Title:	**Contrast Enhanced Ultrasound (CEUS) Detection of Microvascular Perfusion Impairment in COVID-19 Pediatric Patients**
Short Title	CEUS in COVID-19
Drug Name(s):	Sulfur hexafluoride lipid-type A microspheres (Lumason™)
Regulatory Sponsor:	Misun Hwang, MD
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Sponsor-Investigator

Misun Hwang, MD

The Children's Hospital of Philadelphia

Department of Radiology
Philadelphia, PA, 19104
Phone 267.425.7110
email: Hwangm@email.chop.edu

TABLE OF CONTENTS

ABBREVIATIONS AND DEFINITIONS OF TERMS

AE	Adverse Event
Category B Drug	Animal reproduction studies have failed to demonstrate a risk to the fetus and there are no adequate and well-controlled studies in pregnant women
CEUS	Contrast-Enhanced Ultrasound
CFR	Code of Federal Regulations
CHOP	Children's Hospital of Philadelphia
ED	Emergency Department
FDA	Food and Drug Administration
HIPAA	Health Insurance Portability and Accountability Act
ICU	Intensive Care Unit
IV	Intravenous
IRB	Institutional Review Board
Lumason™	FDA-approved for contrast-enhanced ultrasound; the investigational drug
MRI	Magnetic Resonance Imaging
ORC	The CHOP Office of Research Compliance
PI	Principal Investigator
SAE	Serious Adverse Event
US	Conventional Ultrasound Scan
PACS	Picture Archiving and Communication System

ABSTRACT

Context:

Initial data from COVID-19 patients in China and Italy suggests that one of the primary causes of death is significant endothelial injury leading to blood clotting and impaired multiorgan microvascular perfusion. This pilot study uses a safe, convenient bedside imaging tool called contrast-enhanced ultrasound (CEUS) to estimate the extent of microvascular perfusion impairment in the heart, kidneys, and brain of COVID-19 pediatric patients and assess the significance of imaging findings by correlating to clinical outcomes.

Objectives:

Primary: Assess microvascular perfusion of the heart, kidneys and/or brain using CEUS in patients with confirmed or probable diagnosis of COVID-19.

Secondary: Correlate CEUS measures of microvascular perfusion of the heart, kidneys and/or brain to clinical outcomes.

Study Design:

Cross-sectional observational study.

Setting/Participants:

Pediatric subjects at the Children's Hospital of Philadelphia. This is a pilot study with a projected recruitment of 30 children with confirmed or probable diagnosis of COVID-19 at CHOP.

Study Interventions and Measures:

Contrast-enhanced ultrasound scan with a duration of approximately 15 minutes will be performed when a COVID-19 diagnosis has been made (or is highly suspected) according to established clinical procedures. One CEUS will be performed per patient, with up to 2 intravenous injections of the contrast agent. The dosing plan will be weight-adjusted, based on a dose of 0.03 mL/kg (with a maximum dose of 2.4 mL per injection). Organ perfusion will be evaluated in the heart, kidneys, and/or brain. Clinical outcomes during hospital stay will be collected for correlation to CEUS-based measures.

PROTOCOL SYNOPSIS

Study Title	**Contrast Enhanced Ultrasound (CEUS) Detection of Microvascular Perfusion Impairment in COVID-19 Pediatric Patients**
Funder	Children's Hospital of Philadelphia
Clinical Phase	Pilot study.
Study Rationale	Initial data from COVID-19 patients in China and Italy suggests that one of the primary causes of death is significant endothelial injury leading to blood clotting and impaired multiorgan microvascular perfusion. This pilot study uses a safe, convenient bedside imaging tool called contrast-enhanced ultrasound (CEUS) to estimate the extent of microvascular perfusion impairment in the heart, kidneys and brain of COVID-19 pediatric patients and assess the significance of imaging findings by correlating to clinical outcomes.
Study objectives	Primary: Assess microvascular perfusion of the heart, kidneys and/or brain using CEUS in patients with confirmed or probable diagnosis of COVID-19. Secondary: Correlate CEUS measures of microvascular perfusion of the heart, kidneys and/or brain to clinical outcomes.
Test Article(s) *(If Applicable)*	Sulfur hexafluoride lipid-type A microspheres (Lumason™, Bracco Inc) is an FDA-approved ultrasound contrast agent which consists of active ingredients including Sulfur hexafluoride (strength 60.7 mg in 1 mg), Distearoylphosphatidylcholine, DL- (strength 0.19 mg in 1 mg), 1,2-Dipalmitoyl-Sn-Glycero-3-Phospho-(1'-Rac- Glycerol), Sodium Salt (0.19 mg in 1 mg). Inactive ingredients include Polyethylene Glycol 4000 (strength 24.56 mg in 1 mg) and Palmitic Acid (0.04 mg in 1 mg). The Sulfur hexafluoride lipid microspheres are composed of SF_6 (molecular weight 145.9) gas in the core surrounded by an outer shell monolayer of phospholipids consisting of 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC) and 1,2-Dipalmitoyl-sn-glycero-3-phosphoglycerol, sodium salt (DPPG-Na) with palmitic acid as stabilizer. Sulfur hexafluoride lipid-type A microspheres fall under Category B, that is, animal reproduction studies have failed to demonstrate a risk to the fetus and there are no adequate and well-controlled studies in pregnant women.1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC), with empirical formula C44H88NO8P, has a molecular weight of 790.6. 1,2-Dipalmitoyl-sn-glycero-3-phospho-rac-glycerol sodium (DPPG-Na),

	with empirical formula C38H74 NaO10P, has a molecular weight of 745.
Study Design	Cross-sectional observational study.
Subject Population key criteria for Inclusion and Exclusion:	**Inclusion Criteria** 1. Patient (male/female) hospitalized at CHOP. 2. Patient 17 years of age or younger. 3. Diagnosis of COVID-19 or high clinical suspicion for COVID-19 despite negative tests (according to the definition of probable case by the ECDC). 4. Patients have evidence of cardiovascular compromise, myocardial injury, acute kidney injury, and/or new-onset neurological symptoms. 5. Parental/guardian permission (informed consent) **Exclusion Criteria** 1. Medical history of Lumason hypersensitivity
Number of Subjects	Total recruitment: 30 patients with confirmed or probable diagnosis of COVID-19.
Study Duration	CEUS has a total duration of approximately 15 minutes including contrast agent injection and image completion.
Study Visit	Subjects will be imaged at bedside at CHOP for approximately 15 minutes.
Efficacy Evaluations	CEUS scans of diagnostic quality, qualitative/quantitative evaluation of microvascular perfusion of heart, kidneys and/or brain in COVID-19 patients, correlation between CEUS and clinical outcomes of COVID-19.
Pharmacokinetic Evaluations	There are no pharmacokinetic evaluations.
Safety Evaluations	All subjects receiving at least one injection of investigational drug will be included in the safety analysis. The frequencies of AEs by type, severity, and temporal relationship to the CEUS scan will be summarized. SAEs (if any) will be described in detail.
Statistical and Analytic Plan	This is a pilot study with a projected recruitment of 30 children with COVID-19. See Section 5.3 for further detail.

DATA AND SAFETY MONITORING PLAN	The safety monitoring for this study is the primary responsibility of the study team. Regular meetings to discuss the outcomes of the study, and of the safety events, will be conducted by the study team. The occurrence of adverse events, serious adverse events and unanticipated events will be reported by the study team in accordance with federal and institutional guidelines, as outlined in Section 7 of this clinical study.

1 BACKGROUND INFORMATION AND RATIONALE

1.1 Introduction

Initial data from COVID-19 patients in China and Italy suggests that one of the primary causes of death is significant endothelial injury leading to blood clotting and impaired multiorgan microvascular perfusion. Cardiovascular complications can arise in both patients with or without prior cardiovascular history. High cardiac troponin levels are associated with severe COVID-19. It has been postulated that direct viral effects as well as secondary inflammatory response can predispose to thromboembolic diseases. Therefore, thrombotic events affecting target organs, such as the heart and kidneys, are common (1). One of the most recently reported complications of COVID-19 infection in children is known as Multisystem Inflammatory Syndrome in Children (MIS-C). Key findings of this syndrome include prominent cardiac dysfunction, enteropathy, and relative thrombocytopenia. Those patients frequently present with cardiogenic shock or acute left ventricular dysfunction in the setting of a multisystem inflammatory state (*1*). Similarly, patients with COVID-19 also present with neurological symptoms. Anosmia and ageusia are very common in adults but not very well elucidated in children. Abdel-Mannan et al (*2*) reported 4 cases of children without respiratory symptoms and new-onset neurological symptoms including encephalopathy, headaches, brainstem and cerebellar signs, muscle weakness, and reduced reflexes. Those patients had signal changes in the corpus callosum. There have been other cases reporting encephalopathy, encephalitis and cerebrovascular disease in COVID-19 patients (*3*).

The current study uses a safe, convenient bedside imaging tool called contrast-enhanced ultrasound (CEUS) to measure the extent of microvascular perfusion impairment in the heart and kidneys of COVID-19 pediatric patients and, as exploratory analysis, to assess the significance of imaging findings by correlating to clinical outcomes. To the investigator's knowledge, this is the first study evaluating microvascular perfusion of the heart and kidneys in COVID-19 pediatric patients.

The completion of this initial study will be important to obtain preliminary descriptive statistics which includes means, standard deviations, medians, minimum maximum and the 95% confidence intervals (CI) for continuous variables. Frequencies, proportions, and the 95% CIs necessary for designing a future trial. In the future, validation of such microvascular perfusion based biomarker for prognostication of severe COVID-19 related complications such as death, mechanical ventilation, myocardial infarction, stroke or renal failure will be tremendously helpful. In addition, the presence of an easily attainable *in vivo* biomarker of disease will help with resource allocation in the ICU when faced with limited supplies and staff. Whereas computed tomography and magnetic resonance imaging contrast agents can potentially be nephrotoxic, ultrasound contrast agents are not cleared through the kidneys and can be used for patients with renal failure, which is prevalent in COVID-19. CEUS can also be performed at the bedside at low cost, without the need to transport patients to CT or MRI suite, which reduces infection risk and provides real-time, rapid decision-making for clinicians.

According to the European Centre for Disease Prevention and Control (ECDC), COVID-19 cases can be classified as possible, probable, or confirmed. A case is possible if the person

meets the clinical criteria (at least one of the following symptoms: cough, fever, shortness of breath, or sudden onset anosmia, ageusia/dysgeusia). A case is probable if the person meets the clinical criteria with an epidemiological link or if the person meets the diagnostic criteria (radiologic evidence showing lesions compatible with COVID-19. A case is confirmed if the person meets the laboratory criteria (detection of SARS-CoV-2 nucleic acid in a clinical specimen). It is noteworthy that test accuracy highly depends on specimen collection by swabs among other variables (including intrinsic test accuracy). For that reason, patients with high clinical suspicion (probable cases as per the ECDC definition) for COVID-19 despite negative tests will be considered as subjects in our study (*4*).

Microvascular Injury in COVID-19

Initial data from COVID-19 patients demonstrates that poor outcomes from the disease is primarily due to hypercoagulability and multiorgan microvascular perfusion impairment (*5-7*). Whereas pulmonary symptoms of COVID-19 are well known, less publicized are widespread incidence of multiorgan failure due to blood clots which are as common as pulmonary disease in deceased patients (*5-10*). An international collaborative review was recently presented to outline the pathogenesis, epidemiology, and management of COVID-19 patients with thrombotic events *(9)*. Other studies have reported the biochemical mechanisms that trigger thromboembolic diseases *(10, 11)*.

The novel coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus (SARS-CoV)-2 utilizes angiotensin-converting enzyme 2 (ACE2) as the host cellular receptor for virus spike (S) protein according to structural analysis and *in vivo* experiments (*11*). It has been shown that the intestine displays the highest expression of ACE2, followed by testis and kidney which explains viral detection in feces and urine. The expression of ACE2 in the heart is lower than that in the intestine and kidney, but higher than that in the lung which serves as a main target organ of this virus, indicating a potential infection susceptibility of the human heart. Epidemiological data indicates that, over two-thirds of patients who died from COVID-19 had diabetes or cardiovascular disease (*12*), potentially due to the preexisting vascular disease with enhanced vulnerability to COVID-19 related vascular insult.

In blood samples of COVID-19 positive patients, increased coagulation factors are commonly observed. In a cohort of 799 patients from China, marked derangement in coagulation factors was commonly observed in 113 deceased patients (*5*). Median prothrombin time was significantly longer in deceased patients (15.5) than in recovered patients (13.9). D-dimer concentrations were markedly greater in deceased patients (4.6 μg/mL) than in recovered patients (0.6 μg/mL). In addition, concentrations of procalcitonin, high sensitivity C-reactive protein, and ferritin, as well as erythrocyte sedimentation rate, were significantly higher in deceased patients than in recovered patients. A very recent study by Yao et al. (*7*) (published online ahead of print) confirms that SARS-CoV-2 infection, in addition to the lungs, damages vessels of the kidney and other organs, and hyaline thrombi were found in small vessels in different organs. Interestingly, Middle East respiratory syndrome (MERS) which first emerged on the Saudi Arabian peninsula in 2012 caused by MERS coronavirus (MERS-CoV) was also shown to cause microvascular thrombosis (*9*), suggesting the possible generalizability of using microvascular perfusion as a marker of disease burden, prognosis, and therapy guidance in viral outbreaks/pandemics.

Beyond the pulmonary involvement, the most commonly reported co-morbidities in COVID-19 include acute kidney injury (AKI), cardiac damage and abdominal pain likely due to the tropism of SARS-CoV-2 for these organs given the high affinity of SARS-CoV-2 to ACE2 in the kidney (*13*). A recent study reported that the human kidney is a specific target for SARS-CoV-2 infection (*9*). Diao and colleagues (*9, 10*) after examining the viral nucleocapsid protein *in situ* in the kidney post-mortem found that SARS-CoV-2 antigens accumulated in kidney tubules, suggesting the possibility of direct viral infection of the kidney prior to inducing AKI. CT scan of the kidneys showed reduced density, suggestive of inflammation and edema (*14*). Cardiac involvement of the disease in COVID-19 patients of poor outcomes has also been documented, with findings ranging from angina, arrhythmia, to acute cardiac injury (*6, 15*). Note that in the heart, ACE2 was found to be highly expressed in pericytes, which are distributed on the abluminal sides of endothelial cell of capillaries and part of venules, and play a critical role in myocardial microcirculation (*6*).

Recent histopathological evidence from COVID-19 autopsy cases suggests that vascular coagulopathy is the predominant finding in the lungs, heart, and kidneys. Worsening respiratory failure is increasingly believed to be caused by pulmonary microvascular thrombosis (*16*) (Figure 1). In the lungs, diffuse thickening and fibrin deposition are seen in alveolar capillaries and serving as the nidus for entrapment of inflammatory cells (*17*); this contrasts with the finding that large arteries such as the pulmonary artery lacked thrombi. Small vessel thrombi were seen in the periphery of the lungs in the majority of cases (in keeping with radiographic appearance of peripheral predominant infiltrates). The majority of cases, with the exception of the immunocompromised, do not demonstrate neutrophilic infiltrates or evidence of infection in the airways or the interstitium. Another report details that in severe COVID-19 patients who become critically ill, generalized thrombotic microvascular injury is seen (*18*). This article reports striking septal capillary injury with extensive deposits of the terminal complement components C5b-9 (membrane attack complex), C4d, and mannose binding lectin (MBL)-associated serine protease (MASP) 2; furthermore, it was found that COVID-19 spike glycoproteins co-localized with C4d and C5b-9 in the inter-alveolar septa and the cutaneous microvasculature.

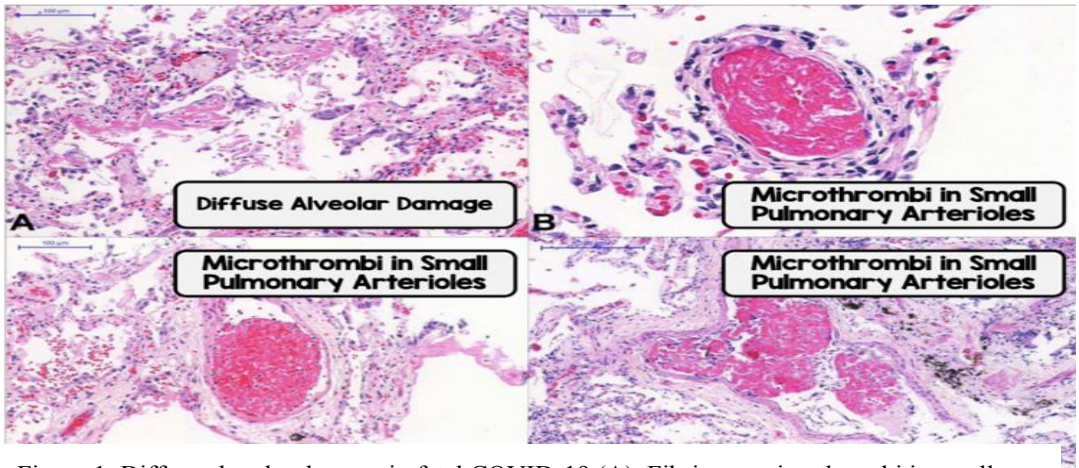

Figure 1. Diffuse alveolar damage in fatal COVID-19 (A). Fibrinous microthrombi in small sized pulmonary arterioles, observed in 8 out of 10 patients (B-D). Marisa D et al. Journal of Thrombosis and Hemostasis 2020 [Epub ahead of print]

It is also interesting to see the sporadic reports of previously healthy young adults suddenly dying of COVID-19 or developing strokes. In such cases, a sudden thrombosis-related event seems plausible. A previously healthy COVID-19 patient has presented with deep vein thrombosis (*19*). Sudden deaths of infants and pediatric patients, while rare, also raise the same concern. Recent reports detail infant death with underlying heart condition (*20*) and without pre-existing conditions (*21*), in neither of which the cause of death is clear. The question as to whether and how viral infection causes sudden infant death has previously been raised (*22*), and is still unknown. In discussion with a CHOP pathologist, we have a COVID-19 positive fetal demise case at HUP in which fibrin/thrombi deposition in the placental vessels is evident. This suggests that while there is paucity of literature on infants/pediatric patients, it is plausible that the virus causes death in a similar way to adults.

Note that current imaging tools cannot detect micron scale microcirculatory dysfunction; here, we have a unique tool and expertise to assess microvascular dysfunctions that may be the key factor leading to significant mortality and morbidity in these patients.

Contrast-Enhanced Ultrasound (CEUS) for Microvascular Flow Assessment

CEUS uses intravascularly injected microbubbles of 2-3 μm in size, smaller than red blood cells, to depict perfusion abnormalities down to 10 μm resolution. Organ perfusion abnormalities including focal or diffuse infarct can readily be detected with CEUS but not with Doppler ultrasound. It is FDA-approved for pediatric and adult applications for the evaluation of the heart and liver and used clinically at CHOP. CEUS has previously been used to evaluate cardiac/renal microvascular perfusion (*23-28*).

The PI has pioneered the application of CEUS in the pediatric population (*29-34*) and is conducting two Investigational New Drug-approved clinical trials in the most vulnerable patients including critically ill, unstable infants with hypoxic ischemic injury and necrotizing enterocolitis. CEUS can be performed conveniently at bedside without the need for patient transport, it is easily repeatable, and cost-effective, which are critical aspects of resource allocation during the COVID-19 pandemic. Furthermore, there is no current technology that can accurately assess organ microvascular perfusion. Color Doppler, also performed at the bedside, is limited to macrovascular flow (Figure 2).

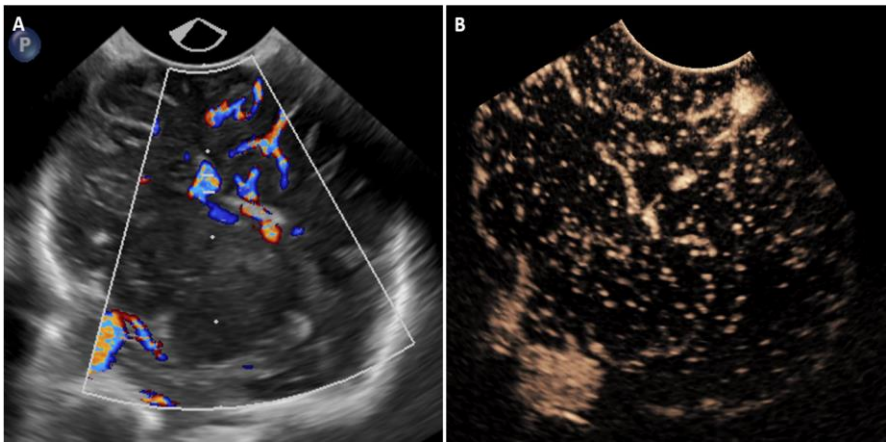

Figure 2. Doppler versus CEUS Assessment of Brain Microvascular Flow. Shown are Doppler (A) and CEUS (B) images of the porcine brain in coronal plane demonstrating the lack of microvessels depicted with Doppler as compared to delineation of < 10 μm microbubbles throughout the brain on CEUS.

CEUS images can be assessed in different ways. The degree of perfusion defect can be categorized as mild, moderate, or severe. Advanced quantitative analysis of perfusion can be done either by intensity analysis or microbubble tracking methods. (Figure 3).

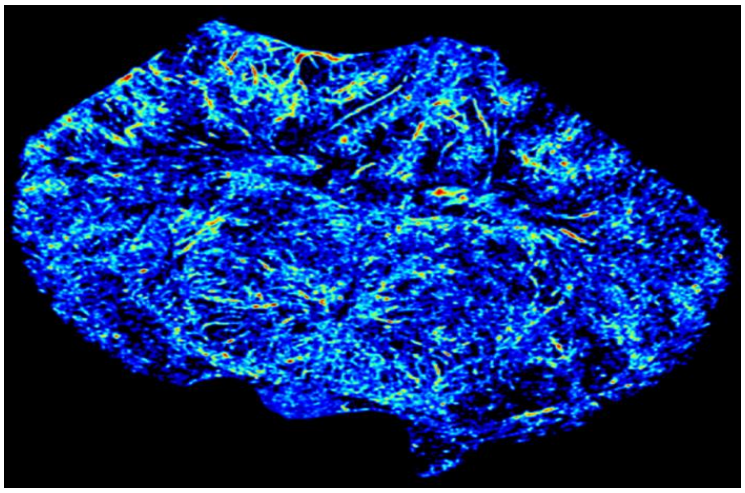

Figure 3. Shown to the left is a post-processed CEUS image of the same porcine brain depicting microvasculature down to the < 10 µm resolution. Microvascular flow trajectory and velocity can also be calculated from this data.

Even upon visual examination, organ ischemia is readily evident on CEUS, whereas it is difficult to assess with grayscale or Doppler ultrasound alone (Figure 4). At CHOP, CEUS is routinely done as part of clinical practice, and up to 4-5 CEUS exams are performed per day.

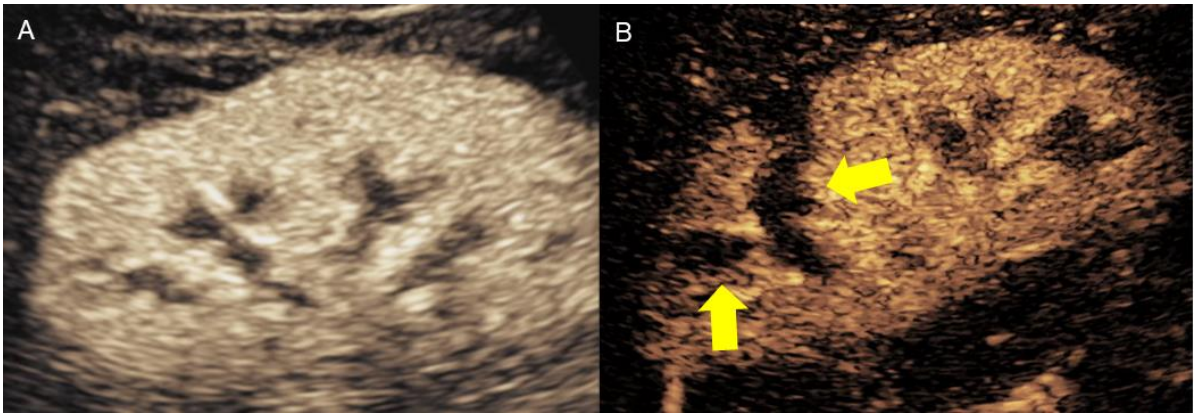

Figure 3. CEUS of the kidneys. Shown are sagittal CEUS images of the kidneys, (A) demonstrating homogeneous perfusion of normal right kidney with high signal due to intravascular microbubbles and (B) demonstrating low signal regions (yellow arrows) consistent with ischemia.

CEUS Quantification: It should be noted that CEUS gives a wide range of quantifiable, continuous parameters reflective of tissue perfusion. Injection of contrast results in increased acoustic signal quantifiable as signal intensity in decibels (dB). Spatiotemporal changes in signal intensity are then measured to reflect tissue perfusion, flow rate, blood volume, and many other parameters that can be derived from the *time intensity curve (TIC)* analysis. In TIC analysis, signal intensity in a given region of interest is plotted over time and parameters are extracted from the curve (for e.g. time to peak or TTP, wash-in area under the curve or WiAUC, rise time or RT, wash-in rate or WiR, wash-in perfusion index or WiPi). This is a well-accepted conventional approach to CEUS quantification previously used by the PI in the report of the first clinical guidelines for application of brain CEUS in infants (*29*), quantitative detection of hypoxic ischemic injury using brain CEUS (*30*), and quantification of tumor microvessel density (*35*). More advanced analysis involves the particle tracking method, in which individual intravascular microbubbles are tracked across thousands of ultrasound frames to quantify microbubble velocity, spatial distribution, trajectory (and other sophisticated parameters such as shear stress, acceleration-deceleration, etc.) (*36*). The conventional time intensity curve and particle tracking method combined would yield more than >30 continuous CEUS microvascular perfusion parameters that can each, or in combination, be correlated to clinical outcomes in this proposal.

1.2 Name and Description of Investigational Product or Intervention

Sulfur hexafluoride lipid-type A microspheres (Lumason™, Bracco Inc) is FDA-approved ultrasound contrast agent which consist of active ingredients including Sulfur hexafluoride (strength 60.7 mg in 1 mg), Distearoylphosphatidylcholine, DL- (strength 0.19 mg in 1 mg), 1,2-Dipalmitoyl-Sn-Glycero-3-Phospho-(1'-Rac-Glycerol), Sodium Salt (0.19 mg in 1 mg). Inactive ingredients include Polyethylene Glycol 4000 (strength 24.56 mg in 1 mg) and Palmitic Acid (0.04 mg in 1 mg). The sulfur hexafluoride lipid microspheres are composed of SF_6 (molecular weight 145.9) gas in the core surrounded by an outer shell monolayer of phospholipids consisting of 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC) and 1,2-Dipalmitoyl-sn-glycero-3-phosphoglycerol, sodium salt (DPPG-Na) with palmitic acid as stabilizer. Sulfur hexafluoride lipid-type A microspheres fall under Category B, that is, animal reproduction studies have failed to demonstrate a risk to the fetus and there are no adequate and well-controlled studies in pregnant women.1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC), with empirical formula C44H88NO8P, has a molecular weight of 790.6. 1,2-Dipalmitoyl-sn-glycero-3-phospho-rac-glycerol sodium (DPPG-Na), with empirical formula C38H74 NaO10P, has a molecular weight of 745. In pediatric patients, after reconstitution 0.03 mL per kg is administered intravenously. The weight-based dose of 0.03 mL per kg will be repeated twice during a single examination. Following each injection, an intravenous flush of 0.9% Sodium Chloride is injected.

1.3 Compliance Statement

This study will be conducted in full accordance with all applicable Children's Hospital of Philadelphia Research Policies and Procedures and all applicable Federal and state laws and regulations including 45 CFR 46, 21 CFR Parts 50, 56, and 312, and the HIPAA Privacy Rule. Any episode of noncompliance will be documented.

The investigators will perform the study in accordance with this protocol, will obtain consent and assent (unless a waiver is granted), and will report unexpected problems in accordance with The Children's Hospital of Philadelphia IRB Policies and Procedures and all federal requirements. Collection, recording, and reporting of data will be accurate and will ensure the privacy, health, and welfare of research subjects during and after the study.

2 STUDY OBJECTIVES

2.1 Primary Objective

Assess microvascular perfusion of the heart, kidneys and/or brain using CEUS in patients with confirmed or probable diagnosis of COVID-19.

2.2 Secondary Objectives

Correlate CEUS measures of microvascular perfusion of the heart, kidneys and/or brain to clinical outcomes.

3 INVESTIGATIONAL PLAN

3.1 General Schema of Study Design

This is a pilot study in which 30 subjects with confirmed or probable COVID-19 will undergo CEUS of heart, kidneys and/or brain.

The dosing plan will be weight-adjusted, based on a dose of 0.03 mL/kg. Each patient will receive up to two contrast injections as per the product label/FDA approved guidelines, with a maximum dose of 2.4 mL per injection. Examinations (heart, kidneys, and/or brain) will be made at the same time, as soon as the first product intravenous injection takes place. The examination will last approximately 15 minutes but no more than one hour.

As exploratory analysis, CEUS findings on these patients will be correlated to clinical outcomes (death, mechanical ventilation, heart attack, stroke, and renal failure).

3.1.1 Study Procedures

The study team will screen hospitalized patients for COVID-19 probable or confirmed cases. Recruitment will occur via medical chart review and/or physician referral.

Study Phase	**Screening (telephone)**	**CEUS Study Encounter**
Informed Consent	X	X
Review Inclusion/Exclusion Criteria	X	X
CEUS safety	X	X
Demographics/Medical History	X	X
Clinical Laboratory Evaluation		X

Lumason IV administration		X
CEUS examination		X
Acute Adverse Event Assessment (<20 minutes after contrast administration)		X

3.2 Study Duration, Enrollment and Number of Sites

3.2.1 Duration of Study Participation

Study participation will occur during the inpatient stay. Study duration per participant will include telephone screening lasting approximately 5-10 minutes to confirm eligibility for the study and CEUS safety.

The CEUS exam will last approximately 15 minutes but no more than one hour. Only one CEUS scan will be performed per patient.

Clinical outcomes during hospitalization will be documented via chart review. No outpatient follow-up will be performed.

3.2.2 Total Number of Study Sites/Total Number of Subjects Projected

This is a pilot study with a projected recruitment of 30 children with confirmed or probable diagnosis of COVID-19 at the Children's Hospital of Philadelphia.

3.3 Study Population

3.3.1 Inclusion criteria

1. Patient (male/female) hospitalized at CHOP.
2. Patient 17 years of age or younger.
3. Diagnosis of COVID-19 or high clinical suspicion for COVID-19 despite negative tests (according to the definition of probable case by the ECDC).
4. Patient has evidence of cardiovascular compromise, myocardial injury, acute kidney injury, and/or new-onset neurological symptoms).
5. Parental/guardian permission (informed consent)

3.3.2 Exclusion Criteria

1. Medical history of Lumason hypersensitivity.

4 STUDY PROCEDURES

Screening will be performed via medical chart review and/or clinical referral of pediatric subjects at the Children's Hospital of Philadelphia. Subjects who fulfill eligibility criteria will be included.

CEUS scan will be performed by CHOP study team members and/or dedicated research ultrasonographers. Subjects will undergo one CEUS exam. The dosing plan will be weight-adjusted, based on a dose of 0.03 mL/kg. Up to two injections per study will be performed as per product label/FDA guidelines. Subjects' and personnel safety will be handled according to clinical protocol for COVID-19.

Organ perfusion will be evaluated in the heart, kidneys and/or brain, provided there is evidence of cardiovascular compromise, myocardial injury, acute kidney injury, and new-onset neurological symptoms. Brain ultrasound will occur if there are new-onset neurological symptoms. We will use a low energy approach to minimize the risk of thermal injury or disruption of the blood-brain-barrier (mechanical index < 0.2 is safe).

Analysis of CEUS imaging by the Principal Investigator will be performed, in addition to medical chart review for clinical outcomes (exploratory analysis).

4.1 Study Visit

Upon patient entry into this study we will:

- Review inclusion/exclusion criteria
- Obtain written informed consent in a private setting.
- Perform CEUS exam
- Department policies concerning COVID19 safety will be followed

4.2 Image interpretation

Images will be assessed by the Principal Investigator (see Primary Endpoint in Section 5.1). Results of image interpretation will not be reported nor impact clinical management. No personnel beyond the PI and study personnel will have access to the master list with identifiable findings.

4.2.1 Follow-up

The medical chart of subjects who underwent CEUS examination will be reviewed to assess clinical outcomes (see Secondary Endpoint in Section 5.2).

4.3 Subject Completion/Withdrawal

Subjects may withdraw from the study at any time without prejudice to their care. They may also be discontinued from the study if they become anxious during this research examination. No sedation will be given. The Investigator may also withdraw subjects who violate the study plan, or to protect the subject for reasons of safety or for administrative reasons. It will be documented whether each subject completes the research study. If the Investigator becomes aware of any serious, related adverse events after the subject completes or withdraws from the study, they will be recorded in the source documents.

4.4 Data variables

4.4.1 EPIC

- Name
- MRN
- Date of birth
- Sex
- Chief complaint
- Symptoms
- Duration of symptoms
- Hospital stay
- Medications being used during hospitalization
- Laboratory tests during hospitalization
- Presence of myocardial infarction during hospitalization
- Presence of stroke during hospitalization
- Presence of renal failure during hospitalization
- Time to recovery
- Need for oxygen
- Need for mechanical ventilation
- Vascular complications

4.4.2 PACS

- Accession number
- All images obtained using CEUS

5 STATISTICAL CONSIDERATIONS

5.1 Primary Endpoint

1. Estimate the proportion of patients with normal perfusion versus area(s) of hypoperfusion in heart, kidneys and/or brain.

5.2 Secondary Endpoints

1. Evaluate heart, kidneys and/or brain perfusion using time to peak (TTP) and wash-in area under the curve (WiAUC).
2. Death within 28 days of imaging or mechanical ventilation within 14 days of imaging as reported in the medical chart.
3. Myocardial infarction, stroke, or renal failure as reported in the medical chart.

5.3 Sample Size and Statistical Analysis Plan

We will enroll 30 subjects (15 male/15 female) in this pilot study. As part of clinical care, CHOP Radiology also performs approximately 4-5 contrast-enhanced ultrasound cases in a given day, which is higher in volume than any other children's hospital in the country.

A sample size of 30 should provide reasonable estimates of the perfusion metrics. For example, a sample size of 30 participants produces a two-sided 95% CI with a width equal to 0.3 when the estimated proportion is as small as 0.3 (95% CI=0.1, 0.5). Also, a two-sided 95% CI with a width equal 0.6 when the estimate of Pearson's product-moment correlation is 0.4 (95% CI= 0.05, 0.7).

Based on microangiopathy/ischemia observed in COVID-19 patients *(9-11),* we expect our cohort to display delayed perfusion, hypoperfusion or no perfusion of the myocardium, kidneys, and/or brain. Results of the myocardial, renal and brain perfusion will be assessed by two experienced researchers in the field of CEUS and report it qualitatively as either normal or abnormal. In case of diagnosis disagreement between the two raters, a third experienced researcher will confirm the correct diagnosis. Hence, the proportion and frequency of myocardial, renal, and/or brain perfusion abnormalities will be documented. As additional primary analysis, we will also assess quantitative CEUS and derive time-to-peak (TTP) and wash-in area under the curve (WiAUC).

Demographic characteristics and descriptive statistics (such as measures of frequency, central tendency, dispersion, and position) will be summarized for the sample and by male and female sex. The percentage of COVID-19 patients with an abnormal clinical exam will be computed. Time intensity curve analysis will provide further details on the nature of perfusion abnormalities. The following presents the variable names, output, and the reported statistics for the primary and secondary aims.

	Measured Variables	**Output and statistics**
Primary Analysis	Perfusion defect (hypo- or absent perfusion)	Proportion of COVID-19 patients with perfusion defect
Primary Analysis	Perfusion quantified using time-to-peak (TTP) and wash-in area under the curve (WiAUC) from the time-intensity curve of contrast signal	Summary statistics for TTP and WiAUC, for e.g. mean, standard deviation, 95% CI
Secondary Analysis	Clinical outcomes from chart review etc.	Correlation of CEUS metrics to clinical outcomes

6 STUDY DRUG

6.1 Description

Lumason™ is currently FDA approved for use in the evaluation of heart and liver in the pediatric and adult population. Recommended intravenous dose for Lumason™ is weight-based, 0.03mL/kg as an intravenous injection, up to a maximum of 2.4mL per injection. The dosing plan will be weight adjusted, based on a dose of 0.03 mL/kg. Subjects will be monitored for potential side effects for 60 minutes following the final study drug dose. It is currently used clinically at CHOP radiology department for routine diagnostic ultrasound studies.

6.1.1 Drug Handling and Accountability

Lumason™, will be maintained as a separate supply from the Radiology Department for accountability. For each subject, the Lumason™ lot number, expiration date, number of vials used, disposition of unused Lumason™, and discard procedure, will be recorded as part of the study record.

7 SAFETY MANAGEMENT

As is standard clinical practice at CHOP, we will follow all current safety standards for CEUS established by the FDA and ACR to provide adequate protection from potential hazards or injuries to subjects. To assure the safety of the participants, all subjects will be monitored for adverse events during the procedure by direct communication through an intercom system and by visual observance. Data will only be transferred between members of the study team, and no identifiable data will be shared with non-CHOP or CHOP individuals who are not on the study team.

7.1 Clinical Adverse Events

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

7.2 Adverse Event Reporting

Unanticipated problems related to the research involving risks to subjects or others that occur during this study (including SAEs) will be reported to the IRB in accordance with CHOP IRB SOP 408: Unanticipated Problems Involving Risks to Subjects. AEs that are not serious but that are notable and could involve risks to subjects will be summarized in narrative or other format and submitted to the IRB at the time of continuing review.

7.3 Definition of an Adverse Event

An adverse event is any untoward medical occurrence in a subject who has received an intervention (drug, biologic, or other intervention). The occurrence does not necessarily have to have a causal relationship with the treatment. An AE can therefore be any unfavorable or unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

All AEs (including serious AEs) will be noted in the study records and on the case report form with a full description including the nature, date and time of onset, determination of non-serious versus serious, intensity (mild, moderate, severe), duration, causality, and outcome of the event.

7.4 Definition of a Serious Adverse Event (SAE)

We will follow IRB SOP 802 for reporting SAEs, should one occur.

7.4.1 Relationship of SAE to study drug or other intervention

The relationship of each SAE to the study intervention should be characterized using one of the following terms in accordance with CHOP IRB Guidelines: definitely, probably, possibly, unlikely or unrelated.

7.5 IRB/IEC Notification of SAEs and Other Unanticipated Problems

The Investigator is responsible for promptly notifying the IRB of all on-site AEs that are (1) serious, (2) unexpected and (3) related to the research intervention or other study procedures and any other unanticipated problems involving risk to subjects or others using the CHOP Internal SAE reporting form and in accordance with the following timeline. External SAEs that are both unexpected and related to the study intervention should be reported as they are received using the External SAE form (if applicable).

Type of Unanticipated Problem	Initial Notification (Phone, Email, Fax)	Written Report
Internal (on-site) SAEs Death or Life Threatening	24 hours	Within 2 calendar days
Internal (on-site) SAEs All other SAEs	7 days	Within 7 business days
Unanticipated Problems Related to Research	7 days	Within 7 business days
All other AEs	N/A	Brief Summary of important AEs may be reported at time of continuing review

7.5.1 Follow-up report

If an SAE has not resolved at the time of the initial report and new information arises that changes the investigator's assessment of the event, a follow-up report including all relevant new or reassessed information (e.g., concomitant medication, medical history) should be submitted to the IRB. The investigator is responsible for ensuring that all SAE are followed until either resolved or stable.

7.6 Investigator Reporting of a Serious Adverse Event to Sponsor

Reporting must be consistent with regulatory, sponsor or GCRC requirements.

8 STUDY ADMINISTRATION

8.1 Data Collection and Management

Confidentiality: All subjects will be assigned a number unrelated to their medical record number or name and this will be kept in a master list. The master list will contain patient identifiers and a link to the research-specific code. The master list will be kept in the PI and Study coordinator's password protected computer at CHOP, on the secure storage network

on the secure Hospital server. Co-investigators will have access to patient identifier's (name, MRN) during the optimization phase of their study. When the optimization finishes, or the article is published all the identification will be erase and only kept in the master list at the PI and Study coordinator computer. The collection of data from EPIC, ChartMaxx, and/or iSite Radiology will be using the MRN or name. Data will only be transferred between members of the study team; no identifiable data will be shared with non-CHOP or CHOP individuals who are not on the study team.

Security: All files (master list and coded data) will be password protected and will be stored on a password protected computer at CHOP, or in a password-protected hard drive. The hard drive will meet CHOP IT standards for encryption and password protection. The only way we will use to transfer internal information will be [send secure] emails using the study members' @email.chop.edu accounts. Access to the medical records and diagnostic studies to collect the data requires patient identifiers such as: medical record number and name. Without these, we would be unable to retrieve and review any information previously collected. For that reason, during the data collection process, we could send identifiable data using the protected CHOP-email.

Image data will be given a unique code and will have no information that can identify the subjects. Information that can identify subjects or image data may be kept in the master list at the PI and study coordinator's computer. Only the study team (co-investigators, PI, study coordinator, research assistants, etc) will be able to see information that can identify the subjects. If the subject leaves the study, he/she can ask to have the image data collected to be removed or destroyed. Imaging data will be archived to permanent storage media at the CHOP secure PACS system.

The study team will share unique research data accrued from this pilot clinical trial to enhance the value and further the advancement of research. The study team will make available such data after study completion and the data will only be provided to those investigators who agree to adhere to a signed research data use agreement. The data and associated documentation will be available to users only under a data-sharing agreement that provides for: (1) a commitment to using the data only for research purposes; (2) a commitment to securing the data using appropriate computer technology; and (3) a commitment to destroying or returning the data after analyses are completed. The data obtained will be periodically reviewed (once every month) by the study team for discussion and improvement as well as planning for dissemination of results to the scientific community.

8.2 Confidentiality

All information gained about the individual patients will remain confidential. When data is used for reports on the usefulness of the studies it will be compiled into statistical summaries from many studies, and the data will not be identified with any patient. After study closure, all data, including identifiers, gathered from the study will be archived for 6 years in a password-protected folder on the primary computer of the PI, which complies with CHOP policy A-3-9 for data retention. As per the FDA regulations, all study data (including PHI) will be kept for 2 years after last marketing approval for the drug. In keeping with CHOP policy A-3-9 and FDA requirements, the data will be destroyed based on whichever period of time is longer.

All HIPAA and IRB guidelines will be followed. The records generated during this study will be kept confidential and the data will only be used for the purpose of conducting the study. Safeguards are described under Section 8.1 Data Collection and Management.

8.3 Regulatory and Ethical Considerations

8.3.1 Data and Safety Monitoring Plan

The safety monitoring for this study is the primary responsibility of the study team. The occurrence of adverse events, serious adverse events and unanticipated events will be reported by the study team in accordance with federal and institutional guidelines, as outlined in Section 7 of this clinical study.

8.3.2 Risk Assessment

The potential risks associated with contrast-enhanced ultrasonography have been extensively studied, and the risk associated with the technique is less than that of CT or MRI contrast agents. The risk of adverse events is the lowest of all contrast agents available, with CT contrast being the highest (0.6%), followed by MR contrast (0.0088%) and ultrasound contrast (0.0086%). The safety profile of Lumason™ has been documented in a large cohort including 23,188 adults, with no fatal event and only 29 (3 severe, 3 moderate, and 23 mild) adverse events. Further studies detail the safety profile of ultrasound contrast agents in children and have shown minor adverse events including nausea, tinnitus, lightheadedness, altered taste sensation. One documented severe reaction in a child documented symptoms of generalized pruritus, nausea, hypotension with tachycardia initially then bradycardia [6]. Management in this instance consisted of oxygen, intravenous epinephrine, and fluids (0.9% normal saline) with resolution of symptoms in two hours. Treatment of both minor, mild, and severe adverse reactions post Lumason™ administration are the same as that of CT or MRI contrast agents. In comparison to CT or MRI contrast agents, however, ultrasound contrast agents have proven to be much safer in children with only one serious adverse event over decades of its use to date (contrasting to approximately 15-20 adverse events per 2000 children if CT contrast agent were to be used). No serious adverse event has been reported in a neonate since its clinical use in this population. Animal studies on its toxicity profile also validate no fetal toxicity and the ultrasound contrast agent belongs to category B. However, a few serious adverse reactions in older patients have been reported, such as anaphylactoid/hypersensitivity reactions, arrhythmias and hypertensive episodes, commonly within 30 minutes of Lumason administration, some of them causing death.

Risks of the administration of the study drug are considered a minor increase above minimal risk, with no prospect of direct benefit for subjects. Adverse effects are not dose-dependent, thus risk is not increased by modifying the timing of interventions or increasing the number of contrast-agent injections.

Another risk of the study includes the insertion of a peripheral IV line. This is a no greater than minimal risk procedure, with the main risks of discomfort, bruising, and infection which are generally self-limited. There is a no greater than minimal risk of breach of confidentiality, which is minimized by having all of the study personnel undergo HIPAA training.

8.3.3 Potential Benefits of Trial Participation

There is no prospect for direct benefit. However, improved understanding of viral impact on microvascular perfusion in relation to outcomes will be tremendously helpful for scientific knowledge and therapeutic directions.

The potential finding that CEUS can detect microvascular perfusion abnormalities in the heart/kidneys, and the proportion of those with microvascular perfusion abnormalities in the heart/kidneys is high in COVID-19 pediatric patients. This will lead to larger observational or interventional trials that can advance this tool for detection of microvascular perfusion dysfunction, prognostication, and guidance of therapies.

To the investigator's knowledge, there is no study to date that has correlated microcirculatory dysfunction *in vivo* to outcomes in COVID-19 despite the strong link between microvascular perfusion impairment and high mortality/morbidity of the disease.

8.3.4 Risk-Benefit Assessment

The benefit to society outweighs the risks of this study.

8.4 Recruitment Strategy

Patients will be recruited via chart review and/or clinical referral.

8.5 Informed Consent/Assent and HIPAA Authorization

Approved members of the study team will obtain parental/guardian consent (from both parents or legal guardians) prior to the proposed study in a private setting. The investigators will assure that parents/guardian comprehend the nature of the study, the study procedures and the risks and benefits of participation, steps that will be taken to avoid coercion and documentation of consent.

This study requires two parent signatures documented on the consent form. After full explanation of the study, and if the two parents/legal guardians are present at the hospital, they will sign the informed consent form (ICF) in the presence of the approved member of the study team who did the disclosure.

However, the study team anticipates this to be a challenge, as (either or) both parents/guardians are not always present at the bedside. Given the critically ill subject population and the need to image patients as early in the disease process as possible, we will send a copy of the ICF in advance to the parent(s)/legal guardian(s) and obtain in either one of the following ways:

- Consent conferences with either or both parents may happen in person or over the telephone.

- Either or both parents may sign the consent form in the physical presence of the study team, or remotely.

- Study team may obtain original signed copy (wet signature) or a copy of the original that is scanned/photographed and e-mailed, texted, faxed or mailed.

After the disclosure, the parent(s)/legal guardian(s) will sign the page of the ICF as indicated by the study team member that is consenting. The parent(s)/legal guardian(s) will send a photograph of the signed page by facsimile, text message, or e-mail to the investigator/designee.

If the parent cannot print the ICF, a special provision is made for them to sign on a blank page. This requires a consent conference where either i) a witness not related to the study is present or ii) a recording of the call (with permission from parent(s)) is made. A copy of the consent form will be sent (e.g. via e-mail, fax, etc.) to the parent(s)/legal guardian(s) in advance of the consent conference so they can read it. If they do not have access to e-mail, the informed consent will be read to them.

A standard process will be used to ensure:

i) Identification of call participants.
ii) Review of informed consent document by study team, response to parents' questions and parents' verbal confirmation that their questions were answered.
iii) Verbal confirmation by the parent/legal guardian that they have agreed to provide permission for the child's participation and verbal confirmation by the parent/legal guardian that they signed and dated a blank piece of paper with a written statement that they voluntarily agree to provide permission for the child's participation in the study, noting both the Protocol 'NUMBER' and brief protocol title.
iv) The parent sends a photograph of the signed and dated statement by facsimile, text message, or e-mail to the investigator/designee; OR returns the document to the investigator by e-mail, facsimile, or mail at a later date, or at a future study visit that might occur in person.

Per §46.408(b) / §50.55(e)(2), if the second parent is reasonably unavailable (deceased, incarcerated, in active military service, etc), the study team will enroll the subject with just one parental/legal guardian signature.

No amendments will be done on the ICF once it is signed by both parents/legal guardians. A combined HIPAA consent-authorization document will be used.

We plan to enroll children 17 years or younger who will be seriously ill. In the event that they turn 18 while hospitalized, we anticipate that they will be unable to re-consent for themselves. In this case, we will re-consent through their Legally Authorized Representative.

A waiver of assent was requested since subjects will likely be very sick and unable to provide assent. However, we will obtain verbal assent from children seven years old or older who are able to provide it. Assent confirmation (or inability to assent) will be documented by the study team on the consent form.

A copy of the signed consent form will be provided to the parent(s)/legal guardian(s).

Subjects with limited English proficiency (LEP) will be consented in person or remotely. When parents/guardians consent in person, a witness/interpreter through CHOP Language

Services will be present in person or remotely. If the interpreter is remote, their reference number will be noted on the signature line. When parents/guardians consent remotely, a witness/interpreter provided through CHOP Language Services will join the consent meeting in person or by video/phone call. If remote, the witness/interpreter's reference number will be noted on the signature line. Apart from using a witness/interpreter, LEP subjects consented remotely will follow the same plan of English-fluent subjects consented remotely.

8.6 Payment to Subjects/Families

Families will be compensated with a $25 gift card.

9 PUBLICATION

We plan to publish the data collected in a scientific journal. Data may also be presented as abstract, podium presentation, or poster presentations at scientific meetings and conventions. No patient identifying information will be used in publication.

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