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Multicentre prospective observational study exploring the predictive value of functional echocardiographic indices for early identification of preterm neonates at risk of developing chronic pulmonary hypertension secondary to chronic neonatal lung disease

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ABSTRACT

Introduction Although chronic pulmonary hypertension (cPH) secondary to chronic neonatal lung disease is associated with increased mortality and respiratory and neurodevelopmental morbidities, late diagnosis (typically ≥36 weeks postmenstrual age, PMA) and the use of qualitative echocardiographic diagnostic criterion (flat interventricular septum in systole) remain significant limitations in clinical care. Our objective in this study is to evaluate the utility of relevant quantitative echocardiographic indices to identify cPH in preterm neonates, early in postnatal course and to develop a diagnostic test based on the best combination of markers.

Methods and analysis In this ongoing international prospective multicentre observational diagnostic accuracy study, we aim to recruit 350 neonates born <27 weeks PMA and/or birth weight <1000 g and perform echocardiograms in the third week of age and at 32 weeks PMA (early diagnostic assessments, EDA) in addition to the standard diagnostic assessment (SDA) for cPH at 36 weeks PMA. Predefined echocardiographic markers under investigation will be measured at each EDA and examined to create a scoring system to identify neonates who subsequently meet the primary outcome of cPH/death at SDA. Diagnostic test characteristics will be defined for each EDA. Pulmonary artery acceleration time and tricuspid annular plane systolic excursion are the primary markers of interest.

Ethics and dissemination Ethics approval has been received by the Mount Sinai Hospital Research Ethics Board (REB) (#16-0111-E), Sunnybrook Health Sciences Centre REB (#228-2016), NHS Health Research Authority (IRAS 266498), University of Iowa Human Subjects Office/Institutional Review Board (201903736), Rotunda Hospital Research and Ethics Committee (REC-2019-008), and UBC Children's and Women's REB (H19-02738), and is under review at Boston Children's Hospital Institutional Review Board. Study results will be disseminated to participating families in lay format, presented to the scientific community at paediatric and critical care conferences and published in relevant peer-reviewed journals.

Trail registration number NCT04402645.

Strengths and limitations of this study

- ► In a large prospective cohort of extremely premature neonates, quantitative echocardiographic markers are being acquired longitudinally using a standardised methodology.
- ► Simple and reliable point-of-care tests to characterise pulmonary vascular resistance (pulmonary artery acceleration time) and right ventricular function (tricuspid annular plane systolic excursion) are being systematically evaluated and shared with the clinical and scientific community to facilitate early diagnosis of chronic pulmonary hypertension.
- ► A robust sample size calculation to guide the recruitment strategy and 'blinded' analysis of echocardiograms is an integral part of the study design.
- ► The final diagnosis of chronic pulmonary hypertension, against which new early diagnostic tests are being evaluated, is based on qualitative diagnostic criteria, as per current standard of care.

INTRODUCTION

Background

Chronic pulmonary hypertension (cPH) is a debilitating and often life limiting condition

in which sustained vasoconstriction, vascular hypoplasia and arterial wall remodelling are associated with high and often progressive increases in pulmonary vascular resistance (PVR) and pulmonary arterial pressure, resulting in chronic exposure of the right ventricle to high afterload and ultimately leading to right ventricular (RV) failure.[1–4] The data from studies in adults and children indicate that early diagnosis and management focused on preservation of RV performance is a major determinant of treatment success and longevity.[5–10] Recognising its importance, the US National Heart, Lung and Blood Institute has recently identified 'determinants of RV function and failure' as a key priority for research.[5 9 10] In neonates, cPH is inextricably linked with developmental disorders of the lung, most commonly chronic neonatal lung disease (CNLD), a frequent complication following extreme preterm birth. It is characterised by inflammation and impaired alveolar development and pulmonary angiogenesis.[11–13] CNLD accounts for ~12% of all cases of cPH diagnosed during childhood and for the vast majority during infancy.[4 14 15] In Canada, CNLD affects 50%–60% of all extremely low birth weight neonates (ELBW, birth weight <1000 g) resulting in more than 800 new cases each year, of which 20%–30% will be diagnosed with cPH during their stay in the neonatal intensive care unit (NICU).[16]

Over the last decade, several retrospective and a few prospective studies consistently demonstrated an association of CNLD/cPH with higher predischarge and postdischarge mortality, longer duration of mechanical ventilation oxygen therapy, hospitalisation and higher rates of adverse neurodevelopmental outcomes compared with ELBW survivors with CNLD alone.[3 17–24] The incidence, severity and resultant mortality of cPH in CNLD is generally proportional to the severity of parenchymal lung disease.[25] A recent meta-analysis reported the pooled incidence of cPH to range from 4% (95% CI: 1% to 7%) in mild CNLD (ie, oxygen dependency ≥28 days but not at 36 weeks postmenstrual age, PMA), to 33% (95% CI: 21% to 44%) in severe CNLD (ie, oxygen dependency ≥28 days and need for ≥30% oxygen or positive pressure ventilation at 36 weeks PMA).[26] Patients with CNLD/cPH had higher odds of mortality versus only CNLD (OR: 5.29 (95% CI: 2.07 to 13.56)). In stark contrast to the expanding literature on clinical outcomes associated with cPH, there is little data to guide day-to-day management, including best practices regarding diagnostic and therapeutic strategies. To date, there is no treatment trial targeting cPH in preterm neonates. While expert bodies have published consensus-based guidelines to provide some direction to clinicians, it remains unknown whether a particular approach can ameliorate the extra burden of disease imposed by cPH on CNLD.[5] The optimal approach to the surveillance of cPH remains unclear and is a major barrier to timely and effective diagnosis and treatment in this population.[27] With this background, this project was envisioned to fill some of the key knowledge gaps in the diagnostic practices relating to cPH in this population.

Importance of early identification of cases

Although CNLD and its severity correlate with the risk of cPH, the diagnosis of CNLD is typically not made until 36 weeks PMA; currently, this is also the characteristic age when cPH evaluation is undertaken clinically using echocardiography. Late diagnosis, however, is a significant barrier for therapeutic trials to demonstrate clinical benefits. Similarly, early prophylactic treatment of all extreme premature infants is an undesirable approach, as it exposes patients without disease to pharmacotherapies. This will adversely affect the risk-benefit profile for therapies, increase treatment costs and necessitate a larger sample size for clinical trials. Several potential therapies are available, and, despite lack of trial data, are already being used in clinical practice, although often as rescue therapy for severe disease.[1 5 28 29] Day-to-day clinical care is significantly hampered by the absence of a diagnostic test that allows case identification early in disease course. In early stages, the disease may be more functional, determined by sustained pulmonary vasoconstriction and relatively less by anatomical remodelling, and may be more amenable to treatments. Thus, the overall aim of this study is to systematically develop quantitative echocardiographic diagnostic criteria which will allow for the identification of neonates with significant pulmonary vascular disease early in postnatal life (figure 1). A significant reduction in lag between disease onset and diagnosis will offer new avenues for testing therapeutic interventions; a necessary prerequisite for trials to have the maximum chance of success.

Need for quantitative echocardiographic diagnostic test for early cPH diagnosis in premature neonates

Challenges in the clinical evaluation of at-risk premature neonates include associated parenchymal lung disease, which often masks the symptoms of evolving cPH and RV dysfunction,[30 31] non-applicability or delayed appearance of specific clinical signs (eg, raised jugular venous pressure and systemic venous congestion) and non-feasibility of a gold standard test (right heart catheterisation) due to small patient size and high risk for complications.[10 32–34] Cardiac MRI, a clinical reference investigation for the assessment of RV function in older patients, is cumbersome and not feasible during early disease in the majority of patients due to clinical instability and need for out-of-facility transport. When feasible, these investigations can only be performed sporadically, late in the disease course and are not suitable for longitudinal assessments. Two-dimensional echocardiography is a safe, quick, well-tolerated, non-invasive bedside investigation which is ideally suited for longitudinal assessments. Although it is considered the clinical investigation of choice for the assessment of heart function and pulmonary haemodynamics in neonates, it has not been utilised to its full potential in neonatal cPH.

Previous studies have advanced our understanding of cPH in neonates; however, their key limitation is the use of qualitative subjective diagnostic criteria—'flat

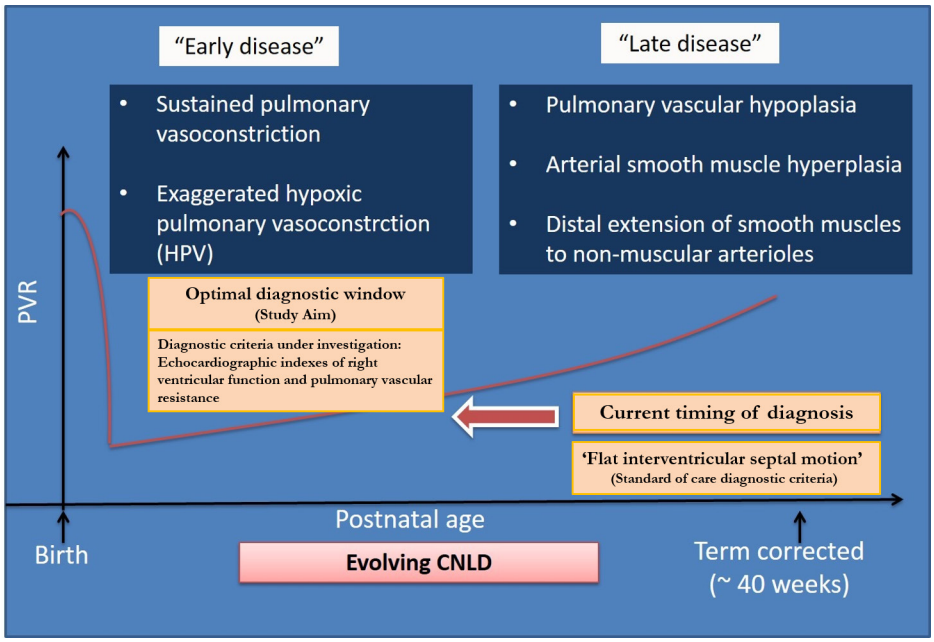

Figure 1 In early stage of chronic pulmonary hypertension (cPH), the disease is expected to be more functional, determined by sustained pulmonary vasoconstriction and relatively less by 'fixed' anatomical remodelling, hence, may be more amenable to treatments. The overall aim of this study is to develop new diagnostic criteria sensitive enough to identify extreme premature neonates with significant pulmonary vascular disease, who subsequently will be diagnosed with cPH secondary to chronic neonatal lung disease. CNLD, chronic neonatal lung disease; PVR, pulmonary vascular resistance

interventricular septum' in the majority of cases. This index represents a qualitative assessment for the loss of normal concave curvature of the septal wall between the right and the left ventricle; this is thought to reflect RV systolic pressure >1/2 systemic systolic pressure.[35 36] Presently, based on above studies, this criterion is used as the standard method to diagnose cPH in NICUs. Though easily assessable in the majority of patients and specific for severe disease, it is unsuitable for early diagnosis or close monitoring of disease progression.[18] For these considerations, these criteria are not usually relied on by clinicians in older patients.[37] Only one-third of neonates eventually diagnosed with cPH could be identified using these criteria in the fourth week of life.[18] Many quantitative echocardiographic markers relevant to the study of cPH are now widely available for use in neonates, in particular those representing RV function and PVR.[38] Several of these have been validated and shown to be of prognostic significance with clearly identified cut-off values for clinical use in adults with cPH.[39] This protocol seeks to address at least three of the domains to improve diagnostic precision and clinical monitoring of cPH in preterm infants recently identified by the Paediatric Pulmonary Hypertension Network.[27] These areas are: (1) the standardisation of echocardiographic characterisation of cPH, (2) the use of echocardiography to identify a risk profile for early prediction of risk, diagnosis and follow-up and (3) enhanced assessment of RV performance and afterload using novel quantitative echocardiographic techniques. This will be the first study systematically examining the predictive value of novel echocardiographic markers to develop a diagnostic test for early diagnosis of cPH in the infants with gestational age (GA) <27 weeks or BW <1000 g.

RESEARCH HYPOTHESIS AND STUDY OBJECTIVES

We hypothesise that quantitative echocardiographic indices of RV function and PVR will allow early diagnosis of cPH prior to 36 weeks PMA in extreme premature infants; thus, reducing the time to diagnosis compared with contemporary clinical practice. More specifically, we hypothesise that pulmonary artery acceleration time (PAAT, a simple marker of PVR validated in adults and children against invasive measurements) and tricuspid annular plane systolic excursion (TAPSE, a routinely used marker of RV function) either alone or in combination will be the most useful parameters for early case identification.

The *primary objective* of this study is to evaluate the ability of relevant quantitative functional echocardiographic indices, in particular PAAT and TAPSE, to identify preterm neonates with cPH early in postnatal life, develop a diagnostic test based on the best combination of parameters and define its diagnostic characteristics (sensitivity, specificity, positive and negative likelihood ratios). Our *secondary objectives* are (1) To test the relationship between the developed diagnostic test and clinical outcomes known to be affected by cPH (mortality and respiratory morbidities). This is important for clinical corroboration of our new criteria and will provide the necessary preliminary data to enable sample size calculations for subsequent therapeutic trials, targeting improvement in clinical outcomes. (2) To longitudinally

study the myocardial maturation pattern in extreme premature neonates and evaluate how it may be affected by occurrence of cPH. This is important as preliminary work indicates that occurrence of CNLD/cPH may negatively impact cardiac maturational patterns in preterm infants.[40] However, this observation needs further validation and the relative impact of CNLD versus cPH on cardiac development is not known. (3) To identify clinical risk factors associated with occurrence of cPH in this cohort. (4) To establish a large representative cohort of high-risk preterm neonates to pursue future long-term follow-up studies to understand their development and respiratory morbidities during childhood.

METHODS AND ANALYSIS

Design, setting and study population

This study is a prospective, multisite, observational cohort study being conducted in five tertiary NICUs across Canada, the USA, UK and Ireland. The study teams from the six participating sites have been trained in the standardised procedure of enrollment, echocardiography and data collection. At its inception, the study was being conducted at two tertiary NICUs in Toronto, Mount Sinai Hospital (start date 1 November 2017) and Sunnybrook Health Sciences Centre (start date 31 January 2018). Subsequently, to help accelerate study completion, the following additional sites were recruited: Liverpool Women's NHS Foundation Trust (start date 6 November 2019), the Rotunda Hospital (start date 20 November 2019), the University of Iowa Stead Family Children's Hospital (start date 6 July 2020) and BC Women's Hospital and Health Centre (start date 7 September 2020). The study protocol is also currently under review at Boston Children's Hospital Institutional Review Board. These additional sites were selected based on their knowledge in similar patient populations and study settings as the primary study sites and their similar echocardiography equipment, analysis software and presence of a formal in-house neonatal echocardiography programme led by an experienced neonatologist with formal training in neonatal echocardiography, all of whom are site collaborators in this study.

Infants are considered eligible for enrollment if they are born at GA <27 weeks and/or with a birth weight (BW) <1000 g, and are cleared by the attending clinical team to approach for informed consent between 10 and 14 days of age. Neonates with major congenital and/or genetic anomalies and congenital heart defects (with the exception of patent ductus arteriosus (PDA), patent foramen ovale (PFO), peripheral pulmonary artery stenosis or small (<3 mm diameter) ventricular septal defects) are not considered eligible for recruitment.

Recruitment and study procedures

Families of eligible neonates are approached for consent between 10 and 20 days postnatal age. All recruited neonates undergo three serial cardiopulmonary evaluations: early diagnostic assessments (EDAs) at two predefined time points (study interventions, between 14 and 21 days of age (inclusive) and again between 32nd and 33rd weeks PMA) and a standard diagnostic assessment (SDA) at ≥36±2 weeks PMA (standard clinical care). Each EDA consists of collecting relevant clinical data and a focused echocardiogram to collect indices representative of PVR and RV functions, while SDA consists of an echocardiogram to allow assigning the final diagnosis of cPH using the current clinical definition (figure 2). Each assessment is carried out by a cardiac sonographer or fellow trained in neonatal haemodynamics, with infants in a non-agitated state and is coordinated with any one of the routine handling times on that day. In the event of an intercurrent illness or procedure, such as sepsis, necrotising enterocolitis or treatment for retinopathy, assessments are deferred until the neonate is deemed to have recovered by the attending neonatologist. Blinded analysis of all EDA echocardiograms to measure parameters under investigation (table 1) is completed by a centralised trained senior research sonographer, at arm's length from the study, who remains blinded to all clinical data. The echocardiogram done for SDA will be analysed by separate personnel to categorise infant as cPH or not based

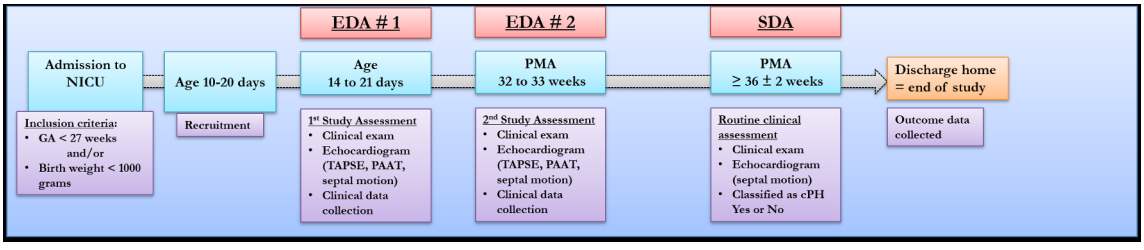

Figure 2 Schematic representation of planned study interventions. Each infant, after obtaining informed parental consent, will undergo two sequential early diagnostic assessments (EDAs) at predefined time points, followed by a standard diagnostic assessment (SDA) to categorise study cohort as chronic pulmonary hypertension (cPH) or no cPH, as per the standard currently used clinical definition. Blinded measurements will be performed for tricuspid annular plane systolic excursion (TAPSE, a marker of right ventricular function) and pulmonary artery acceleration time (PAAT, a marker of pulmonary vascular resistance) at both EDAs to calculate their early diagnostic characteristics (sensitivity, specificity and positive and negative likelihood ratios) to diagnose cPH by comparing to eventual diagnosis made at SDA. GA, gestational age; NICU, neonatal intensive care unit; PMA, postmenstrual age.

Table 1 Echocardiographic variables measured on study echocardiograms

Index name	Description	Measurement	Study specific
Pulmonary artery acceleration time (PAAT) (Primary index)	A marker of pulmonary vascular resistance (inversely related).	Interval between the onset of ejection and peak flow velocity measured from a pulse wave Doppler tracing obtained by placing a 2 mm sample volume in the middle of the main pulmonary artery, at the tip of pulmonary valve leaflets.	In addition, the ratio of total right ventricular (RV) ejection time to PAAT is also measured as a heart rate independent measure of PVR (directly related).
Tricuspid annular plane systolic excursion (TAPSE) (primary index)	A marker of RV longitudinal systolic function.	Measure of the downward displacement of tricuspid annulus during contraction.	From an apical four-chamber view by placing M-mode cursor through the tricuspid valve annulus.
Two-dimensional peak global end-systolic longitudinal strain (pLS)—right ventricle (secondary index)	A measure of myocardial deformation expressed as percentage change in length in systole from the baseline at end diastole.	Performed offline using software, which uses frame to frame tracking of the unique ultrasound speckles within the myocardial wall.	Longitudinal strain will be calculated for RV lateral and inferior wall from RV apical four chamber and three chamber views, respectively.
Fractional area change (FAC-4C) % (secondary index)	A surrogate to ejection fraction and indicative of overall 'pump function'.	Manual tracing of the RV endocardial borders at respective phases of the cardiac cycle.	From an apical four chamber view the end systolic area (ESA) and end diastolic area (EDA) are measured. FAC-4C (%) =((EDA-ESA)/EDA)x 100
Tissue Doppler imaging (TDI) (secondary index)	Allows measurement of velocities directly from the myocardium to assess longitudinal systolic and diastolic RV function.	Measure the peak systolic and diastolic velocities, and duration of various phases of a cardiac cycle.	Measurements obtained at the RV basal segment.
RV peak systolic pressure (RVsP) (secondary index)	A direct measure of pulmonary artery systolic pressure.	Calculated by measuring peak velocity (v) of tricuspid regurgitation using the modified Bernoulli equation. RVsP=$4v^2$+right atrial pressure.	Calculated whenever feasible. Right atrial pressure is predefined as 5 mm Hg in all cases.
Assessment of shunts (secondary index)	Patent ductus arteriosus and patent foramen ovale/atrial septal defect.	Visualised on colour Doppler assessment.	Shunt diameter in millimetre and flow direction documented.
Flow patterns in main and branch pulmonary arteries (secondary index)	Presence of notching is considered a marker of high downstream resistance	Visualised on pulse wave Doppler	Presence or absence of midsystolic notching in the Doppler profile.

PVR, pulmonary vascular resistance.

on the currently used definition, as noted above. If study participants have clinically indicated scans after 36 weeks PMA, the data regarding outcome of cPH are collected. Clinical data, including baseline demographics, respiratory illness severity during first 3 weeks of age and exposure to relevant but unrelated pre-existing morbidities and treatments are also collected in addition to data regarding common neonatal outcomes at discharge and from the infants' 18-month follow-up neurodevelopmental clinical assessment. At the end of the recruitment, all deidentified data will be sent for analysis to a statistician blinded to all other aspects of this study.

Rationale for primary parameter selection

PAAT and TAPSE are simple, reliable, bedside tests, which, in older patients, have been validated[30 41–49] and correlate with prognosis in cPH.[30 50–53] The normal data for neonates are well established and these markers are commonly used in NICUs, including study NICUs, for longitudinal monitoring; however, no specific diagnostic cut-offs currently exist. In previous work, we have established the reproducibility and normal values for various markers of ventricular function and PVR in neonates;[54 55] intraclass correlation coefficient for PAAT and TAPSE was 0.90 and 0.97, respectively. We then confirmed the ability

of PAAT to detect reduction in PVR in healthy neonates during first day after birth.[56] Among RV markers, TAPSE was found sensitive to these changes,[57] while no change was seen on septal flattening.

Study definitions

CPH: infants will be classified as having significant cPH if they meet any of the following features at SDA:[17]

1. Right ventricular systolic pressure (RVSP) ≥0.5 systolic blood pressure.
2. Bidirectional (right-left in systole) or a right to left shunt across a PDA.
3. A flat interventricular septal motion in end-systole.

Infants who meet the above criteria on EDAs will only be classified as cPH if the findings persist until SDA or if severity increases enough for clinicians to initiate cPH treatment.

CNLD: infants are assessed at 36 weeks PMA to classify the severity of CNLD based on standard well-defined objective clinical criteria:[58]

1. Mild CNLD: supplemental oxygen requirement for ≥28 days but off all respiratory support at 36 weeks PMA or discharge, whichever is earlier.
2. Moderate CNLD: supplemental oxygen requirement for ≥28 days and at 36 weeks PMA but fractional inspired oxygen concentration (FiO_2) <0.30.
3. Severe CNLD: supplemental oxygen requirement for ≥28 days and at 36 weeks PMA with fractional inspired oxygen concentration (FiO_2) ≥0.30 or dependent on any positive pressure ventilatory support.

Infants who are discharged from study NICU before 36 weeks PMA will be classified based on respiratory status at discharge.

Outcome measures

Primary clinical outcome

The primary clinical outcome is final diagnosis of cPH, made based on above definition at SDA. Our intention is to develop a risk score using a combination of echocardiographic markers, at each EDA, predicting the primary clinical outcome. A cut-off point of the risk score will then be determined using Youden's method to define a new diagnostic test at each EDA. The sensitivity and specificity of the new diagnostic test for early prediction of cPH will be estimated.

Secondary clinical outcomes

Key prematurity-related clinical outcomes including composite of predischarge mortality or CNLD, CNLD, total duration of need for respiratory support, length of hospital stay, discharge on home oxygen and neurodevelopmental status at 18–24 months corrected age. These secondary clinical outcomes are those which are well known to be worsen in relation to the diagnosis of cPH in preterm neonates. These will be used to corroborate and test the clinical validity of our newly identified early cPH diagnostic test, as we anticipate that the infants identified as cPH with the new diagnostic test developed by this study would demonstrate more adverse clinical outcomes than those without cPH.

Statistical methods

Sample size calculation:

The incidence of cPH in the study population is estimated to at least 20%. The mean and SD or sensitivity and specificity of echocardiographic markers during the EDA time points are not known, making specific sample size calculations non-feasible. Presuming a disease prevalence of 20%, to estimate new diagnostic test with a sensitivity of 85% and an absolute precision of 0.07, a sample size of approximate 350 babies will be required.[59] The actual incidence of cPH at SDA will be evaluated at the halfway stage in the recruited cohort, to confirm representativeness of the estimated incidence. The sample size calculation may be revised if the incidence in the recruited cohort is found to be significantly higher or lower than the estimate.

Analysis

- The study population will be summarised descriptively. The baseline infant characteristics (including BW z-score), clinical measurements and echocardiographic indices (ie, PAAT and TAPSE), as well as the presence of shunts such as atrial septal defect, at each EDA will be compared between two groups: infants with/without cPH using the χ^2 test for categorical variables and the student t-test or Wilcoxon Rank-Sum test for continuous variables, as appropriate. Changes over time in echocardiographic indices will also be compared using two-way repeat measures analysis of variance.
- We will conduct a multiple logistic regression at each EDA using backward variable selection procedure with variable stay criterion of p<0.1. All echocardiographic indices associated with cPH identified in the bivariate analysis (p<0.1) or based on clinical expertise, including the presence of shunts if relevant, will be included in the full model. Denote the final model as

  $$\text{logitPr}\left(\text{cPH} = \text{yes}\right) = a0 + a1x1 + a2x2 + \ldots + akxk \quad \ldots (1)$$

  where x1, …, xk are the echocardiographic indexes that remain. A risk score (RScore) will then be defined as Rscore= a0 + a1x1 + a2x2 + . . . + akxk. A new diagnostic test can be defined as DTest=(Rscore <u0), where DTest=1 predicts cPH and the cut-off u0 is determined based on the Youdon method such that the sum of the sensitivity and specificity of DTest is maximised. The diagnostic characteristics such as sensitivity, the specificity, positive and negative predictive values of the new diagnostic test will be reported for each EDA. We will also analyse each individual echocardiographic index under investigation (ie, PAAT and TAPSE) and report their diagnostic characteristics using similar methods.
- In addition, the association between the neonatal outcomes and the newly developed diagnostic test

will be assessed, by comparing the outcomes between infants with/without cPH defined based on the new diagnostic test using χ^2test. Multivariable regression analysis will also be performed to investigate the association between the new diagnostic test and subsequent occurrence of composite outcome of CNLD or death, adjusted for potential confounders identified in the univariate analysis. Similarly, the association between the outcomes and the key early-diagnostic echocardiographic parameter(s) identified in the analyses mentioned above will be examined.

- Further, since male sex has consistently been shown to be associated with worse neonatal outcomes, we will also include an interaction term between sex and the combination of echocardiographic indices in the model (1). A significant in the interaction indicates that the performance of the diagnostic test may vary between males and females. If this is the case, subgroup analysis stratified by sex will be conducted to develop a sex-specific diagnostic test for cPH.
- To identify risk factors for diagnosis of early cPH, we will also compare demographics and baseline variables, including pre-existing morbidities, between patients who are diagnosed with cPH using the newly developed diagnostic test and those who do not meet the criteria of early cPH diagnosis. Multivariable regression analysis will also be performed to investigate the association between cPH diagnosis and baseline variables.
- Intervariability for the diagnosis of cPH as per currently practiced qualitative criteria will be reported using Cohen's kappa statistic in 40 random deidentified scans (20 each with and without cPH), while reliability for TAPSE and PAAT will reported as intraclass correlation coefficient, Bland Altman analysis and coefficient of variability.

Data management

Maternal and neonatal data are collected in a standardised format (data collection forms approved by the research ethics boards at all participating sites) and stored in a password protected electronic file on a secure server. For the purpose of confidentiality, the data are entered using the participant's study number and all identifying information will be removed prior to data analysis. A separate log is maintained linking the study number to the infants' identifying information. Only the study coordinator at each site has access to this file. Study echocardiograms are stored on a secure hospital network and measurements are performed offline using GE EchoPAC software version 202 (GE Vingmed Ultrasound AS, Horten, Norway). Some recruited infants may not have an SDA due to discharge from the study NICUs prior to reaching eligibility age. Infants discharged in room air to community hospitals with no SDA results will be presumed to have no cPH, as cPH is known to be rare in infants off respiratory support <36 weeks PMA. However, infants discharged on respiratory support without SDA will be excluded and additional participants will be recruited to ensure that all 350 included infants have at least the first EDA (third week of life) and outcome of death or cPH.

PATIENT AND PUBLIC INVOLVEMENT

A range of stakeholders and partners have been actively involved in the design and conduct of this observational study including healthcare providers in participating NICUs, paediatric cardiologists, members of the Canadian Neonatal Network (CNN), respiratory therapists, neonatal nurses and parent groups. Their input was sought during protocol design regarding the selection of echocardiographic markers most suitable for clinical translation and their potential for early detection of cPH. The burden of serial echocardiographic assessments on preterm neonates was also carefully assessed in consultation with these groups. All stakeholders and partners are updated at regular intervals about the study's progress in order to identify facilitators and barriers to study conduct and knowledge translation. Following study completion, we plan to discuss future directions, including a multicentre clinical trial based on study findings, in consultation with neonatologists, paediatricians, healthcare practitioners and parent-led community groups.

ETHICS AND DISSEMINATION

This study was approved for the coordinating site (Mount Sinai Hospital) by the Research Ethics Board at Mount Sinai Hospital on 17 June 2016 (REB ID#16-0111-E). Documented approval was obtained from the Research Ethics Board/Institutional Review Board for all participating centres prior to participant enrollment at that centre. Parents, as the legal substitute decision-maker, have the opportunity to read the approved consent form and ask questions as needed. Once all questions are answered and parents confirm their understanding of study procedures, parents are invited to provide voluntary written informed consent on behalf of their child.

All coauthors will facilitate knowledge dissemination among key stakeholders, as listed above, providing the network necessary to support future multicentre randomised clinical trials. As done previously, we will work with national (CNN, Canadian Paediatric Society, Children's Healthcare Canada) and provincial (the Provincial Council of Maternal and Child Health) child health advocacy organisations, using our research findings to drive evidence-informed changes in clinical practice.[60] The results of this study will also be disseminated to participating families in a lay format and communicated to a wider scientific and healthcare community through end of study meetings, presentations at national and international paediatric and critical care conferences and high-impact peer-reviewed publications. Information regarding the use of specific echocardiographic diagnostic criteria to identify extreme preterm neonates with early significant pulmonary vascular disease will be

shared with clinicians across various disciplines including neonatologists, cardiologists, trainees, developmental paediatricians and other community-based healthcare practitioners. Study results are also intended to be shared with software companies of echocardiography technology in order to inform the development of equipment specific to the needs of preterm infants. Finally, these findings will be communicated to policy-makers to identify priority areas of care, to reduce burden on families and to reduce costs to the healthcare system by organising tailored follow-up services for these neonates.

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Contributors LT is the lead coordinator overseeing all study sites and is responsible for all patient recruitment and acquisition of data in Toronto along with MB. PD, AK, SJ, FZ and DI-R are involved in the completion of all study echocardiographic assessments in Toronto. AS is responsible for patient recruitment and the completion of study echocardiographic assessments in Dublin. AJ, DEW, EN, JT, MC, PL, AFE-K, REG and PJM are the principal investigators at their respective sites and were all extensively involved in the study concept, design and completion of echocardiographic assessments. PSS, XYY, LM and RPJ are involved in the development of the analysis plan. All the authors have extensively reviewed the protocol/final manuscript. All authors read and approved the final manuscript.

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REFERENCES

1 Ambalavanan N, Mourani P. Pulmonary hypertension in bronchopulmonary dysplasia. *Birth Defects Res A Clin Mol Teratol* 2014;100:240–6.
2 Berger RMF, Beghetti M, Humpl T, *et al*. Clinical features of paediatric pulmonary hypertension: a registry study. *Lancet* 2012;379:537–46.
3 Khemani E, McElhinney DB, Rhein L, *et al*. Pulmonary artery hypertension in formerly premature infants with bronchopulmonary dysplasia: clinical features and outcomes in the surfactant era. *Pediatrics* 2007;120:1260–9.
4 Mourani PM, Abman SH. Pulmonary vascular disease in bronchopulmonary dysplasia: pulmonary hypertension and beyond. *Curr Opin Pediatr* 2013;25:329–37.
5 Abman SH, Hansmann G, Archer SL, *et al*. Pediatric pulmonary hypertension: guidelines from the American heart association and American thoracic Society. *Circulation* 2015;132:2037–99.
6 Bogaard HJ, Abe K, Vonk Noordegraaf A, *et al*. The right ventricle under pressure: cellular and molecular mechanisms of right-heart failure in pulmonary hypertension. *Chest* 2009;135:794–804.
7 Bogaard HJ, Natarajan R, Henderson SC, *et al*. Chronic pulmonary artery pressure elevation is insufficient to explain right heart failure. *Circulation* 2009;120:1951–60.
8 Haddad F, Doyle R, Murphy DJ, *et al*. Right ventricular function in cardiovascular disease, part II: pathophysiology, clinical importance, and management of right ventricular failure. *Circulation* 2008;117:1717–31.
9 Voelkel NF, Gomez-Arroyo J, Abbate A, *et al*. Mechanisms of right heart failure-A work in progress and a plea for failure prevention. *Pulm Circ* 2013;3:137–43.
10 Voelkel NF, Quaife RA, Leinwand LA, *et al*. Right ventricular function and failure: report of a national heart, lung, and blood Institute Working group on cellular and molecular mechanisms of right heart failure. *Circulation* 2006;114:1883–91.
11 Thébaud B, Abman SH. Bronchopulmonary dysplasia: where have all the vessels gone? roles of angiogenic growth factors in chronic lung disease. *Am J Respir Crit Care Med* 2007;175:978–85.
12 Abman SH. Bronchopulmonary dysplasia: "a vascular hypothesis". *Am J Respir Crit Care Med* 2001;164:1755–6.
13 Thébaud B, Ladha F, Michelakis ED, *et al*. Vascular endothelial growth factor gene therapy increases survival, promotes lung angiogenesis, and prevents alveolar damage in hyperoxia-induced lung injury: evidence that angiogenesis participates in alveolarization. *Circulation* 2005;112:2477–86.
14 Parker TA, Abman SH. The pulmonary circulation in bronchopulmonary dysplasia. *Semin Neonatol* 2003;8:51–61.
15 Farquhar M, Fitzgerald DA. Pulmonary hypertension in chronic neonatal lung disease. *Paediatr Respir Rev* 2010;11:149–53.
16 NetworkTM CN. Annual report, 2015. Available: http://wwwcanadianneonatalnetworkorg/Portal/LinkClickaspx?fileticket=TfREFhFBcvc%3d&tabid=39
17 An HS, Bae EJ, Kim GB, *et al*. Pulmonary hypertension in preterm infants with bronchopulmonary dysplasia. *Korean Circ J* 2010;40:131–6.
18 Bhat R, Salas AA, Foster C, *et al*. Prospective analysis of pulmonary hypertension in extremely low birth weight infants. *Pediatrics* 2012;129:e682–9.
19 del Cerro MJ, Sabaté Rotés A, Cartón A, *et al*. Pulmonary hypertension in bronchopulmonary dysplasia: clinical findings, cardiovascular anomalies and outcomes. *Pediatr Pulmonol* 2014;49:49–59.
20 Fitzgerald D, Evans N, Van Asperen P, *et al*. Subclinical persisting pulmonary hypertension in chronic neonatal lung disease. *Arch Dis Child Fetal Neonatal Ed* 1994;70:F118–22.
21 Kim D-H, Kim H-S, Choi CW, *et al*. Risk factors for pulmonary artery hypertension in preterm infants with moderate or severe bronchopulmonary dysplasia. *Neonatology* 2012;101:40–6.
22 Slaughter JL, Pakrashi T, Jones DE, *et al*. Echocardiographic detection of pulmonary hypertension in extremely low birth weight infants with bronchopulmonary dysplasia requiring prolonged positive pressure ventilation. *J Perinatol* 2011;31:635–40.
23 Waruingi W, Mhanna MJ. Pulmonary hypertension in extremely low birth weight infants: characteristics and outcomes. *World J Pediatr* 2014;10:46–52.
24 Choi EK, Shin SH, Kim E-K, *et al*. Developmental outcomes of preterm infants with bronchopulmonary dysplasia-associated

pulmonary hypertension at 18-24 months of corrected age. *BMC Pediatr* 2019;19:26.
25 Arjaans S, Haarman MG, Roofthooft MTR, *et al*. Fate of pulmonary hypertension associated with bronchopulmonary dysplasia beyond 36 weeks postmenstrual age. *Arch Dis Child Fetal Neonatal Ed* 2021;106:45–50.
26 Al-Ghanem G, Shah P, Thomas S, *et al*. Bronchopulmonary dysplasia and pulmonary hypertension: a meta-analysis. *J Perinatol* 2017;37:414–9.
27 Levy PT, Jain A, Nawaytou H, *et al*. Risk assessment and monitoring of chronic pulmonary hypertension in premature infants. *J Pediatr* 2020;217:199–209.
28 Krishnan U, Krishnan S, Gewitz M. Treatment of pulmonary hypertension in children with chronic lung disease with newer oral therapies. *Pediatr Cardiol* 2008;29:1082–6.
29 Mourani PM, Sontag MK, Ivy DD, *et al*. Effects of long-term sildenafil treatment for pulmonary hypertension in infants with chronic lung disease. *J Pediatr* 2009;154:379–84.
30 Benatar A, Clarke J, Silverman M. Pulmonary hypertension in infants with chronic lung disease: non-invasive evaluation and short term effect of oxygen treatment. *Arch Dis Child Fetal Neonatal Ed* 1995;72:F14–19.
31 Forfia PR, Vaidya A, Wiegers SE. Pulmonary heart disease: the heart-lung interaction and its impact on patient phenotypes. *Pulm Circ* 2013;3:5–19.
32 Galiè N, Torbicki A, Barst R, *et al*. Guidelines on diagnosis and treatment of pulmonary arterial hypertension. The task force on diagnosis and treatment of pulmonary arterial hypertension of the European Society of cardiology. *Eur Heart J* 2004;25:2243–78.
33 Galiè N, Hoeper MM, Humbert M, *et al*. Guidelines for the diagnosis and treatment of pulmonary hypertension: the task force for the diagnosis and treatment of pulmonary hypertension of the European Society of cardiology (ESC) and the European respiratory Society (ERS), endorsed by the International Society of heart and lung transplantation (ISHLT). *Eur Heart J* 2009;30:2493–537.
34 Bossone E, D'Andrea A, D'Alto M, *et al*. Echocardiography in pulmonary arterial hypertension: from diagnosis to prognosis. *J Am Soc Echocardiogr* 2013;26:1–14.
35 Jone P-N, Ivy DD. Echocardiography in pediatric pulmonary hypertension. *Front Pediatr* 2014;2:124.
36 Ryan T, Petrovic O, Dillon JC, *et al*. An echocardiographic index for separation of right ventricular volume and pressure overload. *J Am Coll Cardiol* 1985;5:918–24.
37 Bellsham-Revell HR, Simpson JM, Miller OI, *et al*. Subjective evaluation of right ventricular systolic function in hypoplastic left heart syndrome: how accurate is it? *J Am Soc Echocardiogr* 2013;26:52–6.
38 Rudski LG, Lai WW, Afilalo J, *et al*. Guidelines for the echocardiographic assessment of the right heart in adults: a report from the American Society of echocardiography endorsed by the European association of echocardiography, a registered branch of the European Society of cardiology, and the Canadian Society of echocardiography. *J Am Soc Echocardiogr* 2010;23:685–713.
39 Naeije R. Assessment of right ventricular function in pulmonary hypertension. *Curr Hypertens Rep* 2015;17:35.
40 Levy PT, El-Khuffash A, Patel MD, *et al*. Maturational patterns of systolic ventricular deformation mechanics by two-dimensional Speckle-Tracking echocardiography in preterm infants over the first year of age. *J Am Soc Echocardiogr* 2017;30:685–98.
41 Kjaergaard J, Petersen CL, Kjaer A, *et al*. Evaluation of right ventricular volume and function by 2D and 3D echocardiography compared to MRI. *Eur J Echocardiogr* 2006;7:430–8.
42 Koestenberger M, Nagel B, Ravekes W, *et al*. Systolic right ventricular function in preterm and term neonates: reference values of the tricuspid annular plane systolic excursion (TAPSE) in 258 patients and calculation of Z-score values. *Neonatology* 2011;100:85–92.
43 Dabestani A, Mahan G, Gardin JM, *et al*. Evaluation of pulmonary artery pressure and resistance by pulsed Doppler echocardiography. *Am J Cardiol* 1987;59:662–8.
44 Kitabatake A, Inoue M, Asao M, *et al*. Noninvasive evaluation of pulmonary hypertension by a pulsed Doppler technique. *Circulation* 1983;68:302–9.
45 Kosturakis D, Goldberg SJ, Allen HD, *et al*. Doppler echocardiographic prediction of pulmonary arterial hypertension in congenital heart disease. *Am J Cardiol* 1984;53:1110–5.
46 Levy PT, Patel MD, Groh G, *et al*. Pulmonary artery acceleration time provides a reliable estimate of invasive pulmonary hemodynamics in children. *J Am Soc Echocardiogr* 2016;29:1056–65.
47 Matsuda M, Sekiguchi T, Sugishita Y, *et al*. Reliability of non-invasive estimates of pulmonary hypertension by pulsed Doppler echocardiography. *Br Heart J* 1986;56:158–64.
48 Urboniene D, Haber I, Fang Y-H, *et al*. Validation of high-resolution echocardiography and magnetic resonance imaging vs. high-fidelity catheterization in experimental pulmonary hypertension. *Am J Physiol Lung Cell Mol Physiol* 2010;299:L401–12.
49 Yared K, Noseworthy P, Weyman AE, *et al*. Pulmonary artery acceleration time provides an accurate estimate of systolic pulmonary arterial pressure during transthoracic echocardiography. *J Am Soc Echocardiogr* 2011;24:687–92.
50 Forfia PR, Fisher MR, Mathai SC, *et al*. Tricuspid annular displacement predicts survival in pulmonary hypertension. *Am J Respir Crit Care Med* 2006;174:1034–41.
51 Grangl G, Pansy J, Burmas A, *et al*. Tricuspid annular plane systolic excursion is reduced in infants with pulmonary hypertension: value of tricuspid annular plane systolic excursion (TAPSE) to determine right ventricular function in various conditions of pediatric pulmonary hypertension. *Echocardiography* 2015;32:883–4.
52 Malowitz JR, Forsha DE, Smith PB, *et al*. Right ventricular echocardiographic indices predict poor outcomes in infants with persistent pulmonary hypertension of the newborn. *Eur Heart J Cardiovasc Imaging* 2015;16:1224–31.
53 Zakaria D, Sachdeva R, Gossett JM, *et al*. Tricuspid annular plane systolic excursion is reduced in infants with pulmonary hypertension. *Echocardiography* 2015;32:834–8.
54 Jain A, El-Khuffash AF, Kuipers BCW, *et al*. Left ventricular function in healthy term neonates during the transitional period. *J Pediatr* 2017;182:197-203.e2.
55 Jain A, Mohamed A, El-Khuffash A, *et al*. A comprehensive echocardiographic protocol for assessing neonatal right ventricular dimensions and function in the transitional period: normative data and Z scores. *J Am Soc Echocardiogr* 2014;27:1293–304.
56 Jain AM, Mertens A, Kavanagh L. Cardio-Pulmonary circulation during the first 24 hours of life – using echocardiography to delineate changes in pulmonary vascular resistance (Pvr) in well human neonates. *American Thoracic Society (ATS) International conference*, 2013.
57 Jain AMA, Mertens L, Kavanagh BP. Cardiac adaptation during the first 24 hours of Life in well human neonates. Pediatric Academic Societies' (PAS) Annual Meeting, San Francisco, May (Abstract) 2017.
58 Jobe AH, Bancalari E. Bronchopulmonary dysplasia. *Am J Respir Crit Care Med* 2001;163:1723–9.
59 Malhotra RK, Indrayan A. A simple nomogram for sample size for estimating sensitivity and specificity of medical tests. *Indian J Ophthalmol* 2010;58:519–22.
60 Elmekkawi A, More K, Shea J, *et al*. Impact of stewardship on inhaled nitric oxide utilization in a neonatal ICU. *Hosp Pediatr* 2016;6:607–15.