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TABLE OF CONTENTS

ABBREVIATIONS AND DEFINITIONS OF TERMS

CE	Contrast-Enhanced
TTE	Transthoracic Echocardiogram
AE	Adverse event
US	Ultrasound
CEUS	Contrast-Enhanced Ultrasound

ABSTRACT

Introduction: Patients with congenital heart disease with residual pulmonary regurgitation, such as repaired tetralogy of Fallot, congenital pulmonary stenosis, pulmonary atresia, and any congenital heart disease requiring an RV-PA conduit, represent a challenging group of patients as they develop progressive right ventricular dilation and failure, increasing the risk for sudden cardiac death and often necessitating pulmonary valve replacement[1]. These patients require close follow-up with serial cardiac imaging; however, the complex three-dimensional structure of these dilated right ventricles renders them difficult to adequately image with traditional two-dimensional echocardiography[2, 3]. Due to these difficulties, cardiac MRI is the current gold standard for assessing right ventricular size and function in these patients[3]. However, cardiac MRI is costly and less accessible for patients than echocardiography[3]. Although initial studies comparing 3D echocardiography with MRI showed that 3D echocardiography underestimates right ventricular size[4], recent advances in three-dimensional imaging technologies which utilize automated ultrasound "speckle-tracking" and artificial intelligence technology are lessening this inherent bias[5-7]. Furthermore, the use of commercially-available ultrasound enhancing agents made of lipid microspheres has improved left ventricular endocardial border detection, inter-rater reliability and correlation of 3D echocardiography obtained estimates of left ventricular size and function compared with CMR[8]. However, their use has yet to be applied to the three-dimensional echocardiographic assessment of the right ventricle despite their ability to improve right ventricular endocardial border detection with two-dimensional echocardiography [9]. We propose the novel integration of two echocardiographic technologies – three-dimensional echocardiography using semi-automated right ventricular analysis coupled with the administration of ultrasound enhancing agents – to change the paradigm of how clinicians assess the right ventricles of patients with repaired congenital heart disease with residual pulmonary insufficiency, such as tetralogy of Fallot. We hypothesize that ultrasound enhancing agents will improve the inter-rater reliability and accuracy of various measures of right ventricular size and function, compared with cardiac MRI, thereby filling an important gap in existing methods for assessing right ventricular function. Lastly, because of the current limitations in assessing right ventricular function in this population, as a secondary aim, we will also assess three-dimensional right ventricular strain -- a novel quantitative surrogate of right ventricular function[10, 11].

Overall Objectives: To shift the paradigm in the assessment of right ventricular size and function by developing a protocol to assess the right ventricles in patients with repaired congenital heart disease with residual pulmonary regurgitation by integrating the use of three-dimensional echocardiography with semi-automated right ventricular analysis software with the intravenous administration of ultrasound enhancing agents.

Specific Aim 1. To compare the accuracy and inter-rater reliability of 3D echocardiography-based measurements of right ventricular end-diastolic volume, end-systolic volume, and ejection fraction, with and without contrast, with MRI derived values in patients with repaired congenital heart disease with residual pulmonary regurgitation. .

Specific Aim 2. To compare the accuracy and inter-rater reliability of 3D echocardiography measurement of right ventricular strain with MRI derived RV strain in patients with repaired congenital heart disease with residual pulmonary regurgitation.

1 BACKGROUND INFORMATION AND RATIONALE

1.1 Introduction

Tetralogy of Fallot is the among the most common congenital heart defects, affecting roughly 4 patients per 10,000 live births [12]. The majority of these patients survive into adulthood, but have increased morbidity and mortality as late sequelae of their initial repair, which often results in significant pulmonary regurgitation[1]. Residual pulmonary regurgitation is a common sequelae in many congenital heart surgeries involving the pulmonary valve, or with surgeries requiring an right ventricle to pulmonary artery conduit. One integral risk factor for poor outcomes in these patients is the severity of right ventricular dilation[13]. This RV dilation can be successfully reversed with pulmonary valve replacement, either surgically or in the catheterization laboratory during transcatheter procedures [14, 15]. Echocardiographic assessment is recommended in adults with tetralogy of Fallot at least every 24 months, but generally provides suboptimal assessment of right ventricular size and function[13]. Right ventricular volumes and ejection fraction cannot be determined with routine two-dimensional echocardiography. Because of these barriers to the assessment of the right ventricle, serial cardiac MRI is needed [3, 13] The routine use of cardiac MRI, however, significantly increases the burden of care and costs on patients as well as health systems.

Three-dimensional echocardiographic software to assess the right ventricle is commercially available, and the sophistication of this software has increased in recent years[5-7]. These software are able to automatically track ultrasound "speckles" in order to provide accurate endocardial detection of the right ventricle for initial image alignment and drawing of ventricular contours. In addition, the software relies on artificial intelligence as it is powered by machine learning from a large database of clinical images and various right ventricular shapes. Nevertheless, because of the complex shape and size of these right ventricles, endocardial detection in some locations may still be suboptimal. The software is able to generate a dynamic three-dimensional surface rendering of the right ventricle and provide several quantitative parameters of right ventricular size and function which are not available from traditional two-dimensional echocardiography.

Ultrasound enhancing agents are a class of commercially available products comprised of lipid microspheres (or microbubbles) which are routinely used in adult echocardiography for improved endocardial border detection, but mainly to image the left ventricle[16]. UEA's are inherently different from contrast agents used for CT and for MRI. UEA's take advantage of the fact that air reflects ultrasound beams. It has long been known that by agitating saline, small air bubbles are created, and when injected it into an IV, the bubbles appear very bright on ultrasound. This property has been utilized for many different ultrasound techniques, but is limited because the air bubbles do not move through the lungs. Additionally, these bubbles are very transient, and it is difficult to fully opacify different cardiac chambers. UEA's like Lumason create microbubbles, which are microscopic bubbles of air suspended in a sulfur hexafluoride lipid sphere for stabilization. These microsphere bubbles are small enough to pass through capillary beds and remain in circulation longer than agitated saline. Most of the product is exhaled through the lungs, and the rest of the components are broken down and excreted in the liver and kidneys. There is about a 1/10,000 risk of anaphylactoid reaction to the medicine, which requires monitoring, as most reactions occur within 30 minutes of injection[22]. Indeed, the only contraindication to use of Lumason is a prior hypersensitivity reaction to any of the ingredients in Lumason. The safety of UEAs from numerous clinical trials and registry data has been documented in several different clinical settings including in patients with pulmonary hypertension, intra-cardiac shunts, and in pediatric patients[17-19]. Data has

also been published demonstrating improved clinical outcomes and increased cost-effectiveness of UEA's in patients with suboptimal echocardiographic imaging in clinical settings [20]. Currently, three UEAs are approved for the use by the federal drug administration for cardiovascular applications. Lumason is currently the only UEA approved for pediatric echocardiography[21].

Ultrasound-enhancing agents have also demonstrated improved ability to image the right ventricular borders in prior studies[9] but only with two-dimensional imaging. By combining improved right ventricular endocardial border detection provided by ultrasound enhancing agents with the latest three-dimensional echocardiographic imaging and analysis technologies, this study will demonstrate the improved value of echocardiography in assessing the right ventricular size and function in this vulnerable patient population. This improved assessment of the right ventricle may lead to earlier diagnosis and more timely intervention for patients with repaired tetralogy of Fallot, potentially resulting in improved clinical outcomes. Successful completion of the aims would result in larger, multicenter follow-up studies to demonstrate the association of contrast three-dimensional echocardiography-derived measures of right ventricular size and function with clinically relevant outcomes.

1.2 Compliance Statement

This study will be conducted in full accordance all applicable Nemours Research Policies and Procedures and all applicable Federal and state laws and regulations including 45 CFR 46, 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 Nemours 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.

Additionally, this study involves the use of Lumason and is therefore FDA-regulated. The use of Lumason in pediatric patients is currently approved in hepatic evaluation via intravenous injection. Our study will comply with the FDA regulations surrounding intravenous use of Lumason in pediatric patients.

2 STUDY OBJECTIVES

2.1 Primary Objective (or Aim)

The primary objective for this pilot study is:

- To compare the accuracy and inter-rater reliability of 3D echocardiography-based measurements of right ventricular end-diastolic volume, end-systolic volume, and ejection fraction, with and without contrast, with MRI derived values in patients with repaired congenital heart disease with residual pulmonary regurgitation. .

2.2 Secondary Objectives (or Aim)

The secondary objectives for this study are:

- To compare the accuracy and inter-rater reliability of 3D echocardiography measurement of right ventricular strain with MRI derived RV strain in patients with repaired congenital heart disease with residual pulmonary regurgitation.

3 INVESTIGATIONAL PLAN

3.1 General Schema of Study Design

Subjects will be drawn from a single population, patients repaired congenital heart disease with residual pulmonary regurgitation includingrepaired tetralogy of Fallot referred to Nemours A.I. DuPont Hospital for Children for clinically indicated cardiac MRI. The rationale for the enrollment of children in this study is that we are comparing cardiac MRI and echocardiography measures, and many of the patients repaired congenital heart disease with residual pulmonary regurgitation who obtain MRIs at Nemours are children.

Lumason is a sulfur hexaflouride gas covered by a microlipid layer. The size of the individual microbubbles are smaller than the size of a red blood cell allowing the microbubbles to pass through the capillary beds of the body without imposing risk to tissues or the microvasculature. The microbubbles are cleared through the lungs without reliance of the renal or hepatic systems. The microbubbles are stable within the circulation for up to 20 minutes. Total dosing is per vial of contrast, which reconstitutes to 4.8 mL per vial of contrast. Each vial is used for a single patient. Patients receive an IV prior to the start of their ultrasound. After use of Lumason, patients are monitored per an outpatient radiology nursing protocol for any signs of an adverse reaction.

Lumason comes stored at room temperature in a kit. Each kit contains the following:

- One Lumason vial of 25 mg lipid-type A white lyophilized powder with headspace fill of 60.7 mg of sulfur hexafluoride
- One prefilled syringe containing 5 mL of Sodium Chloride 0.9% injection, USP (Diluent). Also note that patients must receive a saline flush (max 5 mL) after injections of the study dose.
- A single Mini-Spike will be obtained.
- Lumason will be prepared based upon the packaging recommendations included within each kit. A three-way stopcock will be attached to the patient's IV with the Lumason syringe directly connected (180° location) and a 5 mL saline flush connected at the 90° location.

Prior to the cardiac MRI, a physician member of the team will contact the family by phone to inform them of the study and answer any questions the family might have. If the family is interested, then after the MRI, a physician member of the team will be present to obtain consent. First, the MRI will be obtained under routine care. Once those images are obtained and the clinical study is complete, the patient will be brought to the Echocardiography laboratory treatment room, and heart rate and respiratory rate monitors will be applied. The echocardiography team will perform echocardiographic views. The patient will receive the weight-based dose of Lumason of 0.03 mL/kg per injection, not to exceed 2.4 mL per injection per the FDA and manufacturer recommendations. Dosages and timing of administration as well as patient size and transducer type will be recorded for each patient. The total cumulative dose of Lumason from the clinical study and the research study will not exceed the maximum recommended dose by the manufacturer, 4.8 mL. A single injection will not exceed the maximal FDA-recommended dose of 2.4 mL per single injection.

Following completion of the ultrasound and echocardiogram, patients will be discharged from the study, the IV will be removed and the patient monitored for adverse reactions per the current post-lumason protocol.

3.2 Study Duration, Enrollment and Number of Sites

3.2.1 Duration of Study for Subject

To minimize duration of the study, echocardiographic clips will be limited for primary evaluation of standard views, consisting of a modified apical 4 chamber view focused on the right ventricle. The study will be performed by senior echocardiography technicians with contrast echocardiography experience in adults. We anticipate that total echocardiography imaging time will take 20 to 30 minutes beyond the MRI imaging.

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

The study will be conducted only at the Nemours A.I. DuPont Hospital for Children. De-identified echocardiographic images will be transferred to Thomas Jefferson University for additional analysis by Dr. Mehrotra. We aim to enroll a up to 60 subjects with patient ages 11 or greater. There is no weight limitation for use of Lumason or enrollment in our study.

3.3 Study Population

Subjects will be drawn from a single population:

1. Patients aged 11 or greater who are referred to Nemours A.I. DuPont Hospital for Children for clinically indicated cardiac MRI.

3.3.1 Inclusion Criteria

1. All patients aged 11 or greater with repaired congenital heart disease with residual pulmonary regurgitation including repaired tetralogy of Fallot who are referred to Nemours A.I. DuPont Hospital for Children for clinically indicated cardiac MRI will be eligible for inclusion.
2. Informed written consent of parent or legal guardian.
3. Informed written assent of subject, if appropriate.

3.3.2 Exclusion Criteria

1. Any patients in which Lumason is contraindicated (i.e. prior anaphylactoid reaction) will be ineligible for the clinical ultrasound and ineligible for the study.
2. History of allergic reaction to Lumason, sulfur hexaflouride, sulfur hexafluoride lipid microsphere components, or other ingredients in Lumason (polyethylene glycol, distearoylphosphatidylcholine (DSPC), dipalmitoylphosphatidylglycerol sodium (DPPG-Na), palmitic acid)
3. Pregnant women will be excluded from the study as well since Lumason has not been studied in pregnancy.

3.3.3 Case ascertainment

Children who are referred to the Nemours A.I. DuPont Hospital for Children for clinically indicated cardiac MRI.

3.3.4 Data sources (for existing records)

The following information will be abstracted from the electronic medical record: date of birth, age, height, weight, body surface area, medical history which includes any history of cardiac disease, related surgical history, medications, cardiac catheterization data, prior echocardiographic data, vital

signs (temperature, blood pressure, respiratory and heart rates), and clinic notes. Storage and management of such data is detailed in Section 8.

Additionally, the CE echocardiogram images will be stored on the Syngo Database server. These images will be used as a data source to perform measurements following study completion. All stored images will not contain any identifiers. A master list linking the images to potential identifying information will be stored in the REDCap database as explained in section 9.

4 STUDY PROCEDURES

All eligible participants will undergo a clinically indicated cardiac MRI. The CE-TTE team will be notified of the case and perform consent followed by a CE-TTE following their cardiac MRI. The patient will be brought to the treatment room of the echocardiography laboratory. The echocardiography technician will obtain the images while the cardiology attending will prepare the lumason and be responsible for injecting the lumason. A modified apical 4 chamber view will be obtained, focusing on the right ventricle. The echo will consist of a full volume three-dimensional acquisition of the right ventricle without contrast from a modified apical 4 chamber view. Three-dimensional right ventricular strain will also be measured. Next, a cardiology attending will inject lumason, at 0.03ml/kg per dose, or 2.4 ml per dose, whichever is lower. A full volume three dimensional right ventricular acquisition will then be acquired. The images will be post-processed on the ultrasound machine by the cardiology attending to obtain right ventricular systolic and diastolic volume, and right ventricular strain. The images will be transferred to a core laboratory and re-analyzed offline by a second observer for interobserver variability assessment. All studies will be performed without the use of sedation or anesthesia. The total Lumason dose will not exceed the recommended maximal dose per the manufacturer's or the FDA's recommendations. Patients who are not tolerating the procedure due to discomfort or parental concern will have the CE-TTE terminated, and the machine will be removed from the echocardiography laboratory treatment room.

In addition to the CE-TTE imaging, enrolled patients will have the following information abstracted: date of birth, age, height, weight, body surface area, medical history which includes any history of cardiac disease, related surgical history, medications, cardiac catheterization data, prior echocardiographic data, vital signs (temperature, blood pressure, respiratory and heart rates), and clinic notes. Storage and management of such data is detailed in Section 9.

Should any abnormalities be identified during the CE-TTE, participants will be notified directly by a member of the study team (same day) and with a letter (within 7 business days), and an expedited cardiology referral will be provided.

4.1 Study Visit

Upon patient entry into this study we will:

- Review inclusion/exclusion criteria.
- Obtain informed consent.
- Perform CE-TTE exam.
- IV removal and monitoring per the protocol

4.2 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 provided on the basis of the CE-TTE study. Should patients require sedation for the scheduled clinical MRI, the anesthesiology team will proceed per their protocols.

The Investigator may also withdraw subjects to protect the subject for reasons of safety or for administrative reasons. 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.

5 STUDY ENDPOINTS AND EVALUATIONS

5.1 Primary and Secondary Endpoints

The echocardiography measured RV end diastolic volume, RV systolic volume, RV strain, and RV ejection, with and without contrast, will be compared with MRI derived measurements of the same using intraclass correlation coefficients. The echocardiographic readers will make independent assessments of the measures, and be blinded to the MRI results. Bland Altman plots will be utilized to assess for systemic bias between the imaging modalities. Interobserver variability will also be assessed using intraclass correlation coefficients. This is a convenience sample, but based on prior research, we anticipate that the number of patients will be sufficient to demonstrate a convincing trend that would justify further studies[8].

5.3 Safety Evaluation

- Current safety protocols used for contrast-enhanced ultrasonography within the Department of Radiology at the Alfred I. duPont Hospital for Children will be used for this protocol.
- An accessible code cart is available in the case of an emergency in the echo lab treatment room and patients receiving contrast-enhanced ultrasounds are observed following IV removal prior to discharge from the department.
- Lumason is a well-tolerated contrast enhancing agent and anaphylaxis is a rare complication of lumason. As a precautions for this rare complication, patients will be monitored according to the protocol. Any adverse events will be recorded and reported to the safety monitoring board.

6 MEASUREMENTS AND EVALUATIONS

The echocardiography measured RV end diastolic volume, RV systolic volume, RV strain, and RV ejection, with and without contrast, will be compared with MRI derived measurements of the same using intraclass correlation coefficients.

7 STATISTICAL CONSIDERATIONS

The echocardiography measured RV end diastolic volume, RV systolic volume, RV strain, and RV ejection will be compared with MRI derived measurements of the same using intraclass correlation coefficients. The echocardiographic readers will make independent assessments of the measures, and be blinded to the MRI results. Bland Altman plots will be utilized to assess for systemic bias between the imaging modalities. Interobserver variability will also be assessed using intraclass correlation coefficients. This is a convenience sample, but based on prior research, we anticipate that the number of anticipated patients will be sufficient to demonstrate a convincing trend that would justify further studies[8].

8 LUMASON DRUG INFORMATION & SAFETY MANAGEMENT

As is standard clinical practice at Nemours we will follow all current safety standards for contrast-enhanced ultrasonography scanning to provide adequate protection from potential hazards or injuries to subjects. As this study is only evaluating patients undergoing clinically indicated MRI who will have an IV placed as part of the exam, there is no increased risk to IV placement. There is minimal increased risk to participation in the study. The agent being given, Lumason, has an excellent safety profile, is FDA approved for use in this patient population, and is on the Nemours formulary. The primary risk to contrast exposure is anaphylactoid reactions. In a recent study evaluating the safety of several different CE ultrasound agents, 2,137 patients received Lumason. Of these patients, 14 patients were found to have intracardiac shunts. There were no serious adverse reactions. There was 1 minor adverse reaction. No adverse events occurred in patients with intracardiac shunts.[19] The estimated rate of serious allergic reactions to a CE ultrasound reported in the literature is 1:10,000.[22] All subjects will be monitored for adverse events during and following the procedure per standard protocol. The CE-TTE study may prolong the patient's scheduled visit up to 30 minutes.

We intend to administer the recommended FDA-approved dose of 0.03 mL/kg/dose up to 2.4 mL per dose injection. However, given the significant amount of blood flow with the heart receiving an entire cardiac output we are aware that dose adjustments may be required to optimize images, 0.03 mL/kg/dose may be too much or too little contrast. If 0.03 mL/kg/dose is not adequate, either too

high or too low, the dose may need to be modified as low as 0.01 mL/kg/dose or as high as 0.06 mL/kg/dose, to remain within the FDA guidelines for maximum contrast per examination.

The FDA approved dose is based on experience at a single institution using a single US vendor. The imaging appearance of the contrast agent is known to be affected by the sensitivity of the US software and varies with the probe, depth of imaging and target organ. Although, CEUS has been performed in adult echocardiography, the patient size and transducer type is significantly different in pediatric patients. Notably, prior to FDA approval, a number of publications described the dose for sulfur hexafluoride lipid-type A microspheres. Outside of the United States, Lumason®(sulfur hexafluoride lipid-type A microspheres) is marketed by Bracco Diagnostics Inc under the name SonoVue®(sulfur hexafluoride lipid-type A microspheres). Aside from the name, these contrast agents are identical to one another. In the recent European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB) position statement, the authors summarized that there was no standardized dose scheme for pediatric CEUS.[23] Publications evaluating liver lesions report doses from 0.1 mL to 4.8 mL per injection. The dose reportedly based on age, weight, body surface area, US scanner, transducer, as well as ultrasound software. Reported pediatric dose schemes include:

1. 0.1mL per year of age [24]

2. standard single dose 0.1mL, 0.5 mL, 1 mL, 1.2 mL, 2.4 mL or 4.8 mL [25-28]

3. FDA dose 0.03 mL/kg up to 2.4 mL per injection [21]

A review article described practice of using 0.6 mL in children < 6 years, 1.2 mL in children 6 - 12 years of age, and 2.4 mL in children > 12 years of age.[29]

In the radiology department at the A.I. duPont Hospital for Children, a range of doses have been utilized depending on the indication, organ evaluated, body habitus and transducer. Lumason®(sulfur hexafluoride lipid-type A microspheres) gained FDA approval for IV administration in pediatrics in 2016.

The main serious adverse event reported in the literature is anaphylaxis, which is dose independent. We have outlined in the table below the articles cited above as well as additional articles on pediatric usage including the dose regimen and adverse events reported. As our examinations will be conducted in the echocardiography laboratory treatment room and anaphylaxis is a dose independent adverse event, enrolled patients will have mildly added risk with any subsequent injections. Patients will be monitored following study participation in the same manner as those undergoing a radiology study with contrast-enhancing ultrasound agents without study participation. This study design has been optimized to minimize patient risk.

Table 1: Literature review of dose algorithms and adverse events in pediatric patients.

Author	N	Age	Dose	Adverse Events
Jacob 2013[26]	44	Median age 11.5 (range 4 – 18 years)	Bolus of 1.2 – 2.4mL of SonoVue	Assessed; None
Bonini 2007[27]	30	Mean age 2 years (range 2	Sonovue was administered by intravenous	Assessed; None

		months to 10 years)[€]	bolus at a maximum dose of 0.5 ml (up to three injections).	
Valentino 2008[30]	27	Mean age 8.9 years (range 4 – 13 years)	Two 2.4 mL bolus contrast agent injections (SonoVue)	Assessed; None
Piskunowicz 2015[31]	137 (161 examinations)	Mean age 10.2 years(range 0 – 18 years)[£]	The volume of contrast agent administered was based on the patient's age and ranged from 0.1 – 1.8 mL	Assessed; 1 occurrence of severe anaphylactic shock 43 seconds after IV administration
Knieling 2016[28]	40 patients with "55 investigations" and "79 IV applications"; (6 patients [9 investigations were > 18 years of age], leaving 46 investigations in children < 18 Study Addendum included 14 off-label applications, including 6 IV applications for focal liver diagnostics including 2 patients < 1 year) (At least 3 pediatric patients in addendum, no other information available)	Mean age 11.4 (range 0 – 26 years; 6 subjects > 18 years)[¥]	< 20 kg was 0.4 ± 0.3 ml, (0.05 ± 0.02 ml/kg) and > 20 kg was 1.0 ± 0.4 ml (0.02 ± 0.01 ml/kg)	2 AEs which consisted of nausea or a single wheal; additional severe AE added to study addendum 1 uncertain AE in a patient diagnosed with a dissociative disorder with fixed look, depressed breathing, loss of ability to communicate and pain. Symptoms resolved after IV loraezpam and Piritramid. Thought to have no causal relationship to contrast agent. In the Study Addendum, an 11 year old girl developed a severe anaphylaxis following IV administration of the UCA (ultrasound contrast agent) and required short term inpatient monitoring and treatment with steroids, antihistamines, and fluids.

Menichini 2015[32]	73	Mean age 8.7 years +/-2.8 y, all < 16 years[α]	Two 1.2 ml bolus of Sonovue	Assessed; None
Miele 2016[33]	27	27 "pediatric" of 77 patients Age range 8-61 years	Two 2.4 ml IV boluses	Not clearly assessed; None described
Stenzel 2013[24]	37 (39 examinations)	Average age 11.1 years (range 1 7/12 – 17 11/12 years)[β]	0.1ml / year, repeat dose as needed	Assessed; 1 child with nausea 15 minutes after UCA admin, continued for 30 minutes
Torres 2017[34]	173 (287 exams)	Mean age 11 years (range 0.1-18 years)[f]	0.1ml/kg up to 24kg; ≥24 kg received 2.4mL. Mean dose 2.3mL (0.1-8.1mL)	Assessed; None (One patient was itching a day after but this was attributed to fentanyl)
Yusuf 2017[17]	305 (some were intracavitary ie, not IV assessment. 147 were for liver lesions and 113 were for trauma – these are presumably the IV subcohort, though the number with IV exams could be higher)	1 month – 18 years	**See table below[δ]	Assessed; 2 patients with delayed adverse reactions that did not require intervention (1) tachycardia in a 4 yo undergoing liver imaging 3 hours after CEUS (patient had also received sedation for CT with IV contrast) and (2) a 15 yo male with a splenic laceration had hypertension 1 hour after CEUS.
Riccabona 2012[35]	948 IV examinations (retrospective survey 29 centers)	Newborn – 18 years Mean age 5 y	Not available	Assessed; 5 patients were reported to have had 6 minor side effects after IV contrast administration, with one having two symptoms (i.e. strange taste and skin reaction).

				The symptoms were minor skin reaction (n=2, urticaria or rash), unusual taste (n=3) and hyperventilation (n=1).

Footnotes: [ε]Weight 3 to 30 kg (mean 8kg) with no specific information about number of children <2y; [£]Youngest group for adrenal lesions but number of children <2yo not specifically listed; [¥]Total study group included imaging liver, spleen, kidney (3) and testes (1) where N=2 (0-27 days) with weight 3.4 +/-0.2 and dose 0.2 +/-0 with weight adjusted dose 0.06mL/kg+/-0, N=9 (28d to 23 mo) with weight 6.1 +/- 2.6 and dose 0.25 +/- 0.06 with weight adjusted dose 0.04 +/-0.04, N=5 (2-5y) with weight 15 +/-1.9 and dose 0.73 +/- 0.18 with weight adjusted dose 0.04+/-0.01; [α]No additional age information presented; [β]No additional information about age was provided; [ʃ]36 patients were ≤1 y and 41 ≤10 kg; [δ]Departmental dosing with no specific information given about the number of exams/patients in each category

**IV Dosing (ml) used in Yusuf 2017

age (y)	liver	spleen/kidney	testis/microvascular
0-6	0.6	0.6	4.8
6-12	1.2	1.2	4.8
12-18	2.4	1.2	4.8

The summary in Table 1 provides evidence that Lumason or Sonovue have both been safe within the proposed dosing parameters for this study without significant adverse events described in patients. In almost 1,800 IV administrations, there are only 2 reported cases of anaphylaxis in the pediatric literature. Lumason is on formulary and in use safely within our institution.

8.1 Clinical Adverse Events

Unanticipated problems involving risks to subjects and others will be monitored throughout the study.

8.2 Adverse Event Reporting

If any unanticipated problems related to the research involving risks to subjects or others happen during the course of this study (including SAEs) these will be reported to the IRB: 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. If abnormalities are incidentally identified, a referral to the division of cardiology will be made with appropriate follow up.

9 STUDY ADMINISTRATION

9.1 Data Collection and Management

Upon entry to the study all subjects will be given a study identifier code. The code will be used in all data acquisition (CE-TTE as well as age and other demographic information). The association between subject name and identifier will be kept in a list on a password protected computer in the locked office of Dr. Campbell. A unique research ID will be used when acquiring the imaging data, so that the images cannot be linked to the patient, except by using the master list. The images will be stored on the Syngo database.

Information that can identify subjects or image data may be kept in a separate computer database at A.I. duPont Hospital for Children; identifiable information will be destroyed at the earliest possible opportunity after study completion. Only the study doctors and those working with them on this study 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.

9.2 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 particular patient.

9.3 Regulatory and Ethical Considerations

9.3.1 Data and Safety Monitoring Plan

9.3.2 Risk Assessment

We believe this study to involve minor increase above minimal risk, relating to the use of the intravenous injection of an ultrasound contrast agent called Lumason. Ultrasound contrast is inherently different from MRI contrast and CT contrast agents. Ultrasound contrast agents take advantage of the fact that air reflects ultrasound beams. It has long been known that by agitating saline, small air bubbles are created, and when injected it into an IV, the bubbles appear very bright on ultrasound. This property has been utilized for many different ultrasound techniques, but is limited because the air bubbles do not move through the lungs. Additionally, these bubbles are very transient, and it is difficult to fully opacify different cardiac chambers. Ultrasound contrast agents like Lumason create microbubbles, which are microscopic bubbles of air suspended in a sulfur hexafluoride lipid sphere for stabilization. These microsphere bubbles are small enough to pass through capillary beds and remain in circulation longer than agitated saline. Most (80%) of the product is exhaled through the lungs, and the rest of the components are broken down and excreted in the liver and kidneys. There is about a 1/10,000 risk of anaphylactoid reaction to the medicine, which requires monitoring, as most reactions occur within 30 minutes of injection. Indeed, the only contraindication to use of Lumason is a prior hypersensitivity reaction to any of the ingredients in Lumason. Unlike contrast for CT or MRI, Lumason is not nephrotoxic. The most common adverse reaction is nausea. This agent has been well studied in children with minor complaints of flushing, dizziness, and mild headaches. In some instances this agent can cause an allergy-type reaction requiring medications and may be life-threatening, including hives, difficulty breathing, low blood pressure and vomiting. There will be safety measures in place to provide medical care should the patient have an allergy-type reaction. If the patient cannot tolerate the Lumason during the research, the echo will stop. The cumulative dose will not exceed the manufacturer recommended dose limit of a maximal individual injection dose of 2.4 mL and a maximal total dose of 4.8 mL (one vial of Lumason). All Lumason injections will be limited to a single vial of contrast. The CE-TTE will

likely extend the timing of the scheduled study by up to 30 minutes. Ultrasound technology has been proven to be extremely safe without any radiation risk to patients. No sedation will be required to perform the CE-TTE. Because these patients will already have an IV for their clinically indicated cardiac MRI, there is no increased risk from IV placement or withdrawal. The other risk is loss of privacy.

9.3.3 Potential Benefits of Trial Participation

There is no potential direct benefit to participating patients. However, there are potential benefits to future patients relating to the development of protocols that enhance image quality and improve information content, which may prevent the need for other more invasive imaging studies such as cardiac catheterization, sedation required to perform cardiac MRI, or radiation exposure related to CTA.

9.3.4 Risk-Benefit Assessment

The main risk is the risk of anaphylactoid type reaction to the lumason injection, which is a very rare complication. There will be no additional IV placement and no sedation will be used. There is no risk of kidney or liver damage with the use of this ultrasound-contrast agent.

9.4 Recruitment Strategy (or Case Ascertainment)

Patients with repaired congenital heart disease with residual pulmonary regurgitation who are referred to A.I. duPont Hospital for Children for clinically indicated cardiac MRIs will be recruited. As these patients are identified, the family will be approached prior to the date of the MRI and informed of the study. If the patients voice their assent over the phone, in person consent from the family and in person assent from the patient will be obtained on the day of the visit. On average, the radiology department performs about 60 clinically indicated cardiac MRIs on patients with repaired tetralogy of Fallot per year.

9.5 Informed Consent/Assent

Informed assent will be obtained from all patients who are able to understand the study, and informed consent will be obtained from adult subjects or the parent or guardian of minors. Permission of both parents is required by the Nemours IRB. Written consent may be obtained in person or virtually (by phone and mail/docusign). Consent forms are attached. A study team member will be responsible for obtaining the informed consent. Explanation of the possible risks will be made to the responsible adult subject, parent, or guardian before he or she is asked to sign the consent form.

9.6 Payment to Subjects/Families

Patients who volunteer for this project will receive $100 for time and effort in the study. Theywill they be charged for this aspect of the study.

10 PUBLICATION

As an indirect benefit for subjects enrolled in this study, there is a plan to publish the results of this study providing guidance as to the appropriate dosage of Lumason and echocardiographic settings for pediatric echocardiography of repaired congenital heart disease with residual pulmonary regurgitation. .

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