these participants. All newsletters and cards sent to RECOVER respondents will be targeted for a 5th grade reading-level.

Subject response burden will be monitored in real-time during the study. If burden is found to be excessive, it will be reduced by altering the data collection strategy, such as by increasing the interval of assessments; reducing the number of data elements collected (eliminating rare symptoms); pre-filling prior responses to reduce data entry time for subjects; tightening criteria for Tier 2 and Tier 3 data collection; increasing the availability of home-based Tier 2/3 assessments; and/or increasing subject reimbursement. All such modifications will be approved by the IRB before implementation.

Recruitment and retention data will be monitored on an ongoing basis to compare target versus actual recruitment rates by site (stratified by age, race/ethnicity, sex); compare the number of expected surveys completed and biospecimens collected to target; and subject retention reports indicating the number of participants active, completed, and lost to follow-up.

9.5 Duration of Study Participation

Up to four years from the time of study entry, or from birth in the case of infants born to mothers with SARS-CoV-2 during pregnancy.

9.6 Total Number of Subjects and Sites

Figure 1 provides an overview of the number of study subjects. Up to 20,000 dyads (children and young adults with and without history of SARS-CoV-2 infection and their primary caregiver), including 2500 infants born to mothers with and without SARS-CoV-2 infection during pregnancy (congenitally exposed), 800 children with MIS-C and 200 participants with history of COVID vaccine associated myocarditis. Up to 20,000 other biological parents will also be recruited.

Participants will be recruited from the enrolling sites that comprise the RECOVER Pediatric PASC Investigator Consortium. The RECOVER Pediatric and Prenatal/Infancy PASC Investigator Consortium study sites are listed in Appendix A.

9.7 Participant Withdrawal or Termination

Subjects are free to withdraw from participation in the study at any time upon request. The participant will provide a written notice of withdrawal to the study site PI.

Upon withdrawal, the subject's w data will be excluded from data distribution for future analyses. Patient data will not be excluded from completed analyses.

Once the subjects withdraw participation, no more information will be collected. However, in cases when the data removal will affect the integrity of the study, all previously collected data will not be removed once the subjects withdraw from the study and subjects will be informed during consenting process.

9.7.1 Reasons for Withdrawal or Termination

Participants may be prematurely terminated from the study in the following situations:

- Consent is withdrawn
- Participant is no longer actively participating in study activities
- Any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant

- The participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation
- The study sponsor, the principal investigator, or other body responsible for monitoring the safety of the study has decided to stop the study.

9.7.2 Handling of Participant Withdrawals or Termination

Subjects may withdraw at any time and for any reason by contacting the PI at the study site. If a subject chooses to withdraw from the study, or is otherwise terminated from the study, there will be an effort to obtain permission to record vital status data up to the protocol-described end of follow-up for that subject. Subjects who withdraw will be asked to provide a written communication via mail or email to the study site with their preferences about future contact and use of collected data and biospecimens. Upon withdrawal, a subject's samples in storage at the Mayo Clinic Biorepository and collected study data will be destroyed according to subject request. No future samples will be collected. After a short period of time to allow for validation of the withdrawal with the study site, the RECOVER Participant ID will be unlinked from fully identifying information. It will not be possible to destroy data that has already been distributed. This includes the limited data set available in the RECOVER de-identified data analysis portal managed by the Data Resource Core at Massachusetts General Hospital.

Subjects will be considered lost to follow-up if they fail to respond to three telephone calls over 2 months at their home and mobile phones on record, fail to respond to three telephone calls made to next of kin when possible, and fail to respond to a registered letter sent to their address on record.

There will be no planned replacement for study subjects lost to follow-up. However, enrollment targets may be adjusted based on planned interim analyses.

9.7.3 Premature Termination or Suspension of Study

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to the NIH Sponsor and site investigators. If the study is prematurely terminated or suspended, the site PI will promptly inform the IRB and will provide the reason(s) for the termination or suspension.
Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Emergence of new information from external sources that merits premature termination of the study
- Determination of futility for achievement of study aims that would warrant premature termination
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable

Study may resume once concerns about safety, protocol compliance, and data quality are addressed and satisfy the sponsor and/or IRB.

10 Study Schedule

10.1 Overview of study schedule

A tiered phenotyping approach will be implemented for structured data collection at all study visits. The tiered phenotyping approach will be tailored across the child/young adult lifespan: early childhood including infancy, toddlerhood and pre-school age (ages newborn-5 years), middle childhood (ages 6-11 years), adolescence (ages 12-17 years) and young adulthood (ages 18-25 years). All subjects will provide informed consent (and age-appropriate assent) at the first Tier 1 study contact before participating in any study procedures.

Tier 1 procedures will include baseline questionnaires for collection of sociodemographic information, social determinants of health, medical history and core data elements related to past SARS-CoV-2 infection, possible PASC symptoms, and quality of life that will be collected remotely at study entry in all participants with or without history of SARS-CoV-2 infection (both caregiver and child/young adult subjects). Tier 1 will also include home collection of limited blood biospecimens including dried blood spot for detection of antibodies to SARS-CoV-2, and for storage in the PASC Consortium RECOVER biorepository for future analyses, and home collection of saliva for extraction of DNA for future analyses. If the primary caregiver is a biological parent of the child or young adult, the other biological parent may also be enrolled to provide a home sample of saliva for DNA analysis.

Tier 2 and Tier 3 procedures will include longitudinal questionnaire data and biospecimen collection conducted remotely and at on-site study visits to provide more detailed longitudinal characterization of PASC for up to four years in subjects with a history of SARS-CoV-2 infection, a matched sample of subjects without history of SARS-CoV-2 infection, and caregivers. Common data elements, adapted across the child and young adult lifespan, will be used for all Tiers when available. For acute participants there is an acute phase of Tier 2 (first 8 weeks), and for all Tier 2 participants there is a post-acute phase extending from 6-48 months after study entry.

The Tier numbering represents progressively increased detail and complexity in phenotyping procedures rather than a temporal relationship. The study visit time windows are designed to allow overlap in the timing of Tier 1-3 procedures depending on the whether the enrollment occurs at the time of an acute or post-acute COVID-19 infection, the individual sub-cohort, the individual participant history of COVID-19, and other logistics related to scheduling.

Collection of blood biospecimens in children and young adults will be conducted in accord with Federal regulations (the lesser of 50 mLs or 3 mL/kg in an eight-week period and collection will not occur more frequently than 2 times per week). Clinical laboratory tests will be collected at each site and processed locally. Biospecimens for future analysis will be collected in collaboration with the PASC RECOVER Biorepository Core at the Mayo Clinic in Rochester Minnesota. For stored specimens, the biorepository staff will utilize test-specific standard operating procedures for biospecimen collection and transport, and will supply each site with necessary collection supplies for each study visit.

In addition to structured data collection, EHR data from pre- and post-pandemic time intervals, and historical data from extant cohorts may be incorporated into the research record when feasible. A mobile health platform may be developed for remote unstructured data collection including sensor data from wearable devices, and remote collection of structured and unstructured participant reported outcomes. Mobile health data will not be collected until IRB approval of a future modified protocol is obtained. Biospecimens derived from clinical procedures may be collected when feasible, including cerebrospinal fluid, other body fluids, bronchoalveolar lavage specimens, procedural biopsies, and surgical pathology specimens. Identified clinical biospecimens will be tracked and either transferred from the cohort site to the PASC central RECOVER biorepository or linked by the study UUID to the site biorepository for future access.

Schedules of Events by sub-cohort and age are included in Appendix B. Research procedures listed in the Schedule of Events will occur based on the elapsed time from study entry except for the MIS-C and post-vaccine myocarditis cohorts, for which the testing will occur based on elapsed time from their index hospitalization and index vaccination respectively, and the cohort of infants born to mothers with SARS-CoV-2 infection during pregnancy, for which the testing will occur based on elapsed time from birth. For the main pediatric cohort post-acute visits will occur at 6, 12, 24, 36, and 48 months after study entry.

Scheduled procedures will be conducted at each visit according to the subject age at the time of the visit. The questionnaire format and data granularity vary across age groups, but the domains remain constant to allow roll up of data for longitudinal comparison across age groups for each participant. For young adults who attain age >25 years after enrollment, participation in the study will continue with the procedures as described for the 18-25 years age group.

10.2 Enrollment/Baseline

The study design is a meta-cohort enrolling subjects in an ambidirectional time frame relative to the presence of a SARS-CoV-2 infection (or a control subject without infection selected from the same time frame). The study meta-cohort figure (Figure 2) represents the ambidirectional time frames for the different components of the meta-cohort. The subjects may be enrolled from extant research cohorts, extant clinical cohorts, acute cohorts with SARS-CoV-2 infection, children born to mothers with SARS-CoV-2 infection during pregnancy, and post-acute cohorts with history of past SARS-CoV-2 infection in extant post-acute research cohorts or clinical post-acute cohorts (Figure 2). All subjects will undergo the same Tier 1 study procedures as described below. Subjects will be selected for Tier 2 study procedures and Tier 3 study procedures based on history of SARS-CoV-2 infection and post-acute symptoms consistent with the sequelae of SARS-CoV-2 infection (Figure 1 Overview of Study Procedures).

Enrollment/Baseline Visit (Tier 1)

- Obtain and document consent from participant on study IRB-approved informed consent form, and assent form if applicable
- Verify SARS-CoV-2 infection status based on history
- Verify and document that enrollment criteria are met
- Obtain identify information for generation of UUID
- Obtain information via interview and questionnaires as described for Tier 1 below.
- Collect biospecimens as described for Tier 1 in Section 11.14.

10.3 Intermediate Visits (Tiers 2 and 3)

- Intermediate visits will be planned to occur according to age and sub-cohort per the descriptions in Section 11 and the schedules of assessments in Appendix B. Study procedures will be conducted for Tier 2 and Tier 3 as described below.
- Subjects will be selected for participation in Tiers 2 and 3 based on their history of COVID exposure and PASC symptoms as determined in Tier 1 for post-acute participant and uninfected controls, and at the week 8 visit for acute Tier 2 participants. Subjects will be selected for participation in Tiers 2 and Tiers 3 to achieve the enrollment targets as summarized in Figure 1.
- Scheduling of intermediate visits will differ by sub-cohort as follows:
    - For main pediatric cohort, each subject will be asked participate in visits according to the elapsed time from study entry and continue to end of the funding period.
    - For infants born to mothers with and without SARS-CoV-2 infection, each subject will be asked to participate in visits according to their elapsed time from birth.
    - For MIS-C patients, each subject will be asked to participate in visits according to their elapsed time from their index hospitalization for MIS-C.
    - For post-COVID vaccine myocarditis patients. each subject will be asked to participate in visits according to their elapsed time from index date of vaccination.
- The maximum number of visits for an acute cohort subject enrolled at the start of the funding period would be 10 visits: 5 visits acute Tier 2 (4 remote and 1 on-site) and 5 visits on-site post-acute Tier 2 as described below.
- Intermediate visits may be conducted on-site at the study center and remotely. There will be telephone contact with the subjects during the study window for intermediate visits to assist in the completion of remote questionnaires. Subjects will receive email links for remote online completion of required study questionnaires. Any email message with PHI will be encrypted prior to sending. Patient may opt-in to receive text communications with study staff.
- In the case of newly identified SARS-CoV-2 infection or re-infection after study entry (newly positive antigen test result >90 days after a known prior acute infection or newly positive antibody test result), additional intermediate visits will be scheduled as follows:
    - For identified acute infection or re-infection with SARS-CoV-2 (≤30 days), the participant will undergo 2, 4, and 8-week remote survey assessments according to Tier 2 acute procedures for participant age. Subsequent post acute Tier 2 visits will be conducted without change in schedule. Participants initially enrolled as uninfected controls will be reclassified as part of the infected cohort.

    - For identified post-acute infection or re-infection (>30 days), one additional remote assessment according to Tier 2 post-acute procedures for participant age will be scheduled if the infection becomes known outside of a scheduled study visit window. Remote post-acute Tier 2 visit procedures will be conducted according to participant age. Subsequent post acute Tier 2 visits will be conducted without change in schedule. Participants initially enrolled as uninfected controls will be reclassified as part of the infected cohort.
- Tier 2 and Tier 3 visits may be scheduled contemporaneously or separately according to the specified visit time windows per discretion of the site investigators, and participant preference.

10.4 Final Study Visit

The final study visit will occur 30 days before the end of the funding period according to the schedule of events in Appendix B. Any ongoing AE/SAE at the time of the final visit will be followed for an additional 30 days or until resolution, whichever is shorter. Subjects will be provided with instructions for further follow up of ongoing adverse events, sharing of study results, and for continuation of care with their primary care physician. Aggregate study results will be shared with participants within 6 months of the final study visit.

10.5 Withdrawal Visit

In the event that a subject withdraws early, or has study participation terminated by the investigator, an effort will be made to schedule a final study visit according to the next scheduled visit as listed in appendix B. The study procedures in the final visit may be abbreviated depending on subject preference and safety considerations.

10.6 Unscheduled Visit

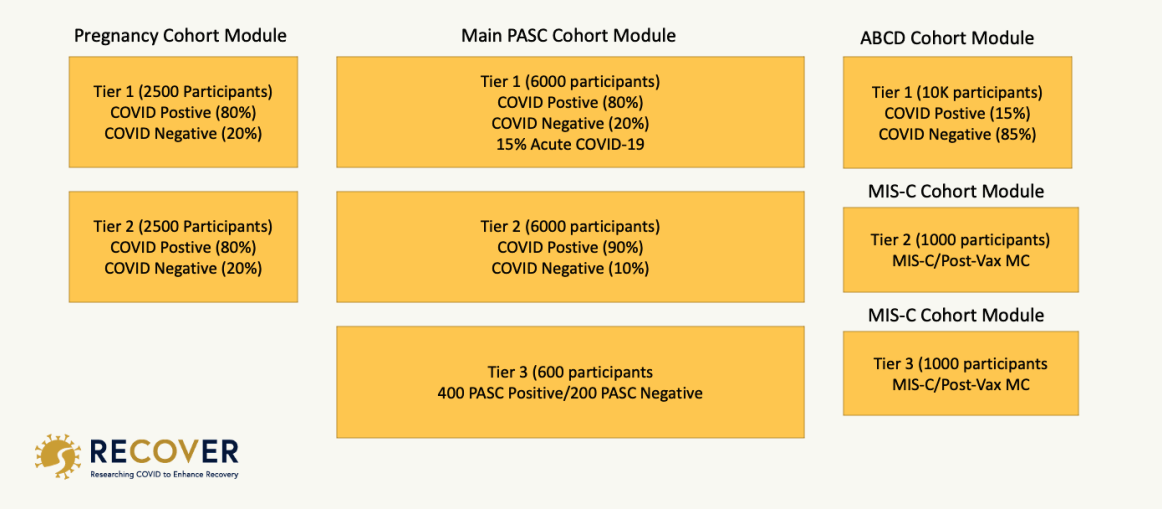
In the event of an unanticipated event as described below, or occurrence of a SAE, subjects may be contacted for an unscheduled remote or onsite visit in order to obtain necessary information for monitoring subject safety and reporting to the IRB and OSMB.

11 Study Procedures/Evaluations

11.1 Tiered phenotyping by sub-cohorts

Tiered phenotyping is customized for each of the sub-cohorts contributing to the RECOVER meta-cohort structure as shown in figure 3. Within each cohort, study procedures are customized based on age of the subject. Subjects participation over time will progress from Tier 1 to Tier 2 to Tier 3 according to sub-cohort criteria within study time windows, but participation in Tier 1 does not obligate participation in Tier 2, and participation in Tier 2 does not obligate participation in Tier 3. Details of the tiered phenotyping procedures are provided by sub-cohorts in the following paragraphs.

Figure 3: Tiered phenotyping by sub-cohort in the RECOVER metacohort



11.2 Main PASC Pediatric Cohort Module (n=6000 infected and uninfected subjects, ages newborn-25 years)

11.3 Main PASC Pediatric Cohort Module Tier 1 Procedures

Tier 1 assessments will be conducted in participants selected from unbiased sources to determine history of COVID-19 infection and history or persistent symptoms post-COVID in order to estimate the incidence rate ratio of PASC. Tier 1 visits will be conducted remotely by caregiver/child self-report (electronic-based, phone-based, or paper-based) or by research staff-assisted data collection (telephone, videoconference) at the time of study entry. Tier 1 questionnaires are adapted according to subject age (newborn-5 years, 6-11 years, 12-17 years, and 18-25 years).

11.3.1 Main Cohort Tier 1 procedures ages newborn-5 years (not including congenital exposure)

For subjects ages newborn to 5 years, the caregiver will be the primary respondent with limited input from the child. Tier 1 data collection may be conducted remotely and or at an on-site study visit according to participant preference. It is anticipated that completion of all Tier 1 questionnaires will require approximately 2 hours. All Tier 1 assessments including initial biospecimen collection will be completed within 1 months.

If Tier 1 enrollment occurs at time of acute COVID-19 and the patient is too ill to participate in the complete baseline assessment, some patient-reported elements may be deferred until recovery but should then be completed as close to the acute infection as possible. Structured data collection will include the following domains:

- Obtain and document consent from participant on study IRB-approved informed consent form,
- Child sociodemographic data and contact information including next of kin
- Child medical history, including history of MIS-C
- Child a vaccination history
- Child health status
    - Child/Young Adult PROMIS Pediatric Global Health measure
- Child history of acute SARS-CoV-2 infection

- Child history of PASC symptoms
- Child COVID health impact
- Household social determinants of health
    - address stability
    - financial stability (weekly stress inventory 5)
    - food insecurity (hunger vital signs)
    - access to health care (Adult Access to Health Care & Utilization Module, Question on Insurance from US Census)
    - perceived discrimination (Major Experiences and Everyday Discrimination Scale)
    - stress (Perceived Stress Scale)
    - social support (RAND-MOS)
    - community cohesion (Neighborhood Collective Efficacy Questionnaire)

The tier 1 study visit(s) will also include remote biospecimen collection (ages 24 months-5 years, <5 ml) as described in Section 11.14. Tier 1 remote biospecimen collection will be performed in acute COVID-19 patients at the 8-week visit of Tier 2. Selected Tier 1 child participants without history of SARS-CoV-2 infection who reside in regions with higher rates of SARS-CoV-2 infection and who have no detectable antibodies on the first test may contribute up to two additional biospecimens for SARS-CoV-2 antibody at 3-month intervals if the test remains negative (total 3 tests over total of 6 months).

Unstructured data collection from the participant EHR may also be recorded for Tier 1 assessments when feasible.

11.3.2 Main Cohort Tier 1 procedures ages 6-17 years

For subjects aged 6 to 17 years, the caregiver will be the primary respondent with optional input from the child Tier 1 data collection may be conducted entirely remotely and may also occur at an on-site study visit according to participant preference. It is anticipated that completion of all Tier 1 assessments will require approximately 2 hours. All Tier 1 assessments including biospecimen collection will be completed within 3 months.

If Tier 1 enrollment occurs at time of acute COVID-19 and the patient is too ill to participate in the complete baseline assessment, some patient-reported elements may be deferred until recovery but should then be completed as close to the acute infection as possible. Structured data collection will include the following domains:

- Obtain and document consent from participant on study IRB-approved informed consent form, and assent form if applicable
- Child sociodemographic data and contact information including next of kin
- Child medical history, including history of MIS-C
- Child a vaccination history
- Child health status
    - Child/Young Adult PROMIS Pediatric Global Health measure
- Child history of acute SARS-CoV-2 infection
- Child history of PASC symptoms
- Child COVID health impact
- Household social determinants of health
    - address stability
    - financial stability (weekly stress inventory 5)
    - food insecurity (hunger vital signs)
    - access to health care (Adult Access to Health Care & Utilization Module, Question on Insurance from US Census)
    - perceived discrimination (Major Experiences and Everyday Discrimination Scale)
    - stress (Perceived Stress Scale)
    - social support (RAND-MOS)
    - community cohesion (Neighborhood Collective Efficacy Questionnaire)

The tier 1 study visit(s) will also include remote biospecimen collection as described in Section 11.14. Tier 1 remote biospecimen collection will be performed in acute COVID-19 patients at the 8-week visit of Tier 2. Selected Tier 1 child participants without history of SARS-CoV-2 infection who reside in regions with higher rates of SARS-CoV-2 infection and who have no detectable antibodies on the first test may contribute up to two additional biospecimens for SARS-CoV-2 antibody at 3-month intervals if the test remains negative (total 3 tests).

Unstructured data collection from the participant EHR may also be recorded for Tier 1 assessments when feasible.

11.3.3 Main Cohort Tier 1 procedures ages 18-25 years

For subjects ages 18-25 years, the young adult will be the primary respondent with optional input from the caregiver. Tier 1 data collection may be conducted entirely remotely and may also occur at an on-site study visit according to participant preference. It is anticipated that completion of all Tier 1 assessments will require approximately 2 hours. All Tier 1 assessments including biospecimen collection will be completed within 3 months.

If Tier 1 enrollment occurs at time of acute COVID-19 and the patient is too ill to participate in the complete baseline assessment, some patient-reported elements may be deferred until recovery but should then be completed as close to the acute infection as possible. Structured data collection will include the following domains:

- Obtain and document consent from participant on study IRB-approved informed consent form
- Young adult sociodemographic data and contact information including next of kin
- Young adult medical history, including history of MIS-C
- Young adult vaccination history
- Young adult health status
    - Young adult PROMIS10 Quality of Life
- Young adult history of acute SARS-CoV-2 infection
- Young adult history of PASC symptoms
- Young adult COVID health impact
- Household social determinants of health
    - address stability
    - financial stability (weekly stress inventory 5)
    - food insecurity (hunger vital signs)
    - access to health care (Adult Access to Health Care & Utilization Module, Question on Insurance from US Census)
    - perceived discrimination (Major Experiences and Everyday Discrimination Scale)
    - stress (Perceived Stress Scale)
    - social support (RAND-MOS)
    - community cohesion (Neighborhood Collective Efficacy Questionnaire)

The tier 1 study visit(s) will also include remote biospecimen collection a as described in Section 11.14. Tier 1 remote biospecimen collection will be performed in acute COVID-19 patients at the 8-week visit of Tier 2. Selected Tier 1 young adult participants without history of SARS-CoV-2 infection who reside in regions with higher rates of SARS-CoV-2 infection and who have no detectable antibodies on the first test may contribute up to two additional biospecimens for SARS-CoV-2 antibody at 3-month intervals if the test remains negative (total 3 tests).

Unstructured data collection from the participant EHR may also be recorded for Tier 1 assessments when feasible.

11.4 Main pediatric cohort acute Tier 2 assessments

Tier 2 assessments will be conducted in participants with acute SARS-CoV-2 infection (≤30 days), and a sample of uninfected control subjects selected contemporaneously from same recruitment pool. The post-acute Tier 2 assessments will be conducted remotely when feasible. Questionnaires are customized for age groups with primary respondent determined by the age of the child/young adult as described for Tier 1. All acute Tier 2 clinical assessments are optional based on the severity of illness, age of the participant and the judgment of the investigator and caregiver. Minimum ages are provided for each procedure. The schedule of assessments for all acute Tier 2 visits will be based on the elapsed time from study entry. Tier 1 biospecimens in acute COVID-19 patients will be collected at the 8 week visit of acute Tier 2.

11.4.1 Main Cohort Acute Tier 2 procedures ages newborn-5 years

For infants, toddlers and pre-school age children with acute post-natal SARS-CoV-2 infection (≤30 days) and uninfected control subjects, Tier 2 assessments will occur at weeks 2, 4, and 8 after study entry. Questionnaires customized for age group newborn-5 years with caregiver as primary respondent will be administered as described for Tier 1. All visits in this age group will be remote with structured data collection at study visits:

- Child Acute COVID testing information
- Child exposure information
- Child Health care resource utilization
- Child Acute COVID medications
- Child Acute COVID symptoms
- Child PASC symptoms
- Child anthropometry and vital signs including oximetry (week 8 ages 3-5 years): Child blood pressure, heart rate, oxygen saturation, oral, axillary or skin temperature, height (length), weight, head circumference, waist circumference and skin fold thickness will be measured as appropriate for age.

A pulse oximeter for home use may be shipped from the study site to acute Tier 2 subjects ages 3-5 years and uninfected control subjects when feasible. The caregiver and child participant will complete a diary or oxygen percent saturation and pulse rate with daily measurements for one week, weekly measurements for the next 3 weeks, and additional measurements at the time of reported symptoms of palpitations or shortness of breath. The diary will be returned to the site for REDCap data entry.

Child biospecimen collection including Tier 1 remote biospecimen collection may be conducted remotely or at on-site study visit at week 8 after start of the SARS-CoV-2 infection as described in Section 11.14.

Unstructured data collection from the participant EHR and from mobile health applications may also be recorded to monitor participant health events and symptoms status during longitudinal post-acute Tier 2 follow-up when feasible. The mobile health applications will not be used until their use is approved by the IRB. If a subject is hospitalized during the acute phase of infection, the electronic health record may be abstracted to capture length of stay, type of hospital unit, use of supplemental oxygen and ventilatory support, presence of MIS-C, and COVID-19 treatments.

No caregiver data will be collected during the acute Tier 2 assessments.

11.4.2 Main Cohort Acute Tier 2 procedures ages 6-17 years

For participants with acute SARS-CoV-2 infection (≤30 days) and uninfected control subjects, Tier 2 assessments will occur at weeks 2, 4, and 8 after study entry. Weeks 2 and 4 will include remote questionnaire assessments. Questionnaires customized for age groups 6-11 and 12-17 years with caregiver as primary respondent will be administered as described for Tier 1. The visit at week 8 will be conducted on-site at the enrolling site facility if possible and will include clinical assessments and biospecimen collection. If the patient is too ill to participate in the scheduled visits, efforts will be made to capture as much data as possible remotely or onsite when patient condition allows. Structured data collection at study visits during this 8-week period will include the following domains:

- Child Acute COVID testing information
- Child exposure information
- Child Health care resource utilization
- Child Acute COVID medications
- Child Acute COVID symptoms
- Child PASC symptoms
- Child anthropometry and vital signs including oximetry (week 8): Child blood pressure, heart rate, oxygen saturation, oral, axillary or skin temperature, height (length), weight, head circumference, waist circumference and skin fold thickness will be measured as appropriate for age.
- Child electrocardiogram (week 8): A 12-lead electrocardiogram will be performed with FDA-approved equipment (optional for ages 5 years and below per judgment of the site investigator and caregiver). Self-sticking pediatric size electrodes will be placed on the extremities and chest for the electrocardiogram recording and will be removed after the recording. The electrocardiogram will be completed in approximately 5 minutes.
- Child Spirometry testing (week 8): An FDA-approved hand-held spirometry system will be used to measure lung function for ages 5-25 years (optional for ages 7 years and below per judgement of the site investigator and caregiver). Subjects will be asked to blow into a single-use tube to capture exhaled gases. Three exhalations will be assessed.

A pulse oximeter for home use may be shipped from the study site to acute Tier 2 subjects and uninfected control subjects when feasible. The caregiver and child participant will complete a diary or oxygen percent saturation and pulse rate with daily measurements for one week, weekly measurements for the next 3 weeks, and additional measurements at the time of reported symptoms of palpitations or shortness of breath. The diary will be returned to the site for REDCap data entry.

Child biospecimen collection including Tier 1 remote biospecimen collection may be conducted remotely or at on-site study visit at week 8 after start of the SARS-CoV-2 infection as described in Section 11.14.

Unstructured data collection from the participant EHR and from mobile health applications may also be recorded to monitor participant health events and symptoms status during longitudinal post-acute Tier 2 follow-up when feasible. The mobile health applications will not be used until their use is approved by the IRB. If a subject is hospitalized during the acute phase of infection, the electronic health record will be abstracted to capture length of stay, type of hospital unit, use of supplemental oxygen and ventilatory support, presence of MIS-C, and COVID-19 treatments.

No caregiver data will be collected during the acute Tier 2 assessments.

11.4.3 Main Cohort Acute Tier 2 procedures ages 18-25 years

For participants with acute SARS-CoV-2 infection (≤30 days) and uninfected control subjects, Tier 2 assessments will occur at weeks 1, 2, 3, 4, and 8 after study entry. Weeks 2 and 4 will include remote questionnaire assessments. Questionnaires customized for age group 18-25 years with young adult as primary respondent will be administered as described for Tier 1. The visit at week 8 will be conducted on-site at the enrolling site facility if possible and will include clinical assessments and biospecimen collection. If the patient is too ill to participate in the scheduled visits, efforts will be made to capture as much data as possible remotely or onsite when patient condition allows. Structured data collection at study visits during this 8-week period will include the following domains:

- Young adult Acute COVID testing information
- Young adult exposure information
- Young adult Health care resource utilization
- Young adult Acute COVID medications
- Young adult Acute COVID symptoms
- Young adult PASC symptoms
- Young adult anthropometry and vital signs including oximetry (week 8): Blood pressure, heart rate, oxygen saturation, oral, axillary or skin temperature, height (length), weight, head circumference, waist circumference and skin fold thickness will be measured as appropriate for age.

- Young adult electrocardiogram (week 8): A 12-lead electrocardiogram will be performed with FDA-approved equipment (optional for ages 5 years and below per judgment of the site investigator and caregiver). Self-sticking pediatric size electrodes will be placed on the extremities and chest for the electrocardiogram recording and will be removed after the recording. The electrocardiogram will be completed in approximately 5 minutes.
- Young adult Spirometry testing (week 8): An FDA-approved hand-held spirometry system will be used to measure lung function for ages 5-25 years (optional for ages 7 years and below per judgement of the site investigator and caregiver). Subjects will be asked to blow into a single-use tube to capture exhaled gases. Three exhalations will be assessed.

A pulse oximeter for home use may be shipped from the study site to acute Tier 2 subjects and uninfected control subjects when feasible. The young adult participant with optional assistance from the caregiver will complete a diary or oxygen percent saturation and pulse rate with daily measurements for one week, weekly measurements for the next 3 weeks, and additional measurements at the time of reported symptoms of palpitations or shortness of breath. The diary will be returned to the site for REDCap data entry.

Young adult biospecimen collection including Tier 1 remote biospecimen collection will be conducted remotely or at on-site study visit at week 8 after start of the SARS-CoV-2 infection as described in Section 11.14.

Unstructured data collection from the participant EHR and from mobile health applications may also be recorded to monitor participant health events and symptoms status during longitudinal post-acute Tier 2 follow-up when feasible. The mobile health applications will not be used until their use is approved by the IRB. If a subject is hospitalized during the acute phase of infection, the electronic health record will be abstracted to capture length of stay, type of hospital unit, use of supplemental oxygen and ventilatory support, presence of MIS-C, and COVID-19 treatments.

No caregiver data will be collected during the acute Tier 2 assessments.

11.5 Main Cohort Post-Acute Tier 2 Assessments

Tier 2 assessments will be conducted in patients with post-acute SARS-CoV-2 infection (>30 days), and a sample of patients without history of SARS-CoV-2 infection who have completed Tier 1 assessments. The post-acute Tier 2 assessments will be conducted remotely when feasible. Questionnaires are customized for age groups with primary respondent determined by the age of the child/young adult as described for Tier 1. All clinical assessments are optional based on the age of the participant and the judgment of the investigator and caregiver. Minimum ages are provided for each procedure. Wide study windows (±2 months) for Tier 2 visits will be implemented to accommodate child and caregiver needs and facilitate completion of all scheduled assessments. The schedule of assessments for all Tier 2 visits will be based on the elapsed time from study entry. The first post-acute Tier 2 visit will be scheduled within 6 months of completion of the Tier 1 visit assessments.

11.5.1 Main Cohort Post-Acute Tier 2 assessments ages newborn-5 years

For infants, toddlers and pre-school age children with post-acute post-natal SARS-CoV-2 infection (>30 days) and uninfected control subjects, Tier 2 assessments will occur at months 6, 12, 18, 24, 36 and 48 after study entry. Questionnaires customized for age group newborn-5 years with caregiver as primary respondent will be administered as described for Tier 1. Study procedures in this age group will be conducted remotely for ages newborn to 2 years and a combination of remote or on-site with structured data collection at on-site study visits for ages 3-5 years.

On-site assessments

- Growth (weight, height, head circumference, skinfolds; measured) at age 36 and 48 months
- Bayley Scales of Infant Development-4 (child development) at age 24 months
- Differential Ability Scales-II, Time (child development) at age 36 and 48 months
- Infant sleep (Brief infant Sleep Questionnaire)
- Child motor development (ages 3-5 years)
    - Neuro-QOL measures of lower extremity mobility and upper body fine motor and ADL

- Child vital signs including oximetry
- Child electrocardiogram (age 3-5 years if feasible based on judgment of site investigator and caregiver, optional after first post post-acute visit)
- Child spirometry (age 3-5 years if feasible based on judgment of site investigator and caregiver, optional after first post-acute visit)

Remote or on-site Assessments

- Child interim medical history
- Child interim vaccination history
- Child interim history of SARS-CoV-2 infection
- Ages and Stages Questionnaire at ages 12, 24 and 36 months of age (child development)
- National Survey of Children's Health Questionnaire at ages 12, 24 and 36 months of age (child health)
- Modified Checklist for Autism in Toddlers at age 18 months (screening test for autism)
- Ages and Stages Questionnaire Social Emotional at age 18 months (child development)
- Growth at ages 12, 18, 24 and 36 months (weight, length, head circumference; self-report or EHR)
- Child Behavior Checklist for Behavioral Problems at ages 24 and 36 months (child development)
- Developmental Profile-4 Time at age 36 months (child development)

Child Tier 2 biospecimen collection (ages 3-5 years, <15 ml) may be conducted at 12, 24, 36, and 48 months after study entry on-site at the pediatrics cohort study locations or by home phlebotomy service as described in Section 11.14.

Unstructured data collection from the participant EHR and from mobile health applications may also be recorded to monitor participant health events and symptoms status during longitudinal post-acute Tier 2 follow-up when feasible. The mobile health applications will not be used until their use is approved by the IRB.

11.5.2 Main Cohort Post-Acute Tier 2 remote assessments ages 6-17 years

For children ages 6-17 years with post-acute SARS-CoV-2 infection (>30 days) and uninfected control subjects, Tier 2 assessments will occur at months 6, 12, 24, 36, and 48 after study entry. Questionnaires customized for age groups 6-11 years and 12-17 years with caregiver as primary respondent will be administered as described for Tier 1. Study procedures in this age group will be conducted remotely and on-site with structured data collection at study visits:

Remote or on-site Assessments

- Child interim medical history
- Child interim vaccination history
- Child interim history of SARS-CoV-2 infection
- Child Adult interim COVID health impact
- Child Adult interim history of PASC symptoms
- Child Adult interim well-being
    - PROMIS Pediatric Global Health measure
    - PROMIS Life Satisfaction
    - PROMIS Meaning and Purpose
    - PROMIS Social Isolation
    - PROMIS Family Relationships
- Child Interim Physical Activity
    - PROMIS- Physical Activity - Parent Proxy/CAT (Child 5 to 7 years)
    - PROMIS - Physical Activity Pediatric/CAT (Child 8+ years)
- Child diet (months 12, 24, 36 48 only)
- Child sleep (PROMIS Sleep-Related Disturbance)
- Child health symptoms
    - PROMIS asthma impact
    - PROMIS fatigue

- Child education status (ages 5 and above)
    - Academic performance
    - Attendance
    - Grade level (months 12, 24, 36 48 only)
    - Accommodations

Onsite Assessments

- Child anthropometry and vital signs including oximetry
- Child electrocardiogram (age 3-25 years, optional after first post post-acute visit)
- Child spirometry (age 5-25 years, optional after first post-acute visit)
- Child Ziopatch recording of heart rhythm for up to 14 days (age 5-25 years, first Tier 2 visit only). Ziopatch is a small wearable device is applied against the left chest using a simple adhesive and fits under normal clothing (the device is approximately 5 inches x 2 inches (including adhesive strips) with a central button that is one-half-inch raised, and it weighs 24.5 grams). The Ziopatch will be sent to the participants with instructions and a return mailer. This procedure is optional for children <7 years of age per judgment of the site investigator and caregiver. The device is designed to record up to 14 days, but the duration of the monitoring can be shortened, or temporarily interrupted to coincide with school days or other activities as per judgment of the site investigator and caregiver.
- Child Beighton Scale for joint flexibility. This scale assesses joint hypermobility for the fifth digits of the hand, thumbs, elbows, knees, and spine with simple maneuvers (age 3-35 years, optional after first post-acute visit).
- Child autonomic dysfunction symptoms assessment (Composite Autonomic Symptom Score, COMPASS-31, age 5-25 years, optional after the first post-acute visit)
- Child detailed cognitive development
    - PROMIS Attention and Executive Function
    - PROMIS Episodic Memory
    - PROMIS Working Memory – age 7 years or more only
    - PROMIS Language
    - PROMIS Executive Function and Attention
    - PROMIS Processing Speed Development-Cognitive-Memory
    - PROMIS Immediate Recall – age 8 years or more only
- Child detailed emotional/mental health development
    - Ages and Stages Questionnaire Social Emotional- 2nd edition (ASQ-SE-2)
    - PROMIS Emotional Distress – Depressive Symptoms (Short form)
    - PROMIS Emotional Distress – Anxiety (Short form)
    - PROMIS Psychological Stress Experiences (Short form)
    - Child Behavior Checklist for Behavioral Problems;
    - PROMIS positive affect (Short form)
    - PROMIS Emotional Distress – Anger (Short form)

Child Tier 2 biospecimen collection may be conducted at 6, 12, 24, 36, and 48 months after start of the SARS-CoV-2 infection on-site at the pediatrics cohort study locations or by home phlebotomy service as described in Section 11.14.

Unstructured data collection from the participant EHR and from mobile health applications may also be recorded to monitor participant health events and symptoms status during longitudinal post-acute Tier 2 follow-up when feasible. The mobile health applications will not be used until their use is approved by the IRB.

11.5.3 Main Cohort Post-Acute Tier 2 remote assessments ages 18-25 years

For young adults ages 18-25 years with post-acute SARS-CoV-2 infection (>30 days) and uninfected control subjects, Tier 2 assessments will occur at weeks months 6, 12, 24, 36, and 48 after study entry. Questionnaires customized for age 18-25 years with young adult as primary respondent will be administered as described for Tier 1. Study procedures in this age group will be conducted remotely and on-site with structured data collection at study visits:

Remote or on-site Assessments

- Young Adult interim medical history
- Young Adult interim vaccination history
- Young Adult interim history of SARS-CoV-2 infection
- Young Adult interim COVID health impact
- Young Adult interim history of PASC symptoms
- Young Adult interim well-being
    - PROMIS 10 Global Health measure
    - PROMIS Life Satisfaction
    - PROMIS Meaning and Purpose
    - PROMIS Social Isolation
    - PROMIS Family Relationships
- Young Adult diet (months 12, 24, 36 48 only)
- Young Adult sleep (PROMIS Sleep-Related Disturbance)

Onsite Assessments

- Young Adult anthropometry and vital signs including oximetry
- Young Adult electrocardiogram
- Young Adult spirometry
- Young Adult Ziopatch recording of heart rhythm for up to 14 days (age 5-25 years, first Tier 2 visit only). Ziopatch is a small wearable device is applied against the left chest using a simple adhesive and fits under normal clothing (the device is approximately 5 inches x 2 inches (including adhesive strips) with a central button that is one-half-inch raised, and it weighs 24.5 grams). The Ziopatch will be sent to the participants with instructions and a return mailer. This procedure is optional for children <7 years of age per judgment of the site investigator and caregiver. The device is designed to record up to 14 days, but the duration of the monitoring can be shortened, or temporarily interrupted to coincide with school days or other activities as per judgment of the site investigator and caregiver.
- Young Adult Beighton Scale for joint flexibility. This scale assesses joint hypermobility for the fifth digits of the hand, thumbs, elbows, knees, and spine with simple maneuvers (age 3-25 years, optional after first post-acute visit).
- Young Adult autonomic dysfunction symptoms assessment (Composite Autonomic Symptom Score, COMPASS-31, age 5-25 years, optional after the first post-acute visit)

Young Adult Tier 2 biospecimen collection may be conducted at 6, 12, 24, 36, and 48 months after study entry on-site at the pediatrics cohort study locations or by home phlebotomy service as described in Section 11.14.

Unstructured data collection from the participant EHR and from mobile health applications may also be recorded to monitor participant health events and symptoms status during longitudinal post-acute Tier 2 follow-up when feasible. The mobile health applications will not be used until their use is approved by the IRB.

11.6 Main Pediatric Cohort Tier 3 assessments

For participants with history of SARS-CoV-2 infection with or without PASC symptoms, all of the listed Tier 3 procedures will be performed if appropriate for age and there is no contraindication to a specific procedure. All Tier 3 testing is optional based on the age of the participant and the judgment of the investigator and caregiver. Tier 3 visits will be conducted twice, first at 3-30 months after study entry and second about one year later, 15-42 months after study entry,

Participants will be recruited to participate in Tier 3 testing based on sampling from Tier 2 participants to maintain diversity in the study population, and in all subjects with history of MIS-C. Wide study windows for Tier 3 will be implemented to accommodate child/young adult and caregiver needs and facilitate completion of all scheduled assessments. It is anticipated that completion of Tier 3 procedures might require up to 3 separate visits (total 12 hours) and may be prioritized based on symptoms reported by the participant. The schedule of assessments for all Tier 3 visits will be based on the elapsed time from study entry.

11.6.1 Main cohort Tier 3 assessments (ages 3-5 years)

For pre-school age participants with history of SARS-CoV-2 infection with or without PASC symptoms, a limited set of Tier 3 assessment may be performed. All Tier 3 testing is optional based on the age of the participant and the judgment of the investigator and caregiver. Tier 3 visits will be conducted twice, first at 3-30 months after study entry, and second about one year later, 15-42 months after study entry.

Structured data collection at Tier 3 study visits may include all or some of the following testing:

- Child Echocardiogram (age 3-5 years). An echocardiogram will be performed according to standard clinical protocols and is optional for age <7 years per judgment of the site investigator and caregiver. Three gel electrodes will be placed on the torso to measure electrocardiogram. Ultrasound gel will be applied to the chest; a hand-held Doppler ultrasound transducer will be applied by a licensed pediatric ultrasound technician to the chest wall to obtain standard images of the cardiac chambers, cardiac valves, Doppler-derived blood flow velocities, and global longitudinal strain. The echocardiogram requires 60 minutes.
- Child Abdominal ultrasound (age 3-5 years). An abdominal ultrasound examination will be performed by a trained technician according to clinical protocols and is optional for age <7 years per judgment of the site PI and caregiver). The liver, pancreas, kidneys, and bladder will be imaged. Total time required is 60 minutes.
- Child Brain MRI (age 3-5 years): A 3T or lower field strength brain MRI will be performed according to standard clinical protocols in children who do not require sedation and is optional for age <9 years per judgment of the site investigator and caregiver. The MRI will be conducted by trained technicians under supervision of licensed radiologists at each study site. The MRI requires up to 60 minutes.
- Awake EEG (age 3-5 years): An electroencepholagram (EEG) will be performed according to clinical protocols with FDA approved equipment and is optional for age <9 years per judgement of the site investigator and caregiver. A trained technician will apply scalp electrodes and record the EEG for up to 60 minutes.
- Child neurocognitive testing. These tests will be conducted by a Child Psychologist or their trained assistant. The main set of measures for the neurocognitive battery come from the Woodcock-Johnson batteries, which is a battery of measures with strong psychometric properties, that can be used with subjects between the ages of 3-5 years (valid for wide age range of 2 to 90 years old). These will include Cognitive measures, Language measures, Achievement measures, and behavioral and psychiatric testing. There are Spanish versions of parts of the Cognitive and Achievement subtests. There are norms developed based upon a large nationally representative sample.

Test category	Test
General Intelligence-Verbal	WJ-IV Oral Language
General Intelligence-Nonverbal	WJ-IV Spatial Reasoning
Executive Function - Attention	NIMH Toolbox - Flanker Test
Executive Function - Inhibitory Control	NIMH Toolbox - Inhibitory Control
Episodic Memory	NIMH Toolbox - Picture Sequence Memory
Language - Receptive	WJ-IV - Understanding Directions
Language - Expressive	WJ-IV - Picture Vocabulary & Rapid Picture Naming
Working Memory - Verbal	WJ-IV Numbers Reversed
Processing Speed	WJ-IV - Pair Cancellation
Verbal Memory	WJ-IV Story Memory
Visual-Motor Integration	Beery Buktenica Test
Motor Speed	Purdue Pegboard
Reading	WJ-IV - Letter Word Identification
Spelling	WJ-IV - Spelling
Mathematics	WJ-IV - Number Sense and Calculation
Emotional and Behavioral Adjustment	CBCL, Strengths and Difficulties Questionnaire
Psychiatric Disorder	K-CAT

- Microbiome biospecimen collection (age 3-5 years). Collection of skin swabs, nasal swabs, oral swabs, urine and stool will be performed and is optional for age <7 years per judgment of site investigator and caregiver. Soft-tipped, sterile cotton swabs will be used for the sample collection.

Microbiome swab collection will require 15 minutes. Stool will be collected at home with remote collection kits mailed to the participant home.

Child Tier 3 biospecimen collection (<15 ml) will be conducted on-site at the pediatrics cohort study locations or by home phlebotomy service as described in Section 11.14.

11.6.2 Main cohort Tier 3 assessments (ages 6-25 years)

For child/young adult participants with history of SARS-CoV-2 infection with or without PASC symptoms, a panel of Tier 3 assessment may be performed. All Tier 3 testing is optional based on the age of the participant and the judgment of the investigator and caregiver. Tier 3 visits will be conducted twice, first at 3-30 months after study entry, and second about one year later, 15-42 months after study entry.

Structured data collection at Tier 3 study visits may include all or some of the following testing:

- Child/Young Adult cardiac structure and function (transthoracic echocardiogram, cardiac MRI)
    - Echocardiogram (age 5-25 years). An echocardiogram will be performed according to standard clinical protocols and is optional for age <7 years per judgment of the site investigator and caregiver. Three gel electrodes will be placed on the torso to measure electrocardiogram. Ultrasound gel will be applied to the chest; a hand-held Doppler ultrasound transducer will be applied by a licensed pediatric ultrasound technician to the chest wall to obtain standard images of the cardiac chambers, cardiac valves, Doppler-derived blood flow velocities, and global longitudinal strain. The echocardiogram requires 60 minutes.
    - Child/Young Adult cardiac MRI (age 5-25 years). A cardiac MRI will be performed according to standard clinical protocols in children who do not require sedation and is optional for age <9 years per judgment of site investigator and caregiver. Children with metallic implants will not be permitted to participate. The MRI will be conducted by trained technicians under supervision of licensed pediatric cardiologists and/or radiologists at each study site. The MRI requires 60 minutes.
- Child/Young Adult pulmonary function tests including diffusing capacity (age 7-25 years). Pulmonary function testing will be completed according to clinical protocols and is optional for age <9 years per judgment of the site investigator and caregiver. Participants will be asked to breath in prescribed patterns to complete the measurements of lung capacity and gas diffusion. Pulmonary function tests require 60 minutes.
- Child/Young Adult sputum induction (ages 10-25 years). A standard of care clinical protocol for sputum induction will be used and is optional for children <12 years per judgment of the site investigator and caregiver. Participant will inhale nebulized hypertonic saline solution (3%) for 5-15 minutes. The saline mist liquefies airway secretions, promotes coughing and allows expectoration of respiratory secretions. Subjects with history of bronchospasm will be excluded. To minimize risk of bronchospasm, children with asthma will be excluded and all children will receive pre-treatment with a bronchodilator (levalbuterol). Levalbuterol is an analog of endogenous catecholamines with established safety profile in studies of >600 children (31). Oxygen saturation will be monitored throughout the procedure. A handheld spirometer will be used to monitor Forced Expiratory Volume at 1 second (FEV1.0) during the procedure at 5-minute intervals. The procedure will be stopped if the FEV1.0 decreased >20% from pre-testing baseline or if the subject has symptoms of shortness of breath. Sputum induction will be performed by an experienced technician under the supervision of a licensed medical professional. Bronchodilator rescue therapy will be readily available if needed.
- Child/Young Adult symptom-limited cardiopulmonary exercise testing (ages 10-25 years). Participants will perform symptom-limited graded exercise on a treadmill or stationary bicycle ergometer according to clinical protocols in children and is optional for children <12 years per judgment of local site investigator and caregiver. Trained technicians under supervision of licensed pediatricians will oversee the test. Pediatric size gel electrodes will be placed to measure the electrocardiogram before, during and after exercise. A mouth tube or facemask will be used to collect expired gases before during and after exercise. Blood pressure will be measured at 1-3 minutes intervals before during and at 2-minute intervals for up to 10 minutes post-exercise. Total time required is 60 minutes.

- Child/Young Adult abdominal ultrasound (age 5-25 years). An abdominal ultrasound examination will be performed by a trained technician according to clinical protocols and is optional for age <7 years per judgment of the site PI and caregiver). The liver, pancreas, kidneys, and bladder will be imaged. Total time required is 60 minutes.
- Child/Young Adult brain MRI (age 5-25 years): A 3T or lower field strength brain MRI will be performed according to standard clinical protocols in children who do not require sedation and is optional for age <9 years per judgment of the site investigator and caregiver. The MRI will be conducted by trained technicians under supervision of licensed radiologists at each study site. The MRI requires up to 60 minutes.
- Awake EEG (age 5-25 years): An electroencepholagram (EEG) will be performed according to clinical protocols with FDA approved equipment and is optional for age <9 years per judgement of the site investigator and caregiver. A trained technician will apply scalp electrodes and record the EEG for up to 60 minutes.
- Child/Young adult neurocognitive testing. These tests will be conducted by a Child Psychologist or their trained assistant. The main set of measures for the neurocognitive battery come from the Woodcock-Johnson batteries, which is a battery of measures with strong psychometric properties, that can be used with subjects between the ages of 6 to 25 years (valid for wide age range of 2 to 90 years old). These will include Cognitive measures, Language measures, Achievement measures and behavioral and psychiatric testing. There are Spanish versions of parts of the Cognitive and Achievement subtests. There are norms developed based upon a large nationally representative sample.

Test category	**Test**
General Intelligence-Verbal	WJ-IV Oral Language
General Intelligence-Nonverbal	WJ-IV Spatial Reasoning
Executive Function - Attention	NIMH Toolbox - Flanker Test
Executive Function - Inhibitory Control	NIMH Toolbox - Inhibitory Control
Episodic Memory	NIMH Toolbox - Picture Sequence Memory
Language - Receptive	WJ-IV - Understanding Directions
Language - Expressive	WJ-IV - Picture Vocabulary & Rapid Picture Naming
Working Memory - Verbal	WJ-IV Numbers Reversed
Working Memory - Visual	WRAML2 - Picture Memory (5 to 18)
Processing Speed	WJ-IV - Pair Cancellation
Verbal Memory	WJ-IV Story Memory
Visual-Motor Integration	Beery Buktenica Test
Motor Speed	Purdue Pegboard
Reading	WJ-IV - Letter Word Identification
Spelling	WJ-IV - Spelling
Mathematics	WJ-IV - Number Sense and Calculation
Emotional and Behavioral Adjustment	Strengths and Difficulties Questionnaire
Psychiatric Disorder	K-CAT

- Microbiome biospecimen collection (age 5-25 years). Collection of skin swabs, nasal swabs, oral swabs, urine and stool will be performed and is optional for age <7 years per judgment of site investigator and caregiver. Soft-tipped, sterile cotton swabs will be used for the sample collection. Microbiome swab collection will require 15 minutes. Stool will be collected at home with remote collection kits mailed to the participant home.

Child Tier 3 biospecimen collection will be conducted on-site at the pediatrics cohort study locations or by home phlebotomy service as described in Section 11.14.

11.7 MIS-C Cohort (n=800, ages 3-25 years)

Since the participants in the MIS-C cohort have existing data collected from participation in other NIH funded research, a limited set of Tier 1 procedures will be performed to avoid duplication with existing data. Visits will be scheduled according to time elapsed from the index date of hospitalization for MIS-C. The initial visit will

be scheduled 3-30 months after the index hospitalization. The second study visit will be scheduled approximately one year later 15-42 months after the index hospitalization. Due to participant burden related to other studies, caregivers will not be enrolled but will be the primary respondents for questionnaires for subjects ages 3-17 years. Tier 1 questionnaires are adapted according to subject age (3-5 years, 6-17 years, and 18-25 years). For subjects ages 3-17 years, the caregiver will be the primary respondent with optional input from children ages 12-17 years). For subjects ages 18-25 years, the young adult will be the primary respondent with optional input from the caregiver.

11.7.1 MIS-C cohort Tier 1 remote assessments

- Sociodemographics and history of MIS-C extracted from research records or health records
- Household social determinants of health
    - address stability
    - financial stability (weekly stress inventory 5)
    - food insecurity (hunger vital signs)
    - access to health care (Adult Access to Health Care & Utilization Module, Question on Insurance from US Census)
    - perceived discrimination (Major Experiences and Everyday Discrimination Scale)
    - stress (Perceived Stress Scale)
    - social support (RAND-MOS)
    - community cohesion (Neighborhood Collective Efficacy Questionnaire)
- Child/Young adult COVID health impact
- Child/Young adult history of PASC symptoms
- Child PROMIS Pediatric Global Health measure or young adult PROMIS10 Quality of Life

11.7.2 MIS-C cohort Tier 2 visits

Participants with MIS-C will undergo a subset of Tier 2 assessments at study entry and annually for two additional years:

- Child/Young Adult history of interim medical history, interim SARS-CoV-2 infection and vaccination history
- Child/Young Adult COVID health impact
- Child/Young Adult interim history of PASC symptoms
- Child PROMIS Pediatric Global Health measure or young adult PROMIS10 Quality of Life
- Child/Young Adult Physical Activity
    - PROMIS- Physical Activity - Parent Proxy/CAT (Child 5 to 7 years)
    - PROMIS - Physical Activity Pediatric/CAT (Child 8+ years)
- Child/Young Adult diet
- Child/Young Adult sleep (PROMIS Sleep-Related Disturbance)
- Child/Young Adult health symptoms
    - PROMIS fatigue

On-site assessments

- Young Adult anthropometry and vital signs including oximetry
- Young Adult autonomic dysfunction symptoms assessment (Composite Autonomic Symptom Score, COMPASS-31, age 5-25 years, optional after the first post-acute visit)

Child or young adult Tier 2 biospecimen collection may be conducted at the time of study entry and at each Tier 2 visit done about a year apart at the pediatrics cohort study locations or by home phlebotomy service as described in Section 11.14

Extant cohort data will be harmonized with RECOVER data collection when possible. Unstructured data collection from the participant EHR will also be recorded for Tier 2 assessments when feasible.

There is no electrocardiogram procedure, spirometry procedure, or blood biospecimen collection in the MIS-C cohort.

11.7.3 MIS-C cohort Tier 3 visits

MIS-C Tier 3 visits will be limited to selection of up to three of the Tier 3 procedures listed for the main cohort. The procedures will be selected based on age, past clinical manifestations and clinical testing for each subject. The procedures will be conducted according to the same procedures as listed for the main pediatric cohort (two Tier 3 visits approximately 1 year apart).

11.8 Post-vaccine Myocarditis Cohort (n=200, ages 3-25 years)

Due to anticipate participation in other NIH and industry-funded research, post-vaccine myocarditis subjects will only participate in Tier 1 and limited Tier 2 remote procedures to avoid duplication of existing data. Tier 3 procedures will not be performed.

11.8.1 Post-vaccine myocarditis remote Tier 1 assessments

Tier 1 visits will be conducted remotely by caregiver/child self-report (electronic-based, phone-based, or paper-based) or by research staff-assisted data collection (telephone, videoconference) at the time of study entry. Study visits will be scheduled according to time elapsed from the index date of vaccination. Tier 1 questionnaires are adapted according to subject age (3-5, 6-11 years, 12-17 years, and 18-25 years). For subjects ages 3-17 years, the caregiver will be the primary respondent with optional input from children ages 5-17 years). For subjects ages 18-21 years, the young adult will be the primary respondent with optional input from the caregiver. Tier 1 data collection may be conducted entirely remotely and may also occur at an on-site study visit according to participant preference. It is anticipated that completion of all Tier 1 assessments will require in approximately 2 hours. Structured data collection will include the following domains:

- Child and caregiver sociodemographic data and contact information including next of kin
- Child and caregiver medical history, including history of MIS-C
- Child and caregiver vaccination history
- Child and caregiver health status
    - Caregiver PROMIS10 Quality of Life
    - Child/Young Adult PROMIS Pediatric Global Health measure
- Child and caregiver history of acute SARS-CoV-2 infection
- Child and caregiver history of PASC symptoms
- Child and caregiver COVID health impact
- Household social determinants of health
    - address stability
    - financial stability (weekly stress inventory 5)
    - food insecurity (hunger vital signs)
    - access to health care (Adult Access to Health Care & Utilization Module, Question on Insurance from US Census)
    - perceived discrimination (Major Experiences and Everyday Discrimination Scale)
    - stress (Perceived Stress Scale)
    - social support (RAND-MOS)
    - community cohesion (Neighborhood Collective Efficacy Questionnaire)

Tier 1 biospecimen collection will be conducted as described in Section 11.14

Unstructured data collection from the participant EHR and from mobile health applications may also be recorded to monitor participant health events and symptoms status during longitudinal post-acute Tier 2 follow-up when feasible. The mobile health applications will not be used until their use is approved by the IRB.

11.8.2 Post-vaccine myocarditis remote post-acute Tier 2 visits

Participants with post-vaccine myocarditis will undergo a subset of Tier 2 remote assessments at study entry and annually for two additional years:

- Child/Young Adult interim history of SARS-CoV-2 infection
- Child/Young Adult interim COVID health impact

- Child/Young Adult interim history of PASC symptoms
- Child PROMIS Pediatric Global Health measure or young adult PROMIS10 Quality of Life
- Child/Young Adult Physical Activity
    - PROMIS- Physical Activity - Parent Proxy/CAT (Child 5 to 7 years)
    - PROMIS - Physical Activity Pediatric/CAT (Child 8+ years)
- Child/Young Adult diet (months 12, 24, 36 48 only)
- Child/Young Adult sleep (PROMIS Sleep-Related Disturbance)
- Child/Young Adult health symptoms
    - PROMIS asthma impact
    - PROMIS fatigue

Tier 2 biospecimen collection will be conducted as described in Section 11.14.

Unstructured data collection from the participant EHR and from mobile health applications may also be recorded to monitor participant health events and symptoms status during longitudinal post-acute Tier 2 follow-up when feasible. The mobile health applications will not be used until their use is approved by the IRB.

11.9 Infants born to mothers with and without history of SARS-CoV-2 during pregnancy (n=2500, ages newborn-48 months)

Tier 1 and 2 procedures for this cohort listed below will be conducted 12, 18, 24, 36, and 48 months after birth. There are no Tier 3 procedures.

11.9.1 Infants cohort Tier 1 assessments (remote)

- Ages and Stages Questionnaire at 12, 24 and 36 months of age (child development)
- National Survey of Children's Health Questionnaire at 12, 24 and 36 months of age (child health)
- Modified Checklist for Autism in Toddlers at 18 months (screening test for autism)
- Ages and Stages Questionnaire Social Emotional at 18 months (child development)
- Growth at 12, 18, 24 and 36 months (weight, length, head circumference; self-report or EHR)
- Child Behavior Checklist for Behavioral Problems at 24 and 36 months (child development)
- Developmental Profile-4 Time at 36 months (child development)

11.9.2 Infants cohort Tier 2 assessments (on-site or remote)

- Growth (weight, height, head circumference, skinfolds; measured) at 36 and 48 months
- Bayley Scales of Infant Development-4 (child development) at 24 months
- Differential Ability Scales-II, Time (child development) at 36 and 48 months
- Infant sleep (Brief infant Sleep Questionnaire)

Tier 2 biospecimens will be collected at age 24 months (<5 ml phlebotomy volume) as described in Section 11.14.

11.10 ABCD cohort (n=10,000, ages 12-17 years)

11.10.1 ABCD Tier 1 assessments

The RECOVER procedures are harmonized to avoid duplication with other study data collection in the ABCD study. Due to participant burden related to the ABCD study, a limited set of Tier 1 assessments will be performed. For ABCD subjects who have evidence of possible PASC symptoms, referral to other RECOVER sites for participation in Tier 2 and Tier 3 assessments will be offered as an option, if geographically feasible.

Tier 1 visits will be conducted remotely by caregiver/child self-report (electronic-based, phone-based, or paper-based) or by research staff-assisted data collection (telephone, videoconference) at the time of study entry. Tier 1 questionnaires are adapted according to subject age for this cohort (12-17 years). For subjects

ages 12-17 years, the caregiver will be the primary respondent with optional input from children ages 12-17 years.

- Child and caregiver sociodemographic data and contact information including next of kin
- Child and caregiver medical history, including history of MIS-C
- Child and caregiver vaccination history
- Child and caregiver health status
    - Caregiver PROMIS10 Quality of Life
    - Child/Young Adult PROMIS Pediatric Global Health measure
- Child and caregiver history of acute SARS-CoV-2 infection
- Child and caregiver history of PASC symptoms
- Child and caregiver COVID health impact
- Household social determinants of health
    - address stability
    - financial stability (weekly stress inventory 5)
    - food insecurity (hunger vital signs)
    - access to health care (Adult Access to Health Care & Utilization Module, Question on Insurance from US Census)
    - perceived discrimination (Major Experiences and Everyday Discrimination Scale)
    - stress (Perceived Stress Scale)
    - social support (RAND-MOS)
    - community cohesion (Neighborhood Collective Efficacy Questionnaire)

The Tier 1 study visit(s) will also include remote biospecimen collection of blood (for the child and caregiver) and saliva (for the caregiver) as described in Section 11.14 (no child saliva collection for DNA as DNA data already exists in the ABCD biobank). Selected Tier 1 child and young adult participants without history of SARS-CoV-2 infection who reside in regions with higher rates of SARS-CoV-2 infection and who have no detectable antibodies on the first test may contribute up to two additional biospecimens for SARS-CoV-2 antibody at 3-month intervals if the test remains negative (total 3 tests).

11.11 Caregiver Assessments

Caregivers will be identified at the time of child/young adult enrollment as described in the study entry criteria for the main cohort, the post-vaccine myocarditis cohort and the ABCD cohort. Caregivers will not be enrolled for the MIS-C cohort or the infants of mothers with history of SARS-CoV-2 during pregnancy (since the mothers will be enrolled RECOVER adult cohort).

11.11.1 Caregiver Tier 1 Assessments

- Caregiver medical history
- Caregiver vaccination history
- Caregiver health status (PROMIS-10 Quality of Life)
- Caregiver history of acute SARS-CoV-2 infection
- Caregiver history of PASC symptoms
- Caregiver COVID health impact

11.11.2 Caregiver Tier 2 Assessments (remote time of Child/Young Adult visits at 12, 24, 36, and 48 months)

- Caregiver interim medical history
- Caregiver interim vaccination history
- Caregiver interim health status (PROMIS-10 Quality of Life)
- Caregiver interim history of acute SARS-CoV-2 infection
- Caregiver interim history of PASC symptoms
- Caregiver interim COVID health impact

Caregiver Tier 1 biospecimen collection will be conducted remotely as described in section 11.14.

11.12 Other Biological Parent Assessments

If the caregiver is a biological parent, the other biological parent may enroll in the study to provide a saliva specimen for biorepository storage and future genetic analyses. PII as required to generate a UUID will be collected.

11.13 Mobile Health Products and Devices Data Collection

Commercial products or devices made by third party companies, including wearable fitness trackers, wearable sleep monitors, mobile apps for use on smartphone and tablets, websites and web apps, and types of computer software that permit screen sharing, record keystrokes, gain access to device files and/or use location tracking technology may be used to collect study data. These devices may be owned by the participant, or provided to the participant by the RECOVER mobile health platform core. These devices will be used in accord with the Terms of Service and/or the End User License Agreements (EULA) provided by the product or device vendor. These products and devices will only be used to collect study data with IRB approval and if the subject has agreed to all applicable Terms of Service.

11.14 Laboratory Procedures/Evaluations

11.14.1 Biospecimen collection overview

There are no screening laboratory assessments. All biospecimens are collected as outcome variables. Blood biospecimens may be collected in children/young adults ages 24 months to 25 years. Blood biospecimens may be collected either remotely (Tier 1 by self-administered dried blood spot collection kits) or via phlebotomy from an upper extremity vein performed at a study site or at home. All clinical laboratory testing will be performed at the local site or nationally accredited laboratory. Before collection of clinical laboratory testing in Tier 2 and Tier 3, the participant electronic health record will be reviewed for clinical laboratory results available within the 30 days prior to the visit if feasible. Only laboratory tests not available in the participant electronic health record in the 30-day period will be collected. Biorepository biospecimens will be processed and shipped as described below. Blood volume will be age adjusted according to the follow table:

Table 1. Phlebotomy maximum volume by age group

Age (years)	Maximum volume single visit blood draw (ml)	% Estimated Total Blood Volume
Newborn-2	5	≤1.4
3-5	15	≤1.6
6-9	25	≤1.5
≥10	38	≤1.5

For Tier 1, the home blood spot biospecimen collection will be conducted with Tasso M-20 blood collection kit. The Tasso-M20 has obtained CE mark certification and is registered with the FDA as a Class I product exempt from premarket notification [510(k)] requirements. This device uses a small lancet to collect 4 samples of 20µL ±5% (total 80µL) from body regions with reduced density of pain receptors (shoulder for ages 6-25 years, shoulder, buttocks, or thigh for ages 2 to 5 years). The device is applied to the skin area with light suction and completes collection of blood in about 2 minutes. The caregiver or young adult will receive the kit at home with instructions for home blood spot collection and an postage-paid pre-addressed envelope for return of the biospecimen to the biorepository core. Dried blood spot samples ship as an exempt human specimen (UN3373 exempt).

Biorepository specimens will be stored indefinitely, or until all of the sample is used up. Biospecimen collections for clinical laboratory testing and biorepository for children, young adults, and caregivers are listed by tier.

11.14.2 Tier 1 biospecimen collections

Children and young adults ages 2-25 years (<5 ml, remote collection at study entry except for acute patients with collection at the 8-week visit)

- Saliva (oragene collection kit) for DNA (biorepository)
- Dried blood spot for SARS-CoV-2 spike and nucleocapsid antibody and biospecimen storage (<5 ml)
- If initial antibody test negative and subject lives in region with high COVID PCR positivity rate, phlebotomy for SARS-CoV-2 spike and nucleocapsid may be repeated up to two additional times at 3-month intervals

Caregivers (remote collection at study entry)

- Saliva (oragene collection kit) for DNA (biorepository)
- Dried blood spot for SARS-CoV-2 spike and nucleocapsid antibody and biospecimen storage

Other biological parent (optional remote collection at study entry)

- Saliva (oragene collection kit) for DNA (biorepository)

11.14.3 Tier 2 Acute biospecimen collections

Children ages 3-5 years (total 15 ml, on-site or remote collection at week 8):

- Saliva (oragene collection kit) for DNA (biorepository)
- Dried blood spot for SARS-CoV-2 spike and nucleocapsid antibody and biospecimen storage (<5 ml)
- Serum: 6 ml Serum Separator Tube (SST) for storage in biorepository
- PBMC: 8 ml in Cell Preparation Tube for storage in biorepository

Children and young adults ages 6-25 years (total 24 ml, on-site or remote collection at week 8):

- Saliva (oragene collection kit) for DNA (biorepository)
- Dried blood spot for SARS-CoV-2 spike and nucleocapsid antibody and biospecimen storage (<5 ml)
- Serum: 6 ml Serum Separator Tube (SST) for storage in biorepository
- Plasma: 10 ml EDTA tube for storage in biorepository
- PBMC: 8 ml in Cell Preparation Tube for storage in biorepository

11.14.4 Tier 2 Post-acute biospecimen collections

Infants of mothers with or without SARS-CoV-2 during pregnancy (age 24 months)

- Dried blood spot collection for SARS CoV-2 antibody and biorepository (<5ml)

First post-acute Tier 2 visit

Children ages 3-5 years (total 15 ml, on-site or remote collection at first visit):

- SARS-CoV-2 antibody (nucleocapsid and spike) (1.0 ml pediatric SST)
- Serum: 6 ml Serum Separator Tube (SST) for storage in biorepository
- PBMC: 8 ml in Cell Preparation Tube for storage in biorepository

Children ages 6-9 years (total blood collection 16 ml, on-site or remote collection at first post-acute Tier 2 visit)

- Complete metabolic panel (1.6 ml SST)
- Complete blood count (0.5 ml pediatric EDTA tube)
- ANA, anti-CCP, anti dsDNA, RF (2.0 ml pediatric SST)
- Lipid panel (2.0 ml pediatric SST)
- Hemoglobin A1c (0.5 ml pediatric EDTA tube)
- Thyroid stimulating hormone and reflex free T4 (1.5 ml pediatric SST)
- 25-hydroxyvitamin D (1.0 mg pediatric lithium heparin tube)
- Serum calcium (0.5 ml pediatric lithium heparin tube)
- Epstein Barr Virus antibody (0.5 ml pediatric SST)
- SARS-CoV-2 antibody (nucleocapsid and spike) (1.0 ml pediatric SST)

- Urine protein:creatinine ratio
- Serum: 6 ml Serum Separator Tube (SST) for storage in biorepository

Children and young adults ages 10 to 25 years (total blood collection 38 ml, on-site or remote collection at first post-acute Tier 2 visit)

- Complete metabolic panel (1.6 ml SST)
- Complete blood count (0.5 ml pediatric EDTA tube)
- ANA, anti-CCP, anti dsDNA, RF (2.0 ml pediatric SST)
- Lipid panel (2.0 ml pediatric SST)
- Hemoglobin A1c (0.5 ml pediatric EDTA tube)
- Thyroid stimulating hormone and reflex free T4 (1.5 ml pediatric SST)
- 25-hydroxyvitamin D (1.0 mg pediatric lithium heparin tube)
- Serum calcium (0.5 ml pediatric lithium heparin tube)
- Epstein Barr Virus antibody (0.5 ml pediatric SST)
- SARS-CoV-2 antibody (nucleocapsid and spike) (1.0 ml pediatric SST)
- Urine protein:creatinine ratio
- Serum: 6 ml Serum Separator Tube (SST) for storage in biorepository
- Plasma: 10 ml EDTA tube for storage in biorepository
- Peripheral blood mononuclear cells (PBMC): 12 ml cell preparation tube for storage in biorepository

Subsequent post-acute Tier 2 visits

Children ages 3-5 years (total 15 ml, on-site or remote collection at first visit):

- SARS-CoV-2 antibody (nucleocapsid and spike) (1.0 ml pediatric SST)
- Serum: 6 ml Serum Separator Tube (SST) for storage in biorepository
- PBMC: 8 ml in Cell Preparation Tube for storage in biorepository

Children age 6 to 9 years (total blood collection 23 ml, onsite collection at subsequent post-acute Tier 2 visits at month 6, 12, 24, 36, and 48)

- SARS-CoV-2 antibody(nucleocapsid and spike) (1.0 ml pediatric SST)
- Serum: 6 ml Serum Separator Tube (SST) for storage in biorepository
- Plasma: 10 ml EDTA tube for storage in biorepository
- Peripheral blood mononuclear cells (PBMC): 6 ml cell preparation tube for storage in biorepository

Children and young adults age 10 to 25 years (total blood collection 29 ml, onsite collection at subsequent post-acute Tier 2 visits at month 6, 12, 24, 36, and 48)

- SARS-CoV-2 antibody(nucleocapsid and spike) (1.0 ml pediatric SST)
- Serum: 6 ml Serum Separator Tube (SST) for storage in biorepository
- Plasma: 10 ml EDTA tube for storage in biorepository
- Peripheral blood mononuclear cells (PBMC): 12 ml cell preparation tube for storage in biorepository

11.14.5 Tier 3 biospecimen collections

Children and young adults age 3 to 25 years (total blood collection 13.1 ml, onsite collection two times approximately 1 year apart)

- Complete metabolic panel (1.6 ml SST)
- Complete blood count (0.5 ml pediatric EDTA tube)
- Lipid panel (2.0 ml pediatric SST)
- Hemoglobin A1c (0.5 ml pediatric EDTA tube)
- Urine protein:creatinine ratio
- D-Dimer (1 ml citrate plasma tube)
- High sensitivity Troponin (2.0 ml lithium heparin tube)

- High sensitivity C-reactive protein (0.5 ml lithium heparin tube)
- Procalcitonin (3 ml no additive tube)
- NT-pro-brain natriuretic peptide (0.5 ml pediatric EDTA tube)
- Insulin C-peptide (1.5 ml pediatric SST)
- Microbiome specimens: sputum, skin swabs, nasal swab, oral swab, urine, stool

11.15 Study Specific Biospecimens

11.15.1 Specimen Collection Procedures

Blood biospecimens may be collected in children/young adults ages 24 months to 25 years. Blood biospecimens may be collected either remotely (Tier 1 by self-administered dried blood spot collection kits) or via phlebotomy from an upper extremity vein performed at a study site or at home. All on-site or home phlebotomy procedures will be performed by an individual with appropriate training and experience for pediatric population. Study staff will follow a standard operating procedure to verify subject identity and proper labeling at the time of phlebotomy.

Blood biospecimens for clinical testing will either be analyzed at the site local accredited laboratory or a national accredited laboratory provider. Blood biospecimens for biorepository will be processed locally as described below and shipped to the Mayo Clinic biorepository. Biorepository specimens will be stored using a UUID with no identifiers. The link to the UUID will be maintained at the local site and/or the central REDCap database. The biospecimens will be stored indefinitely, or until the sample is used up. Stored deidentified biospecimens may be used for future research as approved by the NIH Sponsor and RECOVER scientific leadership. Participants who withdraw from the study will have their stored biospecimens destroyed.

11.15.2 Specimen Preparation, Handling, and Storage

The Mayo Clinic Central biorepository will create blood collection kits for each visit. The kits will contain Blue Refrigerate Biohazard bag, tubes and aliquot tubes pre-labelled biorepository specimen ID collection for local freezing and storage, and return mailing container.

Samples collected in SST tubes will be cold centrifuged and aliquoted in 0.5 ml volumes, and placed in a freezer for short-term storage. Frozen aliquoted serum specimens will be shipped on dry ice in containers provided by Mayo Clinic.

Samples collected in EDTA and CPT tubes will be placed in appropriate packaging for immediately shipment to Mayo clinic biorepository.

If standard phlebotomy is not feasible at Tier 2 visits, dried blood spot samples will be obtained by a lancet-based system and shipped to the biorepository.

11.15.3 Specimen Shipment

Biospecimens will be shipped to the Mayo Biorepository in Rochester Minnesota. Blood spot and saliva collections obtained at home will be shipped directly to the biorepository by the participant. Frozen specimens collected at study sties will be batched and shipped once weekly on dry ice. CPT specimens collected at study sites will be shipped the same day as the study visit. All specimens will be labeled according to the standard operating procedure and shipped in an appropriate biosafe container provided by Mayo Biorepository.

11.16 Questionnaire Administration

The names and domains covered by each of the questionnaires is listed above in the description of the Tier 1-3 procedures. Questionnaires will be administered either remotely via electronic online versions or paper versions, or during an onsite visit. Questionnaire have all been adapted for age ranges across the pediatric and young adult life spectrum. At each study visit the age-appropriate version of the questionnaire will be used. All the selected questionnaires have been previously validated with the exception of the symptom

checklist for post COVID-19 symptoms. There is no validated version of this checklist due to the recent discovery of the post-acute sequelae of COVID-19.

12 Safety and Adverse Events

12.1 Definitions

Unanticipated Problems Involving Risk to Subjects or Others
Any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in nature, severity, or frequency (i.e., not described in study-related documents such as the IRB-approved protocol or consent form, the investigators brochure, etc.)
- Related or possibly related to participation in the research (i.e., possibly related means there is a reasonable possibility that the incident experience, or outcome may have been caused by the procedures involved in the research)
- Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, or social harm).

Adverse Event
An **adverse event** (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

Serious Adverse Event
Adverse events are classified as serious or non-serious. A **serious adverse event** is any AE that is:

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the participant, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

All adverse events that do not meet any of the criteria for serious should be regarded as **non-serious adverse events**.

Preexisting Condition
A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

Post-study Adverse Event
All unresolved adverse events will be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator will instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study. The investigator will notify the study

sponsor of any death or adverse event occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study. The sponsor will also be notified if the investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that has participated in this study.

12.2 Recording of Adverse Events

At each contact with the subject, the investigator will seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events will be recorded immediately in the source document, and also in the appropriate adverse event module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results will be recorded in the source document, though should be grouped under one diagnosis.

All adverse events occurring during the study period will be recorded. The clinical course of each event will be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period will be followed until resolution. Any serious adverse event that occurs after the study period and is considered to be possibly related to study participation will be recorded and reported according to the same criteria as other serious adverse events.

12.3 Reporting of Serious Adverse Events and Unanticipated Problems

Since the participants in the study have known acute and post-acute SARS-CoV-2 infections, known manifestations of SARS-CoV-2 infection will be recorded as endpoints according to pre-specified criteria rather than AE and SAE. These known events include:

- Upper respiratory infection
- Fever
- Flu-like symptoms
- Pneumonitis
- Respiratory failure
- Psychosis and delirium
- Multi-system inflammatory syndrome
- Multisystem organ failure
- Arterial thromboembolic events including stroke and myocardial infarction
- Venous thromboembolic events including deep vein thrombosis, CNS venous thrombosis, and pulmonary embolism
- Signs and symptoms of myocarditis and pericarditis
- Cholelithiasis and cholecystitis
- Acute kidney failure
- Signs and symptoms of autonomic dysfunction
- Headache
- Hair loss
- Tooth loss
- Tinnitus
- Loss of smell and taste
- Fatigue
- Malaise
- Muscle pain and weakness
- Bone pain
- Generalized pain
- Anxiety
- Depression
- Neurological symptoms (loss of concentration, loss of memory)
- Palpitations
- Shortness of breath

- Cough
- Poor appetite
- Nausea, vomiting, diarrhea, abdominal pain
- Glucose intolerance
- Skin Rash
- Thirst
- Raynaud's phenomenon
- COVID (chilblain-like) toes
- Abnormal laboratory tests related to COVID infection, inflammation and organ dysfunction
- Abnormal tests related to COVID-related organ damage or dysfunction
- Change in menstruation
- Death

Investigators will conform to the adverse event reporting timelines, formats and requirements of the various entities to which they are responsible, but at a minimum those events that must be reported are those that are:

- related to study participation,
- unanticipated, and
- serious or involve increased risks to subjects or others.

For Narrative Reports of Safety Events
If the report is supplied as a narrative, the minimum necessary information to be provided at the time of the initial report includes:

- Study identifier
- Study center
- Subject number
- A description of the event
- Date of onset
- Current status
- Whether study treatment was discontinued
- The reason why the event is classified as serious
- Investigator assessment of the association between the event and study treatment

12.3.1 Investigator reporting: notifying the IRB

Federal regulations require timely reporting by investigators to their local IRB of unanticipated problems posing risks to subjects or others. The following describes the NYULMC IRB reporting requirements, though Investigators at participating sites are responsible for meeting the specific requirements of their IRB of record.

Report Promptly, but no later than five (5) working days:
Researchers are required to submit reports of the following problems promptly but no later than five (5) working days from the time the investigator becomes aware of the event:

- Unanticipated problems including adverse events that are unexpected and related
  - Unanticipated**:** An event is "unanticipated" when its specificity and severity are not accurately reflected in the protocol-related documents, such as the IRB-approved research protocol, any applicable investigator brochure, and the current IRB-approved informed consent document and other relevant sources of information, such as product labeling and package inserts.
  - Related to the research procedures**:** An event is related to the research procedures if in the opinion of the principal investigator or sponsor, the event was more likely than not to be caused by the research procedures.
  - Harmful: either caused harm to subjects or others, or placed them at increased risk

Other Reportable events:
The following events also require prompt reporting to the IRB, though ***no later than 10 working days***:

- ***Complaint of a research subject*** when the complaint indicates unexpected risks or the complaint cannot be resolved by the research team.
- ***Protocol deviations or violations*** (includes intentional and accidental/unintentional deviations from the IRB approved protocol) for any of the following situations:
  - one or more participants were placed at increased risk of harm
  - the increased harm event has the potential to occur again
  - the deviation was necessary to protect a subject from immediate harm
- ***Breach of confidentiality***
- ***Incarceration of a participant*** when the research was not previously approved under Subpart C and the investigator believes it is in the best interest of the subject to remain on the study.
- ***New Information indicating a change to the risks or potential benefits*** of the research, in terms of severity or frequency. (e.g., analysis indicates lower-than-expected response rate or a more severe or frequent side effect; Other research finds arm of study has no therapeutic value; FDA labeling change or withdrawal from market)

Reporting Process
The reportable events noted above will be reported to the IRB using a Reportable New Information submission and will include a description of the event with information regarding its fulfillment of the above criteria, follow-up/resolution, and need for revision to consent form and/or other study documentation. Copies of each report and documentation of IRB notification and receipt will be kept in the Clinical Investigator's study file.

13 Study Oversight

13.1 Monitoring Board

Oversight of data and safety is provided by a RECOVER Observational Safety Monitoring Board appointed by NHLBI. The OSMB is composed of experts in longitudinal research methods, clinical experts in the manifestations of COVID-19 in adults, pregnant women, and children, biostatistics, bioethics, and patient/caregiver representatives. The OSMB may also appoint ad hoc members with subspecialty expertise in the diverse array of clinical manifestations of PASC.

A charter of the OSMB will be submitted for IRB review before starting enrollment. The OSMB will meet at least twice a year to review data on AEs, unanticipated events, patient-reported outcomes, data quality, and study recruitment as described in the committee charter, and make recommendations about study conduct to the NHLBI. As the RECOVER Pediatric PASC Investigator Consortium study does not involve any interventions, a pre-specified stopping rule for efficacy or futility is not indicated.

After each OSMB meeting, the OSMB determination letter and a summary report of adverse events will be prepared within 30 days and will be distributed by NHLBI staff to each principal investigator at the RECOVER Clinical Science Core for review. The summary report will contain the following information:

- A statement that a OSMB review of outcome data, adverse events, and information relating to study performance across all centers took place on a given date.
- A statement as to whether or not the frequency of adverse events exceeded what was expected and indicated in the informed consent.
- A statement that a review of recent literature relevant to the research took place.
- The OSMB's recommendation with respect to progress or need for modification of the protocol or informed consent. If the OSMB recommends changes to the protocol or informed consent, the rationale for such changes and any relevant data will be provided.
- A statement that if safety concerns are identified, the NHLBI Program Official will communicate these promptly to the investigators.

13.2 Data Safety Monitoring Plan

The Data and Safety Monitoring Plan for this trial will follow recommended monitoring principles for an observational study of a vulnerable population. Oversight of data and safety is provided by the RECOVER PASC Observational Safety Monitoring Board (OSMB) appointed by NHLBI. The OSMB will be composed of experts in longitudinal research (adult and pediatric populations), clinical experts in adult and pediatric manifestations of COVID-19, biostatistics, bioethics, and patient/caregiver representatives. The OSMB will also appoint ad hoc members with subspecialty expertise in the diverse array of clinical manifestations of PASC. The OSMB will meet at least twice a year as described in Section 13.1.

Each study site lead investigator will assume responsibilities for monitoring and reporting unanticipated events to the single IRB and the Clinical Science Core. The site lead investigator will provide requested follow-up information as requested by the IRB and Clinical Science Core. The Clinical Science Core will also perform routine monitoring of all sites in the RECOVER PASC consortium and issue queries for any protocol deviations. Not for cause audits for all sites in the RECOVER PASC consortium will also be conducted by an outside vendor with report to the IRB and Clinical Science Core.

14 Statistical Considerations

Hypotheses to be tested

- The incidence ratio of PASC between infected and uninfected individuals will be greater than 1.10.
- Risk factors related to demographics, social determinants of health, and co-morbid conditions will be associated with increased risk for development of PASC symptoms after SARS-CoV-2 infection.
- Direct and indirect effects of SARS-CoV-2 infection on organ function will mediate clinical and subclinical manifestations of PASC.

14.1 Sample size determinations

Up to 20,000 participants will be recruited to the RECOVER pediatric cohort, with enrollment targets to include N=9,300 infected participants and 10,200 uninfected participants (**Table 2**). Accordingly, up to 20,000 caregivers will also be enrolled. For the purposes of the power calculations, the following numbers of targets by sub-cohort will be used: N=800 children in the acute infected cohort, N=200 children in the acute uninfected cohort, N=4,000 children in the post-acute infected cohort, N=1,000 children in the post-acute uninfected cohort, N=1,500 children from the ABCD cohort with prior SARS-CoV-2 infection, N=8,500 children from the ABCD cohort without prior SARS-CoV-2 infection, N=2,000 infants born in the context of maternal SARS-CoV-2 infection during pregnancy, N=500 infants not born in the context of maternal SARS-CoV-2 infection during pregnancy, N=800 children in the MIS-C cohort, and N=200 children in the post-vaccine myocarditis cohort.

Table 2. Sample size for the RECOVER Pediatrics Cohort by infection status and type of enrollment

Status	Acute	Post-acute	Total
Infected	800	8,500[a]	9,300
Uninfected	200	10,000[b]	10,200
Total	1,000	18,500	19,500

a. includes post-acute infected, ABCD with prior infection, infants born in the context of maternal infection during pregnancy, MIS-C, and post-vaccine myocarditis
b. includes post-acute uninfected, ABCD without prior infection, and infants not born in the context of maternal infection during pregnancy

Sample size determinations are based on a type 1 error rate of 0.01 and 90% power. In the analytic phase, a false discovery rate adjusted p-value will be used that appropriately accounts for the number of comparisons considered at that time.

In the infant cohort: Assuming a developmental milestone score is standardized to mean 0 and standard deviation 1, the minimum detectable difference between infants born in the context of maternal SARS-CoV-2 infection and infants not born in the context of maternal SARS-CoV-2 infection is 0.19.

In the main acute/post-acute cohort: Assuming the risk of PASC in the uninfected is 10%, the minimum detectable effect size for the difference in risk of PASC as measured at Tier 1 between infected and uninfected participants is 4.1%. Assuming the risk of PASC in the uninfected is 10%, and that 50% of acute/post-acute participants are asymptomatic during the acute phase of SARS-CoV-2 infection, the minimum detectable effect size for the difference in risk of PASC as measured at Tier 1 between asymptomatic infected and uninfected participants is 6.0%. Assuming the prevalence of a given binary Tier 2 feature is 50% among participants with PASC, the minimum detectable effect size for the difference in proportion with the feature between PASC+ and PASC- participants is 5.6%.Assuming the prevalence of a given binary Tier 3 feature is 50% among participants with PASC, the minimum detectable effect size for the difference in proportion with the feature between PASC+ and PASC- participants is 16.4%.

In the MIS-C/post-vaccine myocarditis cohorts: Assuming the prevalence of cardiac manifestations (or any other Tier 1/2/3 feature) is 50% among participants with MIS-C, the precision of a prevalence estimate as described by a 95% confidence interval is ± 4.6%. Assuming the prevalence of a given Tier 1 or 2 feature is 50% among participants with MIS-C, the minimum detectable effect size for the difference in proportion with the feature between participants with MIS-C and participants with post-vaccine myocarditis is 15.0%.

14.2 Statistical Methods

14.2.1 Methods of Data Collection

Structured data elements will be collected remotely by telephone with study personnel, by home visit by study personnel, through a secure encrypted mobile, email, or web-based platform, or (if no other option) by return of printed questionnaire by postal mail. Text messaging may be used with subject permission for collection of data without PHI. If preferred by the participant and participant caregiver, structured data may be collected in person at a study site.

Biospecimen collection will be handled in two ways, depending on stability of the sample:

- Samples for analytes that require rapid freezing will be processed locally and sent to the central RECOVER biorepository on dry ice.
- Other samples will be sent directly to the centralized RECOVER biorepository for processing.
- Off-protocol clinically obtained samples including cerebrospinal fluid, bronchoalveolar lavage specimens, procedural biopsies, and surgical pathology specimens will be tracked and either transferred from study site biorepository to the central RECOVER biorepository or linked by the UUID to the institutional RECOVER biorepository for future access.

14.2.2 Strategies for Study Modifications

This protocol is designed to be pragmatic and flexible in design. A governance structure will evaluate internal and external sources of data on a regular basis and make recommendations to the NIH Executive Committee and OSMB for any proposed protocol modifications. We may undertake the following procedures to guide protocol modifications over time:

1) The frequency of PASC will be monitored in real-time during the study. If the incidence or prevalence is found to be higher or lower than planned, recruitment strategies may be altered to deliberately undersample/oversample PASC cases.
2) Participant response burden will be monitored in real-time during the study. If burden is found to be excessive, it may be reduced by altering the data collection strategy, such as by increasing the assessment interval; reducing the number of data elements collected; increasing the availability of home-based assessments; and/or increasing participant reimbursement.
3) Free text responses to interval assessments will be monitored in real-time during the study. If a new symptom or outcome is being reported at a frequency >15% by participants, the symptom may be added to the data collection tool.

4) Data elements that are not NIH recommended CDE may be modified based on ongoing analysis by DRC; data elements that are not informative to PASC definition models may be removed, with substitution by new data elements.
5) PASC definition may be revised in an iterative manner based on existing PASC data, medical literature, and feedback from patient representatives, participants, and the scientific community. Updated PASC definitions may be used to implement a strategy to modify deeper phenotyping.
6) Tier 2 and Tier 3 assessments may be evaluated for futility at pre-specified intervals; protocol assessments will be adjusted accordingly, and may include elimination of some assessments and introduction of other new assessments.

14.2.3 Overview of Analytic Approach to Aims

Full details of the analytic approach are included in the pediatric statistical analysis plan. The statistical analysis plan will include the following key elements.

Aim 1 is to characterize the incidence and prevalence of long-term sequelae, including clinical and biological features, severity and distinct sub-phenotypes, following SARS-CoV-2 infection. This will be achieved by estimating the incidence of PASC phenotypes among participants with SARS-CoV-2 infection or born to a mother with SARS-CoV-2 infection who are free of PASC-like symptoms and/or diagnoses prior to SARS-CoV-2 infection, compared with uninfected individuals free of PASC-like symptoms prior to the pandemic followed over the same time interval. These analyses will be performed in subgroups as detailed in Table 3 below. To identify PASC phenotypes, sub-phenotypes and severity based on clinical and biologic features, we will statistically compare the incidence among infected and uninfected individuals. Supervised and unsupervised approaches will be applied to characterize sub phenotypes.

Aim 2 is to characterize the clinical course and recovery of acute and post-acute sequelae over time from the time of study entry and to determine associated risk factors for PASC among SARS-CoV-2 infected, PASC positive individuals compared to infected PASC negative individuals and compared to uninfected individuals. We will characterize the patterns of outcomes of acute and post-acute sequelae over time using longitudinal data methods (e.g., general estimating equations and generalized linear mixed models) and functional principal component analysis. Longitudinal trajectories will be compared statistically between infected and uninfected individuals. We will additionally estimate and test the association of pre-infection and peri-infection risk and resiliency factors (e.g., social determinants of health, family dynamics, demographic, behavioral, biological factors and preexisting clinical and subclinical disease) prior to and following SARS-CoV-2 infection with the presence, severity and time to resolution of acute and post-acute sequelae using standard longitudinal and time-to-event models. These analyses will be performed in subgroups as detailed in Table 3 below. We will also estimate the incidence and prevalence of subclinical organ injury/disease after SARS-CoV-2 infection and compare the prognostic significance of subclinical organ injury/disease for incident clinical disease among SARS-CoV-2 infected versus uninfected individuals.

Aim 3 will define the pathophysiology of and mechanisms associated with the development of acute and post-acute sequelae including MIS-C, including the direct and indirect effects of SARS-CoV-2 infection on symptom onset and potential modifiers. We will estimate the direct and indirect effects of SARS-CoV-2 infection on the development of acute and post-acute sequelae, including potential mediation by post-traumatic responses (e.g., severe disease) and caregiver characteristics. We will also determine whether the occurrence of MIS-C after SARS-CoV-2 exposure and post-COVID-19 vaccine myocarditis modifies the trajectory of prior organ dysfunction, and/or risk of developing new organ injury compared with pre-pandemic injury, and identify possible pathophysiological mechanisms using mediation analysis based on longitudinal models (e.g., generalized linear mixed models) including interactions.

For all aims, additional analyses of pre-specified subgroups based on age, demographics, region of index SARS-CoV-2 infection if known, and COVID-19 health status of primary caregiver will be performed as sample sizes allow as detailed in the following Table.

Table 3. Planned subgroup analyses overall and by infection status at time of RECOVER enrollment

Demographics	*Overall (N=)*	*SARS-CoV-2 Infected (N=)*	*SARS-CoV-2 Uninfected (N=)*	*Unadjusted Infected vs. Uninfected*
Female sex – count/total (%)	count/total (%)	count/total (%)	count/total (%)	*p-value*
Age – Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)	*p-value*
Age newborn-2 years (infancy/toddlerhood)	count/total (%)	count/total (%)	count/total (%)	*p-value*
Age 3-5 years (early childhood)	count/total (%)	count/total (%)	count/total (%)	
Age 7-11 years (middle childhood)	count/total (%)	count/total (%)	count/total (%)	
Age 12-17 years (adolescence)	count/total (%)	count/total (%)	count/total (%)	
Age 18-25 years (young adulthood)	count/total (%)	count/total (%)	count/total (%)	
Race/Ethnicity				
White/non-Hispanic	count/total (%)	count/total (%)	count/total (%)	*p-value*
Black/non-Hispanic	count/total (%)	count/total (%)	count/total (%)	
Hispanic	count/total (%)	count/total (%)	count/total (%)	
Asian	count/total (%)	count/total (%)	count/total (%)	
Native Hawaiian/Pacific Islander	count/total (%)	count/total (%)	count/total (%)	
American Indian/Alaska Native	count/total (%)	count/total (%)	count/total (%)	
Other	count/total (%)	count/total (%)	count/total (%)	
Geographic region*				
HHS Region 1	count/total (%)	count/total (%)	count/total (%)	*p-value*
HHS Region 2	count/total (%)	count/total (%)	count/total (%)	
HHS Region 3	count/total (%)	count/total (%)	count/total (%)	
HHS Region 4	count/total (%)	count/total (%)	count/total (%)	
HHS Region 5	count/total (%)	count/total (%)	count/total (%)	
HHS Region 6	count/total (%)	count/total (%)	count/total (%)	
HHS Region 7	count/total (%)	count/total (%)	count/total (%)	
HHS Region 8	count/total (%)	count/total (%)	count/total (%)	
HHS Region 9	count/total (%)	count/total (%)	count/total (%)	
HHS Region 10	count/total (%)	count/total (%)	count/total (%)	
Rural	count/total (%)	count/total (%)	count/total (%)	*p-value*
Caregiver information (at study entry)				
History of SARS-CoV-2 infection	count/total (%)	count/total (%)	count/total (%)	count/total (%)

* https://www.hhs.gov/about/agencies/iea/regional-offices/index.html

14.3 Data Management Plan

14.3.1 Data Categories

RECOVER study data may be divided into two broad categories: Structured and Unstructured. Structured data can be simple (e.g., surveys/lab tests) or complex (e.g., sleep studies). REDCap will be used to capture structured data electronically. REDCap may include PII and PHI data to enable centralized coordination of biospecimen collection information.

14.3.2 Data Types

RECOVER study data may be divided into at least 12 different operational data types.

1. Patient questionnaires (in-person or submitted online; Structured)
2. Clinical site historical data (extant data; Structured→Complex)
3. Clinical site historical data (extant data; Unstructured)
4. EHR repository data (Structured→Complex)
5. Lab test results (Structured→Complex)
    i. Hospital
    ii. Commercial
    iii. Home
6. RECOVER biorepository inventory data (Biospecimens, slides; Structured→Complex)
7. Neuropsychological assessment data (Complex/Structured)
8. Functional assessment data (e.g., exercise testing, pulmonary/liver/kidney function; Structured→Complex)
9. Mobile health/wearable devices and computer software (e.g., fitness trackers, sleep monitors, Zio patch)
10. Advanced imaging data (CT and MRI; Unstructured)
11. Vaccination status data (Structured)
12. Vital signs and physiological testing data (e.g., PFTs, tilt table; Structured→Complex)

14.3.3 Functional data workflow summary

The RECOVER program studies the long-term effects of SARS-CoV-2 based on the study of several cohorts: Pediatric, Adult, Autopsy, and Pregnancy/Pediatric. In all cases, study participants are consented, samples are collected, and data is stored in central REDCap instance as described in the next section. Data may be entered into REDCap Central via four mechanisms: 1) direct entry into REDCap Central by CRCs at the clinic sites and 2) entry of data into surveys by participants in the study (and, in the case of the Pediatric cohort, their caregivers), 3) entry into an instance of REDCap by CRCs at the study sites, with subsequent data transfer to REDCap central, and 4) data transfer from existing research data repositories. The data are validated for quality using SAS within the FISMA envelope that hosts REDCap Central and then flows into a central i2b2 database (“I2b2 Data Hub”) as a limited dataset for analysis. The data is made available to investigators via a front-end built into the i2b2 Data Hub.

14.3.4 Harvard Medical School (HMS) AWS Cloud Environment

RECOVER systems, including REDCap Central (all cohorts), the i2b2 Data Hub, and statistical analysis tools such as R, SAS, SQL Server, SHRINE, and Gitlab will reside on a FISMA Moderate compliant infrastructure. i2b2 will be the primary software component used to centrally store all data and provide investigator tools for querying, reporting and extraction of analysis data sets.

The REDCap Central environment is managed by HMS. It is developed on an existing fully authorized FISMA Moderate environment in Amazon Web Services (AWS) currently in use to support the NHLBI BioData Catalyst (BDC) project, as authorized by the NHLBI in March, 2021. This environment leverages all the management and security systems, controls, change control methodologies, training, documentation, and 3rd party security testing (e.g., Penetration Testing) and assessments (e.g., 3PAO reviews) in place for the HMS BDC project.

14.3.5 Data Storage

Data will be stored in a cloud infrastructure. To comply with the government’s Cloud Smart policy, all PHI will live within a FISMA Moderate cloud environment that has received an ATO from NHLBI based on review by NHLBI’s cybersecurity office.

14.3.6 Data Destruction

When participants withdraw from the study, their data will be destroyed in REDCap using standard REDCap functionality. The record, including its participant ID, will persist, as will records of ICFs that were signed, and the withdrawal record. All other data will be destroyed from REDCap. It will not be possible to restore this data once it is destroyed.

Per the study protocols, data that are stored in the i2b2 Data Hub will not be destroyed at the time of withdrawal.

When the study ends, all data will be de-identified in REDCap Central. The data in the i2b2 Data hub will persist in its existing de-identified form.

14.3.7 Data Integrity
Detailed Quality Control programs will be designed and deployed by the DRC to ensure and audit data integrity

14.3.8 Security Management
Data will be stored in cloud infrastructure. To comply with the government's Cloud Smart policy, all PHI will live within a FISMA Moderate cloud environment that has received an ATO from NHLBI, which includes network firewalls and systems for access control, change control, continuous monitoring, and training. A System Security Plan, which will be reviewed and approved by NHLBI as part of the ATO, describes the cybersecurity and IT management plan in detail.

14.3.9 Source Documents and Access to Source Data/Documents
Source data are all information, original records of clinical findings, observations, or other activities in a study necessary for the reconstruction and evaluation of the study. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the study.

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write "N/D". If the item is not applicable to the individual case, write "N/A".

Access to study records will be limited to IRB-approved members of the study team. The investigator will permit study-related monitoring, audits, and inspections by the IRB/EC, the sponsor, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g., source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g., pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

15 Ethics/Protection of Human Subjects

15.1 Ethical Standard
The investigator will ensure that this study is conducted in full conformity with Regulations for the Protection of Human Subjects of Research codified in 45 CFR Part 46.

15.2 Institutional Review Board
The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. All changes to the consent form will be IRB approved; a determination will be made regarding whether previously consented participants need to be re-consented.

NYU will serve as the sIRB for sites without prior reliance agreements. The single IRB (sIRB) model has been adapted for the RECOVER initiative to permit reliance on existing central IRBs in some of the site networks.

For site networks with existing reliance agreements, each central IRB will execute a memorandum of understanding with the NYU sIRB to standardize protocol document handling and IRB review processes across all sites and maintain harmonization with the local requirements for each existing central IRB. This hybrid model has been reviewed and approved by NIH for the RECOVER initiative.

15.3 Informed Consent Process

Informed consent will be obtained and documented in writing before participation in study procedures. Study sites will identify potential participants in their available recruitment pools (extant cohorts, clinical cohorts, acute cohorts, and/or post-acute cohorts). The research study will be explained in lay terms to each potential research participant/parent/legal guardian in their preferred language. The overall common consent document will include:

1) consent for participation in RECOVER Tier 1 and optional participation in Tier 2 activities;
2) separate consent for participation in RECOVER Tier 3 activities
3) consent for recontact for future participation in research;
4) consent to obtain and link data from electronic health records, regional health information exchanges, non-financial claims data and the National Death Index;
5) consent for broad sharing of deidentified data and specimens through RECOVER databases and specimen repositories (in addition to other NIH-designated repositories).
6) optional return of genetic information and other research laboratory findings, including option to change decision for minor reaching age of majority
7) Subjects who reach the age of majority during study participation will be re-consented.

The potential participant (if above age of majority) or a parent/legal guardian above the age of majority will provide written documentation of informed consent before undergoing any study procedures. Age-appropriate assent will also be obtained. The consent process may be conducted by telephone, secure video conference platform approved for exchange of PHI, or in person. The investigator or suitable designated delegate will conduct a meeting with the study candidate and legally authorized representative (if younger than the age of majority) to review all the required elements of informed consent and to address all questions about the study. Adjunct education materials including flyers, booklets, slide presentations, animations, videos may be made available to the participant in print or electronic media formats to enhance comprehension of the informated consent process and scope of the study. All such materials will be approved by the IRB prior to use. Secure encryption will be used for email delivery of any of these materials. Comprehension of the study procedures and risks will be confirmed with standardized questions to the participant and/or parent/legal guardian. A standardized teach back method will be implemented as needed to ensure understanding of the key aspects of participation before enrollment. Subjects will be provided information on how to contact an appropriate individual for pertinent questions about the research and their rights and whom to contact in the event that they sustain a research-related injury.

Participants and their caregivers will only be approached for consent during periods of clinical stability. If required, the treating physician will be notified prior to the consent process. Consents may be conducted remotely using telephone or electronic audio and audiovisual platforms or in person. The child and caregiver will be at home or other private location during the consent process.

Documentation of consent will be recorded electronically via REDCap. For participants below the age of majority, the consent process will be conducted with the child and the parent/legal guardian. The parent/legal guardian will sign the consent form; the child may sign the age-appropriate assent form. For participants above the age of majority, the consent process will be conducted with the young adult; the young adult will sign the consent form.

The parent/legal guardian or young adult above the age of majority will be sent a link to the REDCap consent form via encrypted email, and potential subjects will be given the phone number of a study team member to call after they have reviewed the consent. The study team member will then explain the consent to the parent/legal guardian or young adult, and answer all questions. The study team member will then administer standardized questions with scoring rubric to assess understanding of key elements of the protocol. The study

team member will re-teach the protocol details for any incorrect responses to the standardized questions, and will repeat the questions to re-assess understanding. The consent process will be temporarily suspended If the parent/legal guardian or young adult continues to offer incorrect responses after 3 attempts of re-teaching. Additional teaching and educational will be provided at a later date. Only parent/legal guardians or young adults who demonstrate understanding of the key elements of the protocol will electronically sign the informed consent document in REDCap. Study personnel will verify identification before sanctioning an individual's electronic signature. An electronic or printed signed copy will be provided to the participant and a copy of the participant's consent to participate will be kept on a password-protected and secure drive at each study site.

For children, the IRB-approved age-appropriate assent document will be made available to the child for review with their parent/legal guardian. The study team will explain the assent document to the child and parent/legal guardian and answer all questions. Child understanding of the key elements of the assent document will be assessed by the study team and parent/legal guardian. The child will be given the opportunity to sign the age-appropriate assent document or provide verbal assent.

The REDCap eConsent link will be sent to the IRB of record before use in the study. Language consistency with the IRB-approved consent will be reviewed and approved by the IRB of record before eConsent is initiated.

If a parent/legal guardian is unable to provide an electronic signature during a remote visit, he or she will be required to sign a paper copy of the informed consent in the presence of a witness. The signature and date of the witness will also be required on the paper copy. A separate record of the required elements of the ICF process will be documented in the participant's study record.

A participant older than the age of majority will provide written informed consent; for participants younger than the age of majority, the informed consent will be provided by the legal authorized representative, with review of the age-appropriate assent document by the participant. If a participant attains the age of majority during participation in the study, the participant will be approached for re-consent Caregivers and other biological parents older than the age of majority will provide written informed consent; for primary caregivers and other biological parents younger than the age of majority, the legally authorized representative for the caregiver or other biological parent will provide written informed consent, with review of the age-appropriate assent document by the caregiver/other biological parent.

15.3.1 Consent/Assent and Other Informational Documents Provided to Participants

Consent forms describing in detail the study intervention, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to starting intervention. The following consent materials are submitted with this protocol:

- Informed consent form Caregiver
- Informed consent form children ages newborn-5 years congenitally exposed (Tiers 1 and 2)
- Informed consent form children ages newborn-5 years post-natally exposed (Tiers 1 and 2)
- Informed consent form children ages 6-17 years (Tiers 1 and 2)
- Informed consent form children ages 6-17 years (Tier 3)
- Informed consent form children ages 3-5 years (Tier 3)
- Informed consent form young adults ages 18-25 years (Tiers 1 and 2)
- Informed consent form young adults ages 18-25 years (Tier 3)
- Informed consent form children ages 3-17 years with history of MIS-C (Tiers 1, 2, and 3)
- Informed consent form young adults ages 18-25 years with history of MIS-C (Tiers 1, 2, and 3)
- Informed consent form children 3-17 years with history of post vaccine myocarditis (Tiers 1 and 2)
- Informed consent form young adults ages 18-25 with history of post vaccine myocarditis (Tiers 1 and 2)
- Informed consent form other biological parent
- Assent for children 7-11 years of age (Tier 1)
- Assent for children 7-11 years of age (Tier 2)
- Assent for children 7-11 years of age (Tier 3)

- Assent for children 12-14 years of age (Tier 1)
- Assent for children 12-14 years of age (Tier 2)
- Assent for children 12-14 years of age (Tier 3)
- Assent for children 15-17 years of age (Tier 1)
- Assent for children 15-17 years of age (Tier 2)
- Assent for children 15-17 years of age (Tier 3)

15.3.2 Consent Procedures and Documentation

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Extensive discussion of risks and possible benefits of participation will be provided to the participants and their families. Consent forms will be IRB-approved and the participant will be asked to read and review the document.

For parents/legal guardians of child participants and young adult participants above the age of majority, the investigator will explain the research study and contents of the consent form and answer any questions that may arise. All parents/legal guardians and young adults will receive a verbal explanation in terms suited to their comprehension of the purposes, procedures, and potential risks of the study and of their or their children's rights as research participants. Parents/legal guardians of child participants and young adult participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The parents/legal guardians and young adult participants should have the opportunity to discuss the study with their surrogates and carefully consider their decision prior to agreeing to participate. The parent/legal guardian of the child participant or young adult participant will sign the informed consent document prior to any procedures being done specifically for the study. The parent/legal guardian or young adult may withdraw consent at any time throughout the course of the study. A copy of the signed informed consent document will be given to the parent/legal guardian or young adult participant for their records. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

For children, the IRB-approved age-appropriate assent document will be made available to the child for review with their parent/legal guardian. The study team will explain the assent document to the child and parent/legal guardian and answer all questions. Child understanding of the key elements of the assent document will be assessed with standard questions with re-teaching as needed. The child will be given the opportunity to sign the age-appropriate assent document or provide verbal assent.

A copy of the signed informed consent document and any signed assent document will be stored in the subject's research record. The consent process, including the name of the individual obtaining consent, will be thoroughly documented in the subject's research record. Any alteration to the standard consent process (e.g., use of a translator, consent from a legally authorized representative, consent document presented orally, etc.) and the justification for such alteration will likewise be documented.

15.3.3 Posting of Clinical Trial Consent Form

The proposed study consent form will be posted on clinicaltrials.gov and a public website at https://www.recovercovidstudies.org/.

15.4 Participant and Data Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study?
- Who will have access to that information and why?
- Who will use or disclose that information ?
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts will be made to obtain permission to collect at least vital status (i.e., that the subject is alive) at the end of their scheduled study period.

Investigators in this research will take all reasonable measures to protect the confidentiality of the medical records of patients and their families. Multiple measures to protect confidentiality are described in the following paragraphs.

15.4.1 REDCap database security

The REDCap instance will be hosted and managed by Harvard Medical School (HMS) in their FISMA Moderate-compliant AWS cloud infrastructure. The security of the HMS infrastructure is governed by alignment with 18 security control families, as defined by FISMA at the Moderate level. In general, as per FISMA, only HMS technical staff and delegates will have access to infrastructure, networking, systems administration, access control, monitoring, and security testing systems. Approved DRC personnel can have applications administrative access to modify REDCap forms, undertake data quality research, and other data processing tasks. Investigators will not be able to have direct access to study data outside of the data entered by their sites. The mechanisms for this access have not yet been determined.

15.4.2 Data storage security

The RECOVER i2b2 Data Hub (the Hub) is the central location where all RECOVER data will exist or be indexed. It will contain the eCRF and some eConsent data from REDCap and some biospecimen data from the Biorepository, as well as all other data, such as EHR data, that is collected on study participants. The i2b2 data hub will live within a FISMA-moderate cloud environment. Only DRC staff will have direct access to the i2b2 Data Hub infrastructure. DRC staff will be responsible for the creation of staff user access. Data in the i2b2 Data Hub will exist as a Limited Data Set with no direct identifiers. There will be a web-based query tool that will be available to privileged study investigators, with proper site credentials and human research participants training, for aggregate queries only.

15.4.3 Storage of Study Materials

Investigators will take all reasonable measures to protect the confidentiality of the study participants through the measures used in all PASC studies, including storage of study materials in locked, secure locations accessible only to study investigators, knowledge of the subject's name only for the minimal time needed for coordination of study logistics, use of a UUID with no personal identifiers to maintain confidentiality and elimination of all PHI in the final study database, and use of secure password protected computer access and encrypted transmission of patient information.

15.4.4 Hashed identifiers

A unique subject hashed identifier (UUID) will be assigned to each study participant. The hashed identifier is a universal subject ID that allows researchers to share data specific to a study participant without exposing personally identifiable information (PII) and at the same time be able to match participants across labs, databases, or research studies. Personal information will not be included in the final research database, but rather we will only generate a unique set of encrypted codes that can then be decrypted to determine if the subject already exists within a data repository. The hashed identifier will allow data from this study to be combined with data from other research studies or databases in an effort to improve outcomes in children and adolescents who have had SARS-CoV-2 infection with or without PASC.

15.4.5 Datavant Tokenization and Privacy Protecting Record Linkage (PPRL) Technology

An important goal of RECOVER is linking data from disparate sources. To achieve this goal, the DRC will guide the implementation of Privacy Preserving Record Linkage (PPRL) using Datavant technology to

securely connect the same individuals across different data sources while maintaining the participants' privacy.

Datavant's de-identification technology replaces private patient information with an encrypted "token" that can't be reverse engineered to reveal the original information. The technology can create the same patient-specific tokens in any data set, so that different data sets can be combined using the patient tokens to match corresponding records without ever sharing the underlying patient information. The process can be thought of in two distinct steps: token creation and privacy protecting record linkage.

Tokenization

Tokens are created from PII and they can be built from many different combinations of PII elements. The specific tokens to be created and PII elements required are specified below.

The processes to be used for RECOVER token creation are still being designed. The token creation may occur locally at the sites or centrally at the DRC. In the first case (and most popular among the site PIs), the site installs Datavant software locally to create the participant tokens using PII. The PII used to create the tokens stays at the local site and does not get sent to the DRC. Only the resulting token is sent to the DRC. In the second case, the site would rely on the DRC to create the token, which requires that the site send PII needed to create the tokens to the DRC. Both processes involve working with Datavant to create the site-specific encrypted tokens.

Linkage

Datavant tokens allow corresponding patient records to be matched across data sets without ever sharing PHI. The matching or linkage will occur at the DRC or at the enrolling site using Datavant software, which will live inside the FISMA-moderate cloud infrastructure. The consortium sites will be trained in the proper implementation of the Privacy Preserving Record Linkage "Hash tokens" consistent with the model being applied to other NIH repositories and programs

15.4.6 Limited Data Set

The data stored in i2b2 Data Hub is a limited data set of identifiable patient information as defined in the Privacy Regulations issued under the Health Insurance Portability and Accountability Act (HIPAA). A limited data set does not include any of the following information:

- Names
- Street addresses or postal address information with the exception of town/city, state and zip code
- Phone/Fax numbers
- E-mail addresses
- Social Security numbers
- Medical records numbers
- Health plan beneficiary numbers
- Other account numbers
- Certificate and license numbers
- Vehicle identifiers and serial numbers, including license plates
- Device identifiers and serial numbers
- URLs and IP addresses
- Biometric identifiers such as fingerprints, retinal scans and voice prints
- Full face photos and comparable images

15.4.7 Reporting of Incidental Findings

Test results determined by CLIA-certified clinical laboratories and imaging and other clinical testing results determined by licensed medical professionals that are analytically valid will be recorded in the participant medical record and will be reviewed by the Principal Investigator or other designated licensed medical professional at each site. If the Principal Investigator or licensed designee determine that the result is clinically significant or medically actionable, the participant will be contacted by telephone or in-person to explain the test findings within one week of the return of the test results. The participant will also be advised to

follow up with their primary care physician. Any additional testing ordered by the primary care physician will be paid by the participant or their insurance company.

Test results determined in research laboratories that cannot be validated in CLIA-certified clinical laboratories will not be recorded in the medial record and will not be returned to the participant.

15.4.8 Reporting of Genetic Testing

The WGS studies for the RECOVER study will be performed in a CLIA-certified laboratory with results interpreted by a board-certified clinical molecular geneticist or molecular genetic pathologist or equivalent. Analytically valid replicated results from the CLIA-certified laboratory that are defined as clinically actionable according to the standards and guidelines defined by the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMB-AMP) guidelines may be disclosed to the participants if all of the following criteria are met:(33-35)

- The genetic finding has important health implications for the participant and the associated risks are established and substantial.
- The genetic finding is actionable, that is, there are established therapeutic or preventive interventions or other available actions that have the potential to change the clinical course of the disease.
- The test is analytically valid and the disclosure plan complies with all applicable laws.
- During the informed consent process or subsequently, the study participant has opted to receive his/her individual genetic results.

All disclosure of clinically actionable genetic results will be guided by the RECOVER WGS Core Laboratory in collaboration with the RECOVER Clinical Science Core. The consent form will inform participants of the potential for return of actionable results from WGS and the potential risks associated with the disclosure of the genetic information. For participants who elect to be informed of their clinically actionable genetic results, the validated, replicated result will be shared with the site PI or designated study personnel, who will use their local process and policies for re-identification of the participant and referral if needed for evaluation and counseling, which may include involvement of their local genetics team and/or the participant's cardiologist or other healthcare providers. Participants who reach the legal age of majority during the study will be re-consented and given the opportunity to opt-in or opt-out of return of genetic information.

Clinical genetic testing targeting known disease-associated variants will not be performed. There is a reasonable possibility that no findings will result from this research effort. If variants are detected, it may be years before any clinical utility of these findings is realized. Further, if samples are "anonymized" prior to release to other investigators for future research, it may not be possible to trace the results back to the participant.

15.4.9 Certificate of Confidentiality

Participant confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

The study monitor, other authorized representatives of the sponsor, or representatives of the IRB may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by local IRB and Institutional regulations.

In accord with NIH policy (https://grants.nih.gov/policy/humansubjects/coc/coc-nih-funded.htm), a Certificate of Confidentiality will be issued automatically as a term of this NIH-funded award, and no physical certificate will be issued. With this Certificate, the researchers of this study cannot be forced to disclose information that may identify a participant, even by a court subpoena, in any federal, state, or local civil, criminal, administrative, legislative, or other proceeding. The Certificate cannot be used to resist a request for information from the United States government when it is used for evaluating federally funded study projects or for information that must be disclosed to meet the requirements of the Food and Drug Administration (FDA). A Certificate of Confidentiality does not prevent a participant or his/her family from voluntarily releasing information about the participant's involvement in this research. If an insurer, employer, or other person obtains a participant's or family's written consent to receive research information, then the researchers will not use the Certificate to withhold that information.

A genome-wide association study performed with samples collected in this study will comply with the NIH Policy for Sharing of Data Obtained in NIH Supported or Conducted GWAS, which calls for investigators funded by the NIH for GWAS to 1) share de-identified genetic (genotypic and phenotypic) data through a centralized NIH data repository; and 2) submit documentation that describes how the institutions have considered the interests of the research participants, such as privacy and confidentiality. Submission of data to the NIH GWAS repository will be consistent with the permissions and limitations delineated on the study consent signed by study participants. Information from DNA analyses and clinical studies or medical records may be placed into a central data repository in the future, such as the National Center for Biotechnology Information repository. Data and samples will be de-identified before submission to this or any other central repository.

15.4.10 Research Use of Stored Human Samples, Specimens, or Data

- Intended Use: Samples and data collected under this protocol may be used to study mechanisms and clinical manifestations of SARS-CoV-2 infection and other disease states in the future. It is anticipated that DNA testing may be performed in the future.
- Storage: Access to stored samples will be limited with policies and procedures requiring multiple reviews prior to release of any samples for analysis. Samples and data will be stored using UUID codes assigned by the investigators until the aliquots are used up. Data will be stored in REDCap. Only investigators authorized by Mayo Clinic and the RECOVER Scientific Leadership will have access to the samples and data.
- Tracking: Data will be tracked using the central research database at the RECOVER PASC Investigator Consortium Data Resource Core at Massachusetts General Hospital. Each specimen will be labeled and tracked with a UUID.
- Disposition at the completion of the study: All stored samples will be sent to the RECOVER PASC biorepository at Mayo Clinic. Study participants who request destruction of samples will be notified of compliance with such request and all supporting details will be maintained for tracking.

15.5 Future Use of Stored Specimens

Biospecimens are defined as any tissues, bodily fluids (such as blood, saliva), and excreta (such as, stool and urine) that are collected as part of the RECOVER cohort protocols. With the participant's approval and as approved by the NYU sIRB and consortium central IRBs, de-identified biological coded biospecimen samples will be stored at the RECOVER PASC biorepository at Mayo Clinic. Participants may be permitted to restrict certain uses of biospecimens. These samples may be used for research into the causes of PASC, complications of PASC, understanding risk factors for PASC, and to develop diagnostic tests and treatments for PASC. These samples may also be used in other areas of research not directly related to PASC, including research conducted by business entities. Whole genome sequencing may be performed, but "true" clinical genetic testing targeting known disease-associated variants will not be performed. The RECOVER PASC biorepository at Mayo Clinic may transfer biospecimens to other biorepositories in the future as designated by the NIH Sponsor. The RECOVER PASC biorepository at Mayo Clinic may also be provided with a UUID that will allow linking the biological specimens with the phenotypic data from each participant, maintaining the masking of the identity of the participant. Only the RECOVER investigators at enrolling sites and the Data Resource Core will have access to the information linking the coded subject ID and subject identity.

During the conduct of the study, an individual participant can choose to withdraw consent to have biological specimens stored for future research. Subjects who reach the age of majority after study entry will have the opportunity to opt-out of storage of biospecimens at that time. However, withdrawal of consent with regard to biosample storage will not be possible after the study is completed, at which time the linking information between the subject coded ID and subject identity has been destroyed and only anonymized samples remain.

Access to stored biospecimens will be provided through the RECOVER PASC biorepository at Mayo Clinic or other designated biorepository as determined by the policies and procedures of the NIH Sponsor.

16 Data Handling and Record Keeping

16.1 Data Collection and Management Responsibilities

Data collection is the responsibility of the study staff at the site under the supervision of the site PI. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Black ink is required to ensure clarity of reproduced copies. When making changes or corrections, cross out the original entry with a single line, and initial and date the change. Do not erase, overwrite, or use correction fluid or tape on the original.

Copies of the electronic CRF (eCRF) will be provided for use as source documents and maintained for recording data for each participant enrolled in the study. Data reported in the eCRF derived from source documents should be consistent with the source documents or the discrepancies should be explained and captured in a progress note and maintained in the participant's official electronic study record.

Research data will be entered into a central REDCap database, and then de-identified and stored as a limited data set in the central data capture system provided by the RECOVER PASC Consortium Data Resource Core at Massachusetts General Hospital. A central instance of REDCap will be used for capture of structured data from enrolling sites. The data capture system meets Federal data security requirements and includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Research data will be entered directly from the source documents or transferred from existing research databases at the enrolling sites. At the end of the study, all identifiers will be removed from the central REDCap database. Identifiers may remain in the local site research database if the participant has provided consent for contact for future research.

16.2 Study Records Retention

Study documents will be retained for the longer of 3 years after close out or 5 years after final reporting/publication. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

16.3 Protocol Deviations

A protocol deviation is any noncompliance with the study protocol and study manual of operations requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

It is the responsibility of the site to use continuous vigilance to identify and report deviations within 5 working days of identification of the protocol deviation. All deviations associated with change in risk to participants or compromise of scientific integrity of the study must be addressed in study source documents, reported to RECOVER program scientific directors at NIH, the Clinical Science Core Principal Investigators at NYU

Langone Health, and the RECOVER PASC DRC Principal Investigators at Massachusetts General Hospital, the IRB or record, and the RECOVER OSMB. Protocol deviations that do not impact risk or scientific integrity must be recorded on note to file and reported to the OSMB at 6-month intervals. Protocol deviations must be reported to the local IRB per their guidelines. The site PI/study staff is responsible for knowing and adhering to their IRB requirements. Further details about the handling of protocol deviations will be included in the MOP.

16.4 Publication and Data Sharing Policy

This study will comply with the NIH Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

17 Study Finances

17.1 Funding Source

This study is financed through a grant from the Other Transactional Authority (OTA) of the US Federal Government. The study is overseen by the National Institutes of Health, NHLBI

17.2 Costs to the Participant

There are no costs to the participant related to participation in the study. The OTA grant will pay for all study related procedures and costs.

17.3 Participant Reimbursements or Payments

Sites will offer patients a nominal reimbursement for participation in the remote interval assessments and more substantial reimbursement for participation in the more complex or time-consuming Tier 2 and Tier 3 testing commensurate with their time and effort.

18 Study Administration

18.1 Study Leadership

The scientific leadership for the study and oversight of sites participating in the study is provided by the RECOVER Clinical Science Core (CSC) at the NYU Grossman School of Medicine. The RECOVER CSC collaborates with the RECOVER Data Resource Core at Massachusetts General Hospital for data management and data storage at the RECOVER biorepository at Mayo Clinic for biospecimen storage. The activity of the RECOVER cohort studies is overseen by a Steering Committee composed of the RECOVER Principal Investigators and NIH program leadership, an Executive Committee composed of NIH Institute leadership, and an OSMB composed of experts in longitudinal observation studies, epidemiology, bioethics, and biostatistics. The Steering Committee, Executive Committee and OSMB will meet at a minimum of twice yearly.

Protocol modifications may be proposed by the RECOVER Clinical Science Core based on interim analysis of study data, safety data, data derived from other RECOVER cohorts, and new information in the medical literature. A study design committee will make recommendations to the RECOVER Steering Committee. The recommendation will be further reviewed by the data safety monitoring board and the RECOVER Executive Committee. If a protocol modification is approved by the Executive Committee and safety monitoring board, a modified proposal, and associated modified consent forms and other protocol documents will be submitted to the NYU sIRB for review. There will be no changes in study procedures until the modification is approved by

the NYU sIRB. Urgent changes in study procedures may be implemented to maintain subject safety. In this instance, the NYU sIRB will be notified of the change within 72 hours.

19 Conflict of Interest Policy

All recipient institutions and investigators in the PASC consortium will comply with the requirements of 42 CFR 50, Subpart F, "Responsibility of Applicants for Promoting Objectivity in Research for which PHS Funding is Sought" (FCOI Regulation), as implemented in the 2011 Final Rule for grants and cooperative agreements.

The requirements promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct, or reporting of research funded under PHS grants or cooperative agreements will be free from bias resulting from any conflicting financial interest of an investigator. An "investigator" is someone defined as the PD/PI and any other person, regardless of title or position who is responsible for the design, conduct, or reporting of research funded by PHS, or proposed for such funding which may include, for example, collaborators or consultants.
Each Institution shall maintain an up-to-date, written, enforced policy on financial conflicts of interest that complies with the regulation and make the policy available via a publicly accessible Web site.

These FCOI requirements do not apply to Federal employees or Federal agencies. Federal agencies have their own set of rules governing financial conflicts of interest for employees.

When submitting a grant application, the signature of the Authorized Organization Representative (AOR) will certify each PASC Consortium applicant institution's compliance with the requirements of 42 CFR 50, Subpart F, including that:

- There is in effect at the Institution an up-to-date, written and enforced administrative process to identify and manage Financial Conflicts of Interest (FCOI) with respect to all research projects for which NIH funding is sought or received;
- The Institution shall promote and enforce Investigator compliance with the regulation's requirements including those pertaining to disclosure of Significant Financial Interests;
- The Institution shall identify and manage FCOIs and provide initial and ongoing FCOI reports to the NIH consistent with this subpart;
- When requested, the Institution will promptly make information available to the NIH/HHS relating to any Investigator disclosure of financial interests and the Institution's review of, and response to, such disclosure, whether or not the disclosure resulted in the Institution's determination of an FCOI;
- The Institution shall fully comply with the requirements of the regulation.

20 APPENDICES

20.1 Appendix A: Enrollment Entry Timepoints

Time point	Status	Cohort	Outcomes
Prior to SARS-CoV-2 infection	Develops SARS-CoV-2 infection	SARS-CoV-2 infected cohort	Define PASC based on excess incidence compared to referent population. Determine clinically interpretable sub-types using supervised or unsupervised learning approach.
	No SARS-CoV-2 infection	Uninfected control cohort	
Current, acute SARS-CoV-2 infection	Active SARS-CoV-2 infection	SARS-CoV-2 infected cohort	To characterize the clinical features, pathophysiology, and mechanisms that influence PASC symptoms and their progression over time and across the childhood lifespan.
Post SARS-CoV-2 infection	Prior SARS-CoV-2 infection	PASC**+** Cases PASC**-** Cases	To characterize the clinical course and recovery of PASC over time

20.2 Appendix B. Overview Schedule of Assessments by Recruitment Pool

Schedule of Assessments Main Pediatric Cohort Tiers 1-3 Ages 6-25 years

All study visits for the main pediatric cohort are timed from study entry. Tier 2 acute visits will have a time window of ± 7 days, and are optional in the event of severe acute illness. Each post-acute Tier 2 visit will have a time window of ±2 months. Post-acute Tier 2 visits will include an on-site component and a remote component for completion of questionnaires. Tier 3 will be conducted over an interval of 3 visits about one year apart, between months 3-30 after study entry and repeated between months 15-42 after study entry.

Tier 1 and Tier 2 Assessments	**BL**		**Acute phase**								**Post-acute phase**								
Demographics	X																		
Social determinants of health	X									X		X		X		X		X	
Medical history	X									X		X		X		X		X	
COVID health impact	X									X		X		X		X		X	
Vaccination status	X		X		X		X			X		X		X		X		X	
History of acute SARS-CoV-2	X		X		X		X			X		X		X		X		X	
PASC Symptoms: ISARIC-WHO	X		X		X		X			X		X		X		X		X	
Tier 1 biospecimen	Z																		
Acute Tier 2 biospecimen							Z												
Anthropometry & vitals							Y			Y		Y		Y		Y		Y	
Electrocardiogram							Y			Y		Y		Y		Y		Y	
Spirometry							Y			Y		Y		Y		Y		Y	
Clinical Laboratory Post-Acute Tier 2																			
PROMIS										X		X		X		X		X	
Educational status										X		X		X		X		X	
Oral health										Y		Y		Y		Y		Y	
Joint flexibility (Beighton Scale)										Y		Y		Y		Y		Y	
Autonomic dysfunction										Y		Y		Y		Y		Y	
Post-Acute Tier 2 biospecimen										Z		Z		Z		Z		Z	
Detailed cognitive development										Y		Y		Y		Y		Y	
Emotional/mental health										Y		Y		Y		Y		Y	
Diet												X		X		X		X	
Tier 3 Assessments																			
Cardiac structure and function												Y		Y					
Lung function (pulmonary function tests)												Y		Y					
Lung microbiome (sputum induction)												Y		Y					
Aerobic capacity												Y		Y					
Abdominal organ structure												Y		Y					
Brain MRI												Y		Y					
Brain EEG												Y		Y					
Neurocognitive testing												Y		Y					
Tier 3 biospecimen collection for microbiome and clinical laboratory												Z		Z					
Weeks	BL	…	1	2	3	4	8	…											
Months	BL					1	2			6		12		24		36		48	

Table Legend: Blue (X) =Questionnaires; Red (Y) =Clinical Assessments; Orange (Z) =Biospecimen Collection
Table Notes:
Visits scheduled base on time elapsed from study entry
Biospecimen collection described in Section 11.14
Electrocardiogram, spirometry and clinical laboratory collection required at first post-acute Tier 2 visit and optional at subsequent post-acute Tier 2 visits

Schedule of Assessments Main Pediatric Cohort Tiers 1-3 Ages newborn-5 years post-natal SARS-CoV-2 infection

Assessments	**Post-Acute**							
Tiers 1 and 2 Assessments								
Demographics	X							
Medical history (NSCH)	X				X		X	
ASQ	X				X		X	
Growth/Exam	X		X		Y		Y	Y
MCHAT			X					
ASQ Social/Emotional			X					
CBCL Behavioral Problems					X		X	
Developmental Profile-4							X	X
Tier 2 biospecimen collection					Z			
Bayley Scales					X			
Differential Ability Scales-II							X	X
Tier 3 assessments								
Echocardiography							Y	Y
Abdominal organ structure							Y	Y
Brain MRI							Y	Y
Brain EEG							Y	Y
Neurocognitive testing							Y	Y
Tier 3 biospecimen collection for microbiome and clinical laboratory							Z	Z
Months	12	…	18		24		36	48

Table Legend: Blue (X) =Questionnaires; Red (Y) =Clinical Assessments; Orange (Z) =Biospecimen Collection
Table Notes:
Visits scheduled base on time elapsed from date of study entry
Assessments at each visit will be conducted according to the age of the child
Biospecimen collection described in Section 11.14

Schedule of Assessments MIS-C Cohort

Assessments	BL		Post-Acute			
Tiers 1 and 2 Assessments						
Demographics	X					
Social determinants of health	X		X		X	
Medical history	X		X		X	
COVID health impact	X		X		X	
Vaccination status	X		X		X	
PASC Symptoms: ISARIC-WHO	X		X		X	
Anthropometry & vitals			Y		Y	
PROMIS			X		X	
Diet			X		X	
Activity			X		X	
Tier 3 Assessments						
Cardiac structure and function			Y		Y	
Lung function			Y		Y	
Lung microbiome			Y		Y	
Aerobic capacity			Y		Y	
Abdominal organ structure			Y		Y	
Brain structure			Y		Y	
Brain activity			Y		Y	
Neurocognitive testing			Y		Y	
Tier 3 Microbiome biospecimen collection			Z		Z	
Months	BL	…	12		24	

Table Legend: Blue (X) =Questionnaires; Red (Y) =Clinical Assessments; Orange (Z) =Biospecimen Collection
Table Notes:
Visits scheduled base on time elapsed from index date of hospitalization
Tier 2 and 3 assessments may be scheduled contemporaneously within specified time windows
Biospecimen collection described in Section 11.14
MIS-C participants will undergo selection of up to three Tier 3 assessments according to participant medical history and symptoms

Schedule of Assessments Post-Vaccine Myocarditis Cohort

Assessments	BL		Post-Acute			
Tiers 1 and 2 Assessments						
Demographics	X					
Social determinants of health	X		X		X	
Medical history	X		X		X	
COVID health impact	X		X		X	
Vaccination status	X		X		X	
PASC Symptoms: ISARIC-WHO	X		X		X	
Tier 1 biospecimen collection			Z		Z	
Anthropometry & vitals			Y		Y	
PROMIS			X		X	
Diet			X		X	
Activity			X		X	
Post-acute Tier 2 biospecimen collection			Z		Z	
Months	BL	…	12		24	

Table Legend: Blue (X) =Questionnaires; Red (Y) =Clinical Assessments; Orange (Z) =Biospecimen Collection
Table Notes:
Visits scheduled base on time elapsed from index date of vaccination
Tier 2 and 3 assessments may be scheduled contemporaneously within specified time windows
Biospecimen collection described in Section 11.14

Schedule of Assessments ABCD Cohort

Tier 1 Assessments	BL
Demographics	X
Social determinants of health	X
Medical history	X
COVID health impact	X
Vaccination status	X
History of acute SARS-CoV-2	X
PASC Symptoms: ISARIC-WHO	X
PROMIS	X
Tier 1 biospecimen collection	Z

Table Legend: Blue (X) =Questionnaires; Red (Y) =Clinical Assessments; Orange (Z) =Biospecimen Collection
Table Note:
Biospecimen collection described in Section 11.14
ABCD cohort will participate in Tier 1 assessments; selected participants may be referred to another RECOVER site for participation in Tier 2 and Tier 3 procedures if geographically feasible

Schedule of Assessments Infants ages newborn-5 years born to mothers with and without SARS-CoV-2 infection during pregnancy

Assessments	Post-Acute								
Tiers 1 and 2 Assessments									
Demographics	X								
Medical history (NSCH)	X				X		X		
ASQ	X				X		X		
Growth/Exam	X		X		Y		Y		Y
MCHAT			X						
ASQ Social/Emotional			X						
CBCL Behavioral Problems					X		X		
Developmental Profile-4							X		X
Tier 2 biospecimen collection					Z				
Bayley Scales					X				
Differential Ability Scales-II							X		X
Months	12	…	18		24		36		48

Table Legend: Blue (X) =Questionnaires; Red (Y) =Clinical Assessments; Orange (Z) =Biospecimen Collection
Table Notes:
Visits scheduled base on time elapsed from birth
Biospecimen collection described in Section 11.14

20.3 Appendix C: Proposed Sample Size by Tier

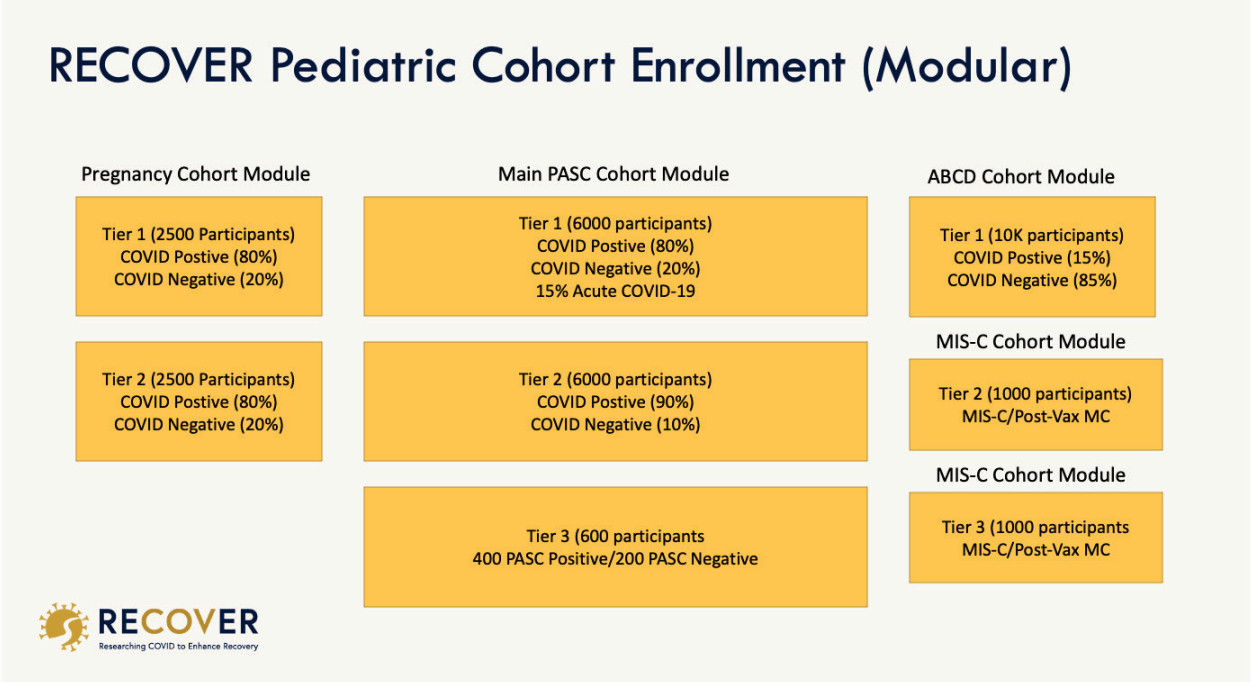
Up to a total of 20,000 children and young adults (age newborn-25 years) may be enrolled. Up to 20,000 caregivers and up to 20,000 may be optionally enrolled with the child or young adult. Based on past history of SARS-CoV-2 exposure and post exposure history, there are 3 distinct modules for participation:

Main PASC Cohort Module. The total number of children and young adults (age newborn-25 years) enrolled will be adjusted to achieve a total of 5400 participants with SARS-CoV-2 infection in Tier 2 of the main PASC Cohort Module (3600 with PASC and 1800 without PASC). This total will include 1000 participants with acute SARS-CoV-2 infection enrolled in Tier 1. The main PASC Cohort Module of Tier 2 will also include 600 participants without SARS-CoV-2 infection. These 600 uninfected control participants will be randomly assigned to Tier 2 follow-up schedule to match the acute infected cohort (n=200) or the post-acute infected cohort (n=400). Approximately 10% of the infected participants in this cohort will participate in Tier 3.

Pregnancy Cohort Module. 2000 infants (age 0-2 years) born to mothers infected with SARS-CoV-2 during pregnancy (congenitally exposed) and 500 infants born to mothers not infected with SARS-CoV-2 during pregnancy will be enrolled and will participate in a separate Tier 1 and Tier 2 assessment schedule.

ABCD Cohort Module (10,000 adolescents) enrolled in the ABCD study will be enrolled for determination of COVID exposure and PASC symptoms in Tier 1 assessments. A subset of subjects will be offered participation in Tiers 2 and 3 based on geographic location.

MIS-C and Post-Vaccine myocarditis. 800 children and young adults with history of MIS-C and 200 children and young adults with history of post-vaccine myocarditis will participate in a subset of Tier 2 and Tier 3 procedures.



20.4 Appendix D: Tier 1: Data Elements

Tier 1 information will be obtained remotely or in-person by participant request. All study participants will complete Tier 1 procedures as described in this table.

Information collected	Description	Source	Interval
Tier 1 – Data Elements			
Caregiver Demographics	Study ID, Name, Date of Birth, Address, Age, Sex, Sexual orientation, Gender identity, Race/ethnicity, Language, Marital status, Caregiver relationship to child, Household composition, Highest level of education, English proficiency, Birthplace, Years in US.	Remote patient report (electronic, paper or telephone assessments)	Baseline assessment
Child Demographics	Study ID, Name, Date of Birth, Address, Age, Sex, Gender identity, Race/ethnicity, Language	Remote patient report (electronic, paper or telephone assessments)	Baseline assessment
Caregiver Other History	Health status pre-COVID, Disability, Medications, COVID vaccination status and date.	Remote patient report (electronic, paper or telephone assessments), EHR	Baseline assessment
Child Other History	Birth history, Health status pre-COVID, Disability, Medications, COVID vaccination status and date.	Remote patient report (electronic, paper or telephone assessments), EHR	Baseline assessment
Household SDOH	Address stability (2 questions: Where was your address 12 months ago? How long have you lived at your current address?) Financial stability (Weekly Stress Inventory 5 Question COVID-19 Related Household Finances), Annual family income from PhenX Employment- RadxUp Food insecurity (Hunger Vital Signs (Update) based on USDA) Access to health care (Adult Access to Health Care & Utilization Module, Question on Insurance from US Census, Since being diagnosed with COVID have you lost or experienced any changes with your health insurance (do not recommend using "diagnosed" consider "symptoms" or "becoming ill") Perceived discrimination (Major Experiences and Everyday Discrimination Scale [everyday subset #10-19] with 1 Follow Up Question) Discrimination impacting likeliness to access care- Was there ever a time when you would have received better medical care if you belonged to a different race/ethnic/identity group? Stress- Perceived stress scale. Social support - RAND MOS Community cohesion (5 Questions on Community Cohesion from 10 Question Neighborhood Collective Efficacy)	Remote patient report (electronic, paper or telephone assessments)	Baseline assessment
Caregiver and Child Behavioral Health	Tobacco Exposure All of Us Research Program Lifestyle Survey	Remote patient report (electronic, paper or telephone assessments) EHR	Baseline assessment

(continued)

20.4 Appendix D: Tier 1: Data Elements (continued)

Information collected	Description	Source	Interval
Caregiver Comorbidities	Diseases present prior to COVID- heart failure, HTN, Cancer, Cardiovascular or cerebrovascular disease chronic liver disease, chronic kidney disease including Dialysis, chronic lung disease (COPD, asthma, plum HTN, plum fibrosis) Rheumatologic disease, Immunocompromised (HIV, AIDS, transplant, immune deficiency), Chronic pain syndrome, POTS/ME/CFS/dysautonomia Mood disorder (anxiety, depression), Obesity, Oral health history (pain, ulcer, easy bleeding, tooth loss, tooth infections, inflammation - Remember tongue in this history.)	Remote patient report (electronic, paper or telephone assessments), EHR	Baseline assessment
Child Past Medical History	Chronic medical problems prior to COVID	Remote patient report (electronic, paper or telephone assessments) EHR	Baseline assessment
Caregiver COVID Infection (including lab confirmation)	Initial SARS-CoV-2 infection (SARS CoV-2 PCR result, SARS CoV-2 antigen result, SARS CoV-2 antibody result, SARS CoV-2 sequencing performed, diagnosed by a doctor based on symptoms, suspected by participant, but not diagnosed by a doctor)	Remote patient report (electronic, paper or telephone assessments) EHR	Baseline assessment
Child COVID Infection (including lab confirmation)	Initial SARS-CoV-2 infection (SARS CoV-2 PCR result, SARS CoV-2 antigen result, SARS CoV-2 antibody result, SARS CoV-2 sequencing performed, diagnosed by a doctor based on symptoms, suspected by participant, but not diagnosed by a doctor)	Remote patient report (electronic, paper or telephone assessments), EHR	Baseline assessment
Caregiver and Child Access to COVID Testing	PhenX Tool kit (Update); adapted from Radxup	Remote patient report (electronic, paper or telephone assessments)	Baseline assessment
Caregiver Severity of SARS CoV-2 Infection	NIH severity level, NIH severity level, Hospitalization	Remote patient report (electronic, paper or telephone assessments)	Baseline assessment
Child Severity of SARS CoV-2 Infection	NIH severity level, NIH severity level, Hospitalization	Remote patient report (electronic, paper or telephone assessments)	Baseline assessment
Caregiver Previous In-Hospital SARS-CoV-2 Treatment Record	Treatment option (Treated with corticosteroids , Treated with hydroxychloroquine, Treated with lopinavir, ritonavir, other antiviral, Treated with monoclonal antibody, Treated with therapeutic anticoagulation, Treated with antibiotics)	Remote patient report (electronic, paper or telephone assessments), EHR	Baseline assessment
Child Previous In-Hospital SARS CoV-2 Treatment Record	Treatment option (Treated with corticosteroids , Treated with hydroxychloroquine, Treated with lopinavir, ritonavir, other antiviral, Treated with monoclonal antibody, Treated with therapeutic anticoagulation, Treated with antibiotics)	Remote patient report (electronic, paper or telephone assessments), EHR	Baseline assessment
Caregiver Health Status	Quality of life-PROMIS-10	Remote patient report (electronic, paper or telephone assessments)	Baseline assessment
Child Health Status	PROMIS Pediatric Global Health measure	Remote patient report (electronic, paper or telephone assessments)	Baseline assessment

(continued)

20.4 Appendix D: Tier 1: Data Elements (continued)

Information collected	Description	Source	Interval
Caregiver Symptoms at the Time of Assessment (Acute symptoms and post-acute symptoms)	Cardiac - Chest pain, tightness, pressure, ache, heaviness, radiating pain. Cardiac - Palpitations, racing heart, arrhythmia, skipped beats Cardiac- Swelling of the lower leg Cardiac- Lightheadedness, dizziness, vertigo, spinning sensation, passing out, or feeling like you will pass out when standing Dermatology- Rash, hair loss Endocrinology- Excessive thirst General Medicine- Fatigue question in PROMIS 10 and 4-item PROMIS fatigue scale General Medicine-Post-exertional malaise, Pain scale in PROMIS 10 General Medicine- Disturbances in sleep (sleeping less, difficulty falling or staying asleep, early awakening, sleeping more, sleepy during the day). General Medicine – Pain anywhere in the body (muscle pain, headache, chest pain, pelvic pain, abdominal pain, joint pain, nerve pain, sore throat, reflux, tooth pain) General Medicine – Weakness GI- Digestion problems (loss of appetite, nausea, early satiety, diarrhea, constipation, reflux). HEENT- Vision problems (light sensitivity, blurry vision, dry eyes, itchy eyes, floaters, difficulty reading, seeing spots, stars, lines, flashing lights, zigzag lines, heat waves, or tiny dots on a gray, white, or black background sometimes referred to as "snow"), Loss or change of smell or taste, Tooth problems (pain, loose teeth, easy bleeding), Problems hearing (hearing loss, ringing in ears) Neuro- Problems thinking (Brain fog, difficulty concentrating, memory loss, attention difficulty, trouble finding the right words, difficulty problem solving, difficulty processing written text, difficulty comprehending speech), Problems with mood (anxiety, stress, depression, irritability, anger, apathy, lability, sense of doom), New psychosis, hallucinations, delirium, nightmares or vivid dreams, Numbness, tingling, burning, electric shock feelings, Tremor, abnormal movements or movement disorder, Seizures, staring spells, lost time, loss of consciousness with jerking of limbs, Can’t move and/or feel one side of body or face, Muscle weakness such as Trouble going up the stairs, brushing hair, getting up from a chair, difficulty opening a jar or hand weakness, etc. Pulmonary- Shortness of breath (at rest, while lying down, standing, or with exertion). Pulmonary – cough Pulmonary – wheeze. Reproductive- Changes to menstrual cycle (new irregularity, heavier), Vaginal symptoms (itch, discharge).	Remote patient report (electronic, paper or telephone assessments)	Baseline assessment

(continued)

20.4 Appendix D: Tier 1: Data Elements (continued)

Information collected	Description	Source	Interval
Child Symptoms at the Time of Assessment (Acute symptoms and post-acute symptoms)	ISARIC-WHO Health and Wellness Survey for Children and Young People: Assess symptoms: Nasal Congestion, Trouble breathing, Pain when breathing, Chest pain, Palpitations/heart racing, Dizziness/lightheadedness, Fainting, Change in hearing/ringing in ears, Blurred vision, Change in smell, Change in taste, Problems with teeth or gums, Tremors/shakiness, Feeling off-balance or unsteady, Feeling tingling or "pins and needles", Seizures/fits, Muscle weakness, Difficulty sleeping, Excessive sleepiness, Fatigue/Low energy, Feeling exhausted after walking, Poor appetite, Stomach pains/cramps, Nausea, Vomiting, Diarrhea, Constipation, Problems with urination, Skin rash, Problems with memory, Problems with concentration, Speech difficulty, Anxiety, Depression, Body pain, Headache, Problems swallowing or chewing, Change in menstruation Assess “Compared to before your Covid-19 illness, how much are you now doing/experiencing the following: eating, sleeping, physical activity, fatigue, spending time with friends in-person, spending time with friends remotely, spending time on screens, etc.”	Remote patient report (electronic, paper or telephone assessments)	Baseline assessment
Tier 1: Biospecimens			
COVID Antibody testing	Clinical lab testing: SARS-CoV-2 antibody (nucleocapsid and spike)	Remote Dried Blood Spot	Baseline assessment
Caregiver and Child Biological Samples	Blood for biobanking	Remote serum collection	Baseline assessment (one tube for each biological parent and one tube for child)
Caregiver and Child Biological Samples	Saliva for biobanking	Saliva for biobanking	Baseline assessment (one tube for caregiver and one tube for child)
Optional collection for other biological parent	Saliva for biobanking		Baseline assessment (one tube for other biological parent)

20.5 Appendix E: Tier 2 and 3: Core Data Elements

Information collected	Description	Source	Interval
Tier 2 – acute remote assessments			
Change in Child Other History	Change in Medications, Change in COVID vaccination status and date.	Remote patient report, in-person patient report, EHR	Weeks 1,2,3,4,8
Change in Child SARS-CoV-2 Treatment Record	Treatment option (Treated with corticosteroids, Treated with hydroxychloroquine, Treated with lopinavir, ritonavir, other antiviral, Treated with monoclonal antibody, Treated with therapeutic anticoagulation, Treated with antibiotics)	Remote patient report, in-person patient report, EHR	Weeks 1,2,3,4,8
Change in Child Symptoms at the Time of Assessment	ISARIC-WHO Health and Wellness Survey for Children and Young People: Assess symptoms: Nasal Congestion, Trouble breathing, Pain when breathing, Chest pain, Palpitations/heart racing, Dizziness/lightheadedness, Fainting, Change in hearing/ringing in ears, Blurred vision, Change in smell, Change in taste, Problems with teeth or gums, Tremors/shakiness, Feeling off-balance or unsteady, Feeling tingling or "pins and needles", Seizures/fits, Muscle weakness, Difficulty sleeping, Excessive sleepiness, Fatigue/Low energy, Feeling exhausted after walking, Poor appetite, Stomach pains/cramps, Nausea, Vomiting, Diarrhea, Constipation, Problems with urination, Skin rash, Problems with memory, Problems with concentration, Speech difficulty, Anxiety, Depression, Body pain, Headache, Problems swallowing or chewing, Change in menstruation Assess “Compared to before your Covid-19 illness, how much are you now doing/experiencing the following: eating, sleeping, physical activity, fatigue, spending time with friends in-person, spending time with friends remotely, spending time on screens, etc.”	Remote patient report, in-person patient report, EHR	Weeks 1,2,3,4,8
Pulse oximeter	Remote collection to assess oxygen saturation and heart rate	Remote assessment with device mailed to home	Baseline with diary with daily measurements for one week, weekly measurements for 1 month, and additional measurements at the time of reported symptoms of palpitations or shortness of breath.
Tier 2 acute in-person assessments			
Medical History Child: General	Anthropometry: weight, length/height, head circumference (<2 years), skin fold thickness (<2 years)	In-person direct assessment	Week 8
Medical History Child: General	Seated Blood Pressure	In-person direct assessment	Week 8
Medical History Child: General	Oxygen Saturation	In-person direct assessment	Week 8
Medical History Child: General	Resting Heart Rate	In-person direct assessment	Week 8

(continued)

20.5 Appendix E: Tier 2 and 3: Core Data Elements (continued)

Information collected	Description	Source	Interval
Medical History Child: General	Resting Respiratory Rate	In-person direct assessment	Week 8
Medical History Child: Lung	Lung Function: Spirometry	In-person direct assessment	Week 8
Medical History: Child: Cardio	Electrocardiogram	In-person direct assessment	Week 8
Tier 2 – acute biological samples for testing and biobanking			
Biological Samples (age adjusted volume of phlebotomy)	Plasma for RECOVER biorepository Serum for RECOVER biorepository PBMC for RECOVER biorepository	In-person direct assessment or remote	Week 8
Tier 2 – post-acute remote assessments			
Change in Caregiver Other History	Change in Medications, Change in COVID vaccination status and date.	Remote patient report, in-person patient report, EHR	At months 12, 24, 36, and 48 months
Change in Child Other History	Change in Medications, Change in COVID vaccination status and date.	Remote patient report, in-person patient report, EHR	At months 6, 12, 24, 36, and 48 months
Household SDOH	Address stability (2 questions: Where was your address 12 months ago? How long have you lived at your current address?) Financial stability (Weekly Stress Inventory 5 Question COVID-19 Related Household Finances), Annual family income from PhenX Employment- RadxUp Food insecurity (Hunger Vital Signs (Update) based on USDA) Access to health care (Adult Access to Health Care & Utilization Module, Question on Insurance from US Census, Since being diagnosed with COVID have you lost or experienced any changes with your health insurance (do not recommend using "diagnosed" consider "symptoms" or "becoming ill") Perceived discrimination (Major Experiences [everyday subset #10-19] and Everyday Discrimination Scale with 1 Follow Up Question) Discrimination impacting likeliness to access care- Was there ever a time when you would have received better medical care if you belonged to a different race/ethnic/identity group? Stress- Perceived stress scale. Social support - RAND MOS	Remote patient report, in-person patient report	At months 12, 24, 36, and 48 months
Change in Caregiver SARS CoV-2 Treatment Record	Treatment option (Treated with corticosteroids, Treated with hydroxychloroquine, Treated with lopinavir, ritonavir, other antiviral, Treated with monoclonal antibody, Treated with therapeutic anticoagulation, Treated with antibiotics)	Remote patient report, in-person patient report, EHR	At months 12, 24, 36, and 48 months
Change in Child SARS CoV-2 Treatment Record	Treatment option (Treated with corticosteroids, Treated with hydroxychloroquine, Treated with lopinavir, ritonavir, other antiviral, Treated with monoclonal antibody, Treated with therapeutic anticoagulation, Treated with antibiotics)	Remote patient report, in-person patient report, EHR	At months 6, 12, 24, 36, and 48 months
Caregiver Health Status	Quality of life-PROMIS-10	Remote patient report, in-person patient report	At months 12, 24, 36, and 48 months

(continued)

20.5 Appendix E: Tier 2 and 3: Core Data Elements (continued)

Information collected	Description	Source	Interval
Child Health/Wellbeing Status	PROMIS Pediatric Global Health measure; PROMIS Life Satisfaction; PROMIS Meaning and Purpose; PROMIS Social Isolation; PROMIS Family Relationships	Remote patient report, in-person patient report	At months 6, 12, 24, 36, and 48 months
Child Physical Activity	Child 1 to 5 years: PROMIS – Physical Activity Early Childhood Parent Report Child 5 to 7 years: PROMIS- Physical Activity - Parent Proxy/CAT Child 8+ years: PROMIS - Physical Activity Pediatric/CAT	Remote patient report, in-person patient report	At months 6, 12, 24, 36, and 48 months
Child Sleep	Brief infant Sleep Questionnaire (infancy); PROMIS Sleep-Related Disturbance	Remote patient report, in-person patient report	At months 6, 12, 24, 36, and 48 months
Child diet	Dietary Questionnaire	Remote patient report, in-person patient report	At months 6, 12, 24, 36, and 48 months
Change in Caregiver Symptoms at the Time of Assessment	Cardiac - Chest pain, tightness, pressure, ache, heaviness, radiating pain. If yes, Seattle Angina Questionnaire-7. Also, if yes, ask about chest pain when you breathe Cardiac - Palpitations, racing heart, arrhythmia, skipped beats Cardiac- Swelling of the lower leg. If yes, unilateral or bilateral to branch to different Tier 2 (e.g., for DVT vs heart failure) Cardiac- Lightheadedness, dizziness, vertigo, spinning sensation, passing out, or feeling like you will pass out when standing - If Y, COMPASS-31 for dysautonomia Dermatology- Rash, hair loss Endocrinology- Excessive thirst General Medicine- Fatigue question in PROMIS 10. If anything but "None" then 4 item PROMIS fatigue scale General Medicine-Post-exertional malaise, Pain scale in PROMIS 10 General Medicine- Disturbances in sleep (sleeping less, difficulty falling or staying asleep, early awakening, sleeping more, sleepy during the day). If yes, the PROMIS questionnaire included in the global protocol can use the Sleep Disturbance and Fatigue domains of PROMIS-57 Profile v2.1. If Promis not used, suggest the 8-item Epworth Sleepiness Scale (ESS) and 7-item Insomnia Severity Index (ISI) surveys General Medicine- Pain anywhere in the body (muscle pain, headache, chest pain, pelvic pain, abdominal pain, joint pain, nerve pain, sore throat, reflux, tooth pain), - If Y, split out to individual symptoms; if yes to chest pain, Seattle Angina Questionnaire-7; if Y to headache, HIT-6	Remote patient report, in-person patient report, EHR	At months 12, 24, 36, and 48 months

(continued)

20.5 Appendix E: Tier 2 and 3: Core Data Elements (continued)

Information collected	Description	Source	Interval
Change in Caregiver Symptoms at the Time of Assessment (continued)	General Medicine – Weakness - if Y, neuroqol upper and lower weakness, Fever, chills, sweats, flushing, Have you been observed to stop breathing during sleep GI- Digestion problems (loss of appetite, nausea, early satiety, diarrhea, constipation, reflux). If Y, then expand to specific questions and refer to fecal sample collection, gastric emptying study, ab CT depending on specific symptom HEENT- Vision problems (light sensitivity, blurry vision, dry eyes, itchy eyes, floaters, difficulty reading, seeing spots, stars, lines, flashing lights, zigzag lines, heat waves, or tiny dots on a gray, white, or black background sometimes referred to as "snow"), Loss or change of smell or taste, Tooth problems (pain, loose teeth, easy bleeding), Problems hearing (hearing loss, ringing in ears) Neuro- Problems thinking (Brain fog, difficulty concentrating, memory loss, attention difficulty, trouble finding the right words, difficulty problem solving, difficulty processing written text, difficulty comprehending speech), Problems with mood (anxiety, stress, depression, irritability, anger, apathy, lability, sense of doom), New psychosis, hallucinations, delirium, nightmares or vivid dreams, Numbness, tingling, burning, electric shock feelings, Tremor, abnormal movements or movement disorder, Seizures, staring spells, lost time, loss of consciousness with jerking of limbs, Can't move and/or feel one side of body or face, Muscle weakness such as Trouble going up the stairs, brushing hair, getting up from a chair, difficulty opening a jar or hand weakness, etc. Pulmonary- Shortness of breath (at rest, while lying down, standing, or with exertion). If yes, FACIT-DYSPNEA 10-item short form (mMRC 4 dyspnea survey is alternative if need shorter questionnaire). Also, if yes, ask separately about SOB while lying down Pulmonary – cough. If yes, mMRC chronic bronchitis assessment Pulmonary – wheeze. Reproductive- Changes to menstrual cycle (new irregularity, heavier), Vaginal symptoms (itch, discharge).		

(continued)

20.5 Appendix E: Tier 2 and 3: Core Data Elements (continued)

Information collected	Description	Source	Interval
Change in Child Symptoms at the Time of Assessment	ISARIC-WHO Health and Wellness Survey for Children and Young People: Assess symptoms: Nasal Congestion, Trouble breathing, Pain when breathing, Chest pain, Palpitations/heart racing, Dizziness/lightheadedness, Fainting, Change in hearing/ringing in ears, Blurred vision, Change in smell, Change in taste, Problems with teeth or gums, Tremors/shakiness, Feeling off-balance or unsteady, Feeling tingling or "pins and needles", Seizures/fits, Muscle weakness, Difficulty sleeping, Excessive sleepiness, Fatigue/Low energy, Feeling exhausted after walking, Poor appetite, Stomach pains/cramps, Nausea, Vomiting, Diarrhea, Constipation, Problems with urination, Skin rash, Problems with memory, Problems with concentration, Speech difficulty, Anxiety, Depression, Body pain, Headache, Problems swallowing or chewing, Change in menstruation Assess “Compared to before your Covid-19 illness, how much are you now doing/experiencing the following: eating, sleeping, physical activity, fatigue, spending time with friends in-person, spending time with friends remotely, spending time on screens, etc.”	Remote patient report, in-person patient report, EHR	At months 6, 12, 24, 36, and 48 months
Child health symptoms	PROMIS asthma impact; PROMIS fatigue		At months 6, 12, 24, 36, and 48 months
Tier 2: In person assessments			
Medical History Child: General	Anthropometry: weight, length/height, head circumference (<2 years)	In-person direct assessment	At months 6, 12, 24, 36, and 48 months
Medical History Child: General	Blood Pressure; Orthostatic assessment	In-person direct assessment	At months 6, 12, 24, 36, and 48 months
Medical History Child: General	Oxygen Saturation	In-person direct assessment	At months 6, 12, 24, 36, and 48 months
Medical History Child: General	Resting Heart Rate	In-person direct assessment	At months 6, 12, 24, 36, and 48 months
Medical History Child: General	Respiratory Rate	In-person direct assessment	At months 6, 12, 24, 36, and 48 months
Medical History Child: General	Oral health/dental health survey	In-person direct assessment	At months 6, 12, 24, 36, and 48 months
Medical History Child: Lung	Lung Function: Spirometry	In-person direct assessment	At months 6, 12, 24, 36, and 48 months
Medical History: Child: Cardio	ECG	In-person direct assessment	At months 6, 12, 24, 36, and 48 months
Medical History: Child: Cardio	Autonomic Function: Compass-31 autonomic survey	In-person direct assessment	At months 6, 12, 24, 36, and 48 months
Medical History: Child:	Beighton scale joint flexibility	In-person direct assessment	At months 6, 12, 24, 36, and 48 months
Tier 2: post-acute Biological samples for testing and biobanking			
Biological Samples (age adjusted volume of phlebotomy)	Blood: Complete blood count + differential; Chemistry panel; AST, ALT, Total Bili, Direct Bili, Alk Phos, GGT; Lipids: LDL, HDL, total, triglycerides; Hgb A1c; TSH/FT4; 25-hydroxyvitamin D; Calcium; ANA, anti-CCP, anti dsDNA, RF; EBV; SARS-CoV-2 antibody (nucleocapsid and spike)	In-person direct assessment	At first Tier 2 visit

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20.5 Appendix E: Tier 2 and 3: Core Data Elements (continued)

Information collected	Description	Source	Interval
Biological Samples (age adjusted volume of phlebotomy)	SARS-CoV-2 antibody (nucleocapsid and spike)	In-person direct assessment	At months 6, 12, 24, 36, and 48 months
Biological Samples (age adjusted volume of phlebotomy)	Plasma for RECOVER biorepository Serum for RECOVER biorepository PBMC for RECOVER biorepository	In-person direct assessment	At months 6, 12, 24, 36, and 48 months
Tier 2: Neurological/child developmental assessments – training for use of the ASQ-3 and Promis measures is required, but not by a special professional			
Education status	Academic performance/ achievement: Used ASEBA initial questions contained on the Preschool CBCL and the CBCL* *cost associated with this measure - also used for behavior problems below – ages 1.5 to 18 covered	In-person direct assessment	At months 12, 24, 36, and 48 months
Education status	School attendance/absence: Used ASEBA initial questions contained on the Preschool CBCL and the CBCL* *cost associated with this measure -also used for behavior problems below – ages 1.5 to 18 covered	In-person direct assessment	At months 12, 24, 36, and 48 months
Education status	Grade level: Used ASEBA initial questions contained on the Preschool CBCL and the CBCL* *cost associated with this measure -also used for behavior problems below – ages 1.5 to 18 covered	In-person direct assessment	Every 12 months
Education status	Accommodations (IEP, 504): Used ASEBA initial questions contained on the Preschool CBCL and the CBCL* *cost associated with this measure -also used for behavior problems below – ages 1.5 to 18 covered	In-person direct assessment	At months 12, 24, 36, and 48 months
Tier 2: Neurological/child developmental assessments: Ages 5 and under			
Development-Cognitive: – 5 and under.	General: ASQ-3 for child age <6 years; National Survey of Children's Health (At 12, 18 and 24 months) Parts A & B; Developmental Profile-4 (36, 48 and 60 months) Domains tapped: Communication and Problem solving	In-person direct assessment	At months 6, 12, 24, 36, and 48
Development – Cognitive: – 5 and under.	Motor: ASQ-3 for child age < 6 years: two questions asking about any mobility or coordination issues; Neuro-QOL measures of lower extremity mobility and upper body fine motor and ADL Domains tapped: Gross motor and fine motor	In-person direct assessment	At months 6, 12, 24, 36, and 48
Autism	Modified Checklist for Autism in Toddlers (M-CHAT): At 18 months only	In-person direct assessment	Once at 18 months old
Tier 2: Neurological/child developmental assessments: Ages 3 to 18 – NIH Toolbox- Promis Measures			
Development-Cognitive: Attention and Executive Function	PROMIS Attention and Executive Function	In-person direct assessment	At months 6, 12, 24, 36, and 48
Development-Cognitive: Episodic Memory	PROMIS Episodic Memory	In-person direct assessment	At months 6, 12, 24, 36, and 48

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20.5 Appendix E: Tier 2 and 3: Core Data Elements (continued)

Information collected	Description	Source	Interval
Development-Cognitive: Working Memory	PROMIS Working Memory – 7 years or more only	In-person direct assessment	At months 6, 12, 24, 36, and 48
Development-Cognitive-Language	PROMIS Language	In-person direct assessment	At months 6, 12, 24, 36, and 48
Development-Cognitive-Executive Function and Attention	PROMIS Executive Function and Attention	In-person direct assessment	At months 6, 12, 24, 36, and 48
Development-Cognitive-Processing Speed	PROMIS Processing Speed	In-person direct assessment	At months 6, 12, 24, 36, and 48
Development-Cognitive-Memory	PROMIS Immediate Recall – 8 years or more only	In-person direct assessment	At months 6, 12, 24, 36, and 48
Development-Emotional/mental health	Broad screener: Ages and Stages Questionnaire Social Emotional-2nd edition (ASQ-SE-2)	In-person direct assessment	At months 6, 12, 24, 36, and 48
Development-Emotional/mental health	Depression: PROMIS Emotional Distress – Depressive Symptoms (Short form)	In-person direct assessment	At months 6, 12, 24, 36, and 48
Development-Emotional/mental health	Anxiety: PROMIS Emotional Distress – Anxiety (Short form)	In-person direct assessment	At months 6, 12, 24, 36, and 48
Development-Emotional/mental health	Perceived Stress: PROMIS Psychological Stress Experiences (Short form)	In-person direct assessment	At months 6, 12, 24, 36, and 48
Development-Emotional/mental health	Behavior: Child Behavior Checklist for Behavioral Problems;	In-person direct assessment	At months 6, 12, 24, 36, and 48
Development-Emotional/mental health	Positive Affect: PROMIS positive affect (Short form)	In-person direct assessment	At months 6, 12, 24, 36, and 48
Development-Emotional/mental health	Irritability/Anger: PROMIS Emotional Distress – Anger (Short form)	In-person direct assessment	At months 6, 12, 24, 36, and 48
Tier 3 assessments: These assessments will require specialized personnel.			
Medical History: Child: Cardio	Resting transthoracic echocardiography + strain imaging	In-person direct assessment	Months 3-15; repeat once Months 15-27 about one year later
Medical History: Child: Cardio	Cardiac MRI	In-person direct assessment	Months 3-15; repeat once Months 15-27 about one year later
Medical History: Child: Cardio	Cardiopulmonary Exercise Testing	In-person direct assessment	Months 3-15; repeat once Months 15-27 about one year later
Medical History: Child: GI	Abdominal ultrasound: liver, pancreas and kidneys	In-person direct assessment	Months 3-15; repeat once Months 15-27 about one year later
Medical History: Child: Pulm	PFTs including diffusion capacity	In-person direct assessment	Months 3-15; repeat once Months 15-27 about one year later
Medical History: Child: Pulm	Sputum induction procedure	In-person direct assessment	Months 3-15; repeat once Months 15-27 about one year later

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20.5 Appendix E: Tier 2 and 3: Core Data Elements (continued)

Information collected	Description	Source	Interval
Neurodevelopment	Low Field Brain MRI	In-person direct assessment	Months 3-15; repeat once Months 15-27 about one year later
Neurodevelopment	Awake EEG/ERP	In-person direct assessment	Months 3-15; repeat once Months 15-27 about one year later
Tier 3: These neurodevelopment assessments below will require a psychometrician and a supervising psychologist			
Neurocognitive	Testing protocol as detailed in the protocol	In-person direct assessment	Months 3-15; repeat once Months 15-27 about one year later
Tier 3 biospecimen collection			
Biological Samples (age adjusted volume of phlebotomy)	Blood: Complete blood count + differential; Chemistry panel; AST, ALT, Total Bili, Direct Bili, Alk Phos, GGT; Lipids: LDL, HDL, total, triglycerides; Hgb A1c: D-Dimer, Troponin, hs-CRP, NT-proBNP, procalcitonin, insulin C-peptide	In-person direct assessment	Months 3-15; repeat once Months 15-27 about one year later
Biological Samples	Microbiome: Urine, stool, skin swab, oral swab, nasal swab, sputum	In-person direct assessment	Months 3-15; repeat once Months 15-27 about one year later

20.6 Appendix F: Appendix E. List of Study Sites

A list of all study sites will be maintained on file as an administrative attachment with the NYU sIRB.

21 References

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