

## ARTICLE



# The use of milrinone in neonates with persistent pulmonary hypertension of the newborn - a randomised controlled trial pilot study (MINT 1)

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**OBJECTIVE:** To assess the impact of milrinone administration on time spent on nitric oxide (iNO) in infants with acute pulmonary hypertension (aPH). We hypothesized that intravenous milrinone used in conjunction with iNO would reduce the time on iNO therapy and the time spent on invasive ventilation in infants  $\geq 34$  weeks gestation with a diagnosis of aPH. We aimed to assess the practicality of instituting the protocol and contributing to a sample size calculation for a definitive multicentre study.

**STUDY DESIGN:** This was a multicentre, randomized, double-blind, two arm pilot study, with a balanced (1:1) allocation. Infants with a gestation  $\geq 34$  weeks and a birth weight  $\geq 2000$  grams aPH, an oxygenation index of  $\geq 10$ , and commenced on iNO were eligible. Participants on iNO were assigned to either a milrinone infusion (intervention) or a normal saline infusion (placebo) for up to 35 h. The primary outcome was time on iNO and feasibility of conducting the protocol.

**RESULTS:** The trial was terminated early after 4 years of enrollment due to poor recruitment. Four infants were allocated to the intervention arm and 5 to the placebo arm. The groups were well matched for baseline variables. No differences were seen in any of the primary or secondary outcomes.

**CONCLUSION:** Conducting an interventional trial in the setting of acute pulmonary hypertension in infants is not feasible using our current approach. Future studies in this area require alternative trial design to improve recruitment as this topic remains understudied in the neonatal field.

**TRIAL REGISTRATION:** [www.isrctn.com](http://www.isrctn.com); ISRCTN:12949496; EudraCT Number:2014-002988-16

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## INTRODUCTION

Acute pulmonary hypertension (aPH), also referred to as persistent pulmonary hypertension of the newborn (PPHN), occurs in 0.5 to 7 per 1000 live births and results in a mortality ranging between 4% to 33% [1, 2]. The aetiology is multifactorial and current approved therapy is limited to inhaled nitric oxide (iNO) or extra corporeal membrane oxygenation (ECMO) [3, 4]. The widespread use of iNO has resulted in a reduction of the use of ECMO; however, mortality and long-term morbidity remains unchanged. The rate of poor response to iNO in this population remains relatively high with up to 40% non-responders or partial responders [4, 5]. In addition, iNO use does not provide direct support to myocardial performance which is often compromised in the presence of aPH. Due to these challenges, there is a real need to evaluate novel approaches to the management of aPH.

Milrinone is a selective phosphodiesterase 3 (PDE3) inhibitor with pharmacological effects including relaxation of vascular smooth muscle, enhanced myocardial contractility (inotropy) and

improved myocardial relaxation (lusitropy) [6, 7]. Milrinone may also exhibit synergistic effects with iNO in lowering pulmonary vascular resistance (PVR) [8, 9]. Its use in the setting of aPH in neonates is limited to case series demonstrating an improvement in oxygenation when used in infants with aPH failing to respond to iNO [10, 11]. A recent Cochrane review illustrated the lack of randomised controlled trials (RCTs) comparing the use of milrinone versus placebo or as an adjunct to iNO compared with iNO alone in the setting of aPH and recommended limiting the use of milrinone in aPH to the research setting [12]. Given the difficulties in recruiting infants in trials of pulmonary hypertension [13–15], it is important to systematically investigate the efficacy of milrinone in the setting of aPH prior on a pilot basis prior to a larger scale trial.

In this pilot RCT, we hypothesized that intravenous milrinone used in conjunction with iNO results in the reduction in the time on iNO therapy and the time spent on invasive ventilation in infants  $\geq 34$  weeks gestation a diagnosis of aPH. We aim to assess

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the practicality of instituting the protocol and determining a sample size calculation for a definitive multicentre study.

## METHODS

### Study setting

This was a multicentre, randomized, double-blind, two arm pilot study, with a balanced (1:1) allocation that was carried out in level III neonatal intensive care units in 2 centres in Ireland: The Rotunda Hospital Dublin and Cork University Maternity Hospital, and one centre in the Netherlands: Radboudumc Amalia Children's Hospital, Nijmegen. A detailed study protocol including detailed methodology was published previously [16]. This study received ethical approval from the Clinical Research Ethics Committee, University College Cork, Ireland [Ref: ECM 5 (4) 03/03/15 & ECM 3 (bbbb) 09/05/17]. This study obtained Health Product Regulatory Authority Approval [CT Number: CT 900/557/1, Case Number 2190463], and is registered in the ISRCTN and EUDRACT clinical trial registries [ISRCTN:12949496; EudraCT Number:2014-002988-16]. Written informed consent was obtained from all parents prior to enrolment.

### Eligibility and exclusions

All infants born at a gestational age  $\geq 34$  weeks and weight  $\geq 2000$  grams with a clinical diagnosis of aPH, an oxygenation index of  $\geq 10$ , and commenced on iNO within the first 10 days following birth were eligible. The process of iNO initiation in aPH, in addition to the management of hypotension was standardised in the NICUs and is detailed elsewhere [16]. In addition, the infants needed to fulfil the following echocardiography criteria of aPH: Absence of significant congenital heart defect excluding a small atrial septal defect or ventricular septal defect (measuring less than 3 mm) and the presence of any of the following: (i) A tricuspid regurgitant jet with a pressure gradient  $\geq 2/3$  systemic systolic blood pressure; (ii) Intra-ventricular septum flattening or bowing into the left ventricular cavity; (iii) Patent ductus arteriosus bidirectional shunting or predominant right to left shunting; (iv) A pulmonary artery acceleration time  $< 40$  milliseconds.

The following infants were excluded: lethal congenital anomalies or obvious syndrome; bleeding diathesis (abnormal coagulation screen/platelet  $< 100,000/\text{mm}^3$ ); The presence of Intraventricular haemorrhage; diastolic hypotension (defined as a diastolic blood pressure less than the 3rd centile for any given gestation) unresponsive to medical treatment ( $\geq 30$  mL/kg fluid bolus and  $\geq 2$  inotropes); hypoxic-ischemic encephalopathy undergoing therapeutic hypothermia; evidence of renal impairment (creatinine  $> 100$  micromol/l [1.13 mg/dL]); severe hypovolaemia: heart rate  $> 180$  beats per minute, capillary refill time  $> 5$  sec, urine output  $< 0.5$  ml/kg/h, in addition to diastolic hypotension mentioned above.

### Study intervention

Infants in the intervention arm received an intravenous loading dose of milrinone at a dose of  $50 \mu\text{g}/\text{kg}$  administered over 60 min followed by a maintenance infusion, beginning at  $0.375 \mu\text{g}/\text{kg}/\text{min}$  to a maximum  $0.750 \mu\text{g}/\text{kg}/\text{min}$ . The loading dose was given in accordance with the recommendations of dosing regimen in the agent's Summary of Product Characteristics [16]. Treatment was continued until the discontinuation of iNO or a maximum of 35 h in adherence with the summary of product characteristics (SmPC) recommendation. A 10 ml/kg bolus of normal saline was administered with the milrinone bolus over the same 1 h period. Infants in the control arm received an intravenous loading dose of placebo (normal saline) which was administered over 60 min at a rate equivalent to the infusion rate of the milrinone bolus. A bolus of normal saline of 10 ml/kg also accompanied the placebo infusion, as per the intervention arm.

### Randomisation, concealment and blinding

A computer-generated central randomisation scheme was used to assign the infants to the two arms in a 1:1 ratio. Both formulations are clear and colourless. Once a patient was recruited and randomised to either Milrinone or Placebo, the trial pharmacist not involved in recruitment, allocation, or data collection prepared the trial drug or placebo and issued the syringe for infusion to the trial investigator team for administration. The trial participants and their families, the care providers, the data collectors, the echocardiographers, the primary outcome assessors, and the data analysts were all blinded to the allocation.

### Haemodynamic monitoring

Continuous measurements of left ventricular output (LVO) and systemic vascular resistance (SVR) using bio-reactance were facilitated by the NICOM system (Cheetah Medical, USA). Those were obtained at baseline, 6, 12 and 24 h after milrinone administration; an average of 10 readings over 10 min was recorded for each time point. Detailed description of the technique, reproducibility and validation in the neonatal population is published by our group elsewhere [17–19].

The functional protocol for the echocardiography assessment was adapted from the recent American Society of Echocardiography Guideline on neonatal echocardiography [20, 21]. All infants were assessed once established on iNO and in a quiet state using the Vivid E95 or the Vivid S6 (GE Medical, Milwaukee, WI, USA) and a 10 Mhz transducer. Congenital heart disease was ruled out on the first assessment. Offline analysis of all echocardiograms was performed at a dedicated workstation (EchoPAC version 202, GE Medical Systems) by a single operator (AF) who was blinded to the allocations of the infants. Detailed methods for image acquisition and analysis techniques have been published elsewhere [22–28]. An assessment of pulmonary haemodynamics was performed via measurement of pulmonary artery acceleration time (PAAT), RV ejection time (RVET); PAAT to RVET ratio (PAATi) and LV end-systolic eccentricity index. The PAAT is inversely correlated to pulmonary vascular resistance (PVR) and has been validated against right heart catheter-derived measurements in infants and children [25]. The LV end-systolic eccentricity index (LV EI) is reflective of the degree of interventricular septal flattening due to elevated RV pressures. Parameters of myocardial function including right ventricular output (RVO), LV global and RV free wall deformation measurements using speckle tracking echocardiography were recorded.

### Outcome assessment

In this pilot feasibility RCT, we aimed to assess our ability to recruit and retain infants, assess parental acceptability and adherence to the administration of the intervention, determine the feasibility of randomization, identification of optimal recruitment methods, and estimate an effect size that can be used to power a definitive trial. The primary clinical outcome was the duration of iNO treatment in hours. The weaning approach to iNO was standardised across the centres and is described in detail in our prior publication of the study protocol [16]. We also collected secondary outcomes including time on invasive ventilation and oxygen supplementation, duration of hospital stay, the rate of hypotension and the use of adjuvant inotropes, need for ECMO and mortality.

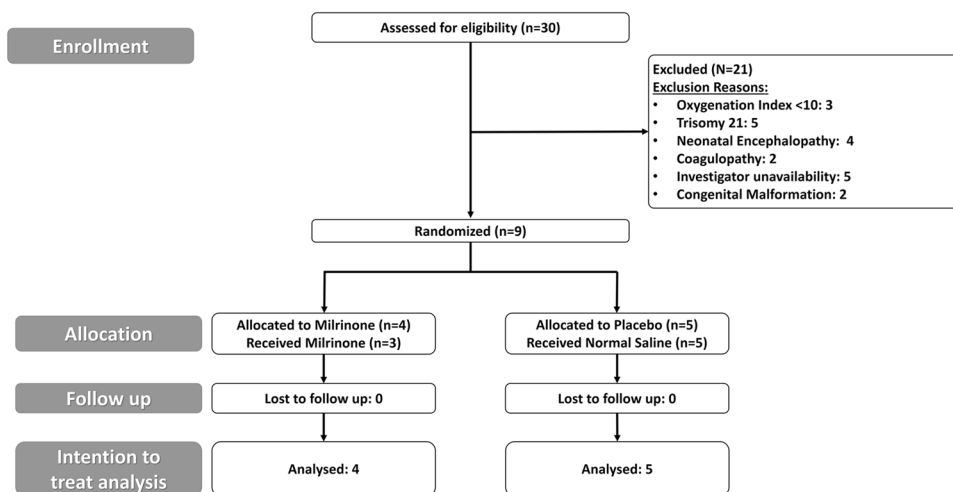
### Sample size and statistical analysis

This study was conducted to determine the feasibility of patient recruitment, instituting the study protocol, randomising and blinding allocation, collecting outcome data and contribute to determine the sample size necessary for a definitive multicentre trial. A sample of 10 infants per arm (a total of 20 infants) was planned for recruitment over a 4-year period. The trial was terminated by the data safety and monitoring board (DSMB) following 4 years of enrollment due to poor recruitment into the trial. A total of nine infants were included in the trial.

Continuous variables were presented as means (standard deviation) or median [inter-quartile range] as appropriate. Dichotomous variables were presented as proportions and summarised in contingency tables. All enrolled infants were grouped and presented on an intention-to-treat basis, including infants that have not continued treatment for any reason. No statistical analysis was conducted due to the smaller number of infants in each group. Data were presented descriptively. We used SPSS (version 25) to perform the analysis.

## RESULTS

Between April 2016 and April 2020, thirty patients from 3 centres were screened for eligibility with a total of 9 infants included in the study (Fig. 1). Four infants were randomised to milrinone (of which three received the drug), and five were randomised to placebo. One infant randomized to milrinone deteriorated, prior to receiving the study drug, and required referral to the ECMO service. There were no differences in baseline demographics between groups, although the pre-enrolment OI appeared higher in the intervention group (Table 1). All infants were in receipt of 20 parts per million of iNO at the time of enrolment.



**Fig. 1 CONSORT diagram of the Study.** Demonstrating the enrollment of subjects, their allocation to treatment, disposition status and how they are analysed in the trial.

**Table 1.** Patient demographics in the two groups.

	Milrinone <i>n</i> = 4	Placebo <i>n</i> = 5
Gestation (Weeks)	40.0 [38.9– 41.6]	40.1 [38.0– 41.1]
Birthweight (Kg)	3.4 [3.2–3.6]	3.9 [3.6–4.1]
Male	1 (25)	2 (40)
Vaginal delivery	2 (50)	3 (60)
Five minute Apgar score	9 [8–10]	8 [4–10]
Surfactant administration	2 (50)	1 (20)
Meconium aspiration	1 (25)	2 (40)
RDS/TTN	1 (25)	1 (20)
Idiopathic	2 (50)	2 (40)
Pre Treatment OI	27 [17–44]	14 [11–19]

Values are presented as medians [Inter-Quartile Range] or count (percent). RDS Respiratory Distress Syndrome, TTN Transient tachypnoea of the newborn, OI Oxygenation index.

**Table 2.** Outcomes in the two groups.

	Intervention <i>n</i> = 4	Placebo <i>n</i> = 5
Time on iNO (Hours)	92 [42 – 161]	48 [28 – 118]
Ventilation days	6 [3–24]	6 [4–12]
Oxygen days	6 [1–13]	6 [3–19]
Hospital days	16 [9–27]	21 [9–31]
Death	0	0
ECMO	1 (20)	1 (25)
Hypotension	3 (60)	3 (75)
Inotrope	3 (60)	3 (75)
Post treatment OI	6 [3–24]	8 [4–27]

Values are presented as medians [Inter-Quartile Range] or count (percent). iNO Inhaled nitric oxide, ECMO extracorporeal membrane oxygenation, OI oxygenation index.

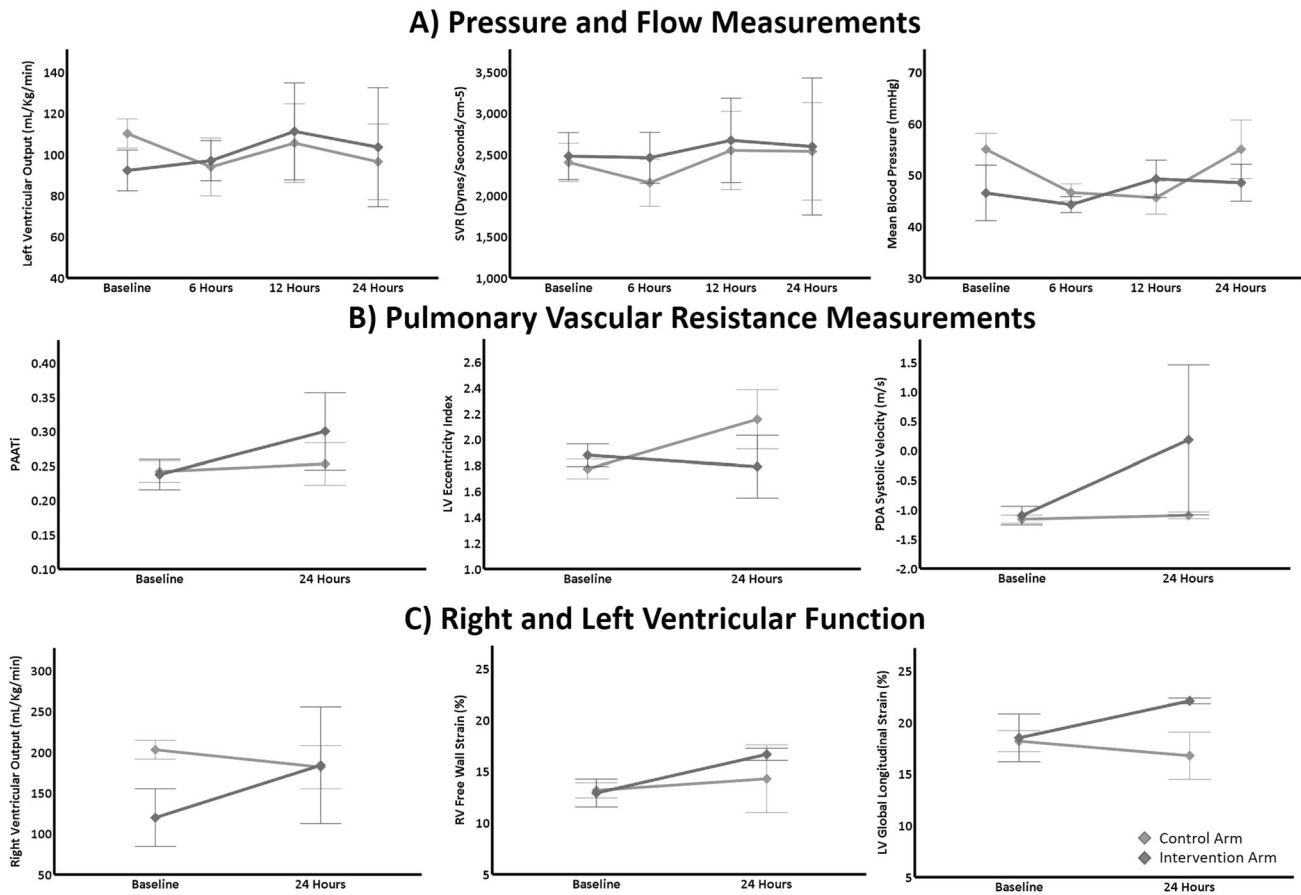
There were no differences in the duration of iNO, ventilation or oxygen administration between the two groups (Table 2). The distribution of adverse events including hypotension, use of inotropes, need for ECMO, or death were comparable between the two groups. There were no differences in the NICOM measured LVO or SVR between the two groups throughout the study period (Fig. 2A). Baseline measures of PVR including PAATi, LV EI and PDA systolic velocity appeared similar between the groups (Fig. 2B). Infants in receipt of milrinone demonstrated a trend of improvement in the surrogate markers of PVR including a higher PAATi, a lower LV EI, and a left to right flow pattern during systole across the PDA. Baseline RVO was lower in the intervention group compared with controls with no differences in the baseline values of RV and LV longitudinal strain (Fig. 2C). Infants in receipt of milrinone appeared to have improved RV strain and LV strain by 24 h following administration.

## DISCUSSION

Despite recent advances in the management of aPH in late preterm and term infants, the risk of mortality and adverse neurological sequelae remains high. LV and RV function is often compromised secondary to increased RV afterload and reduced LV preload mediated by the higher PVR and resultant reduction in

pulmonary venous return [29]. The effects of a pressure-loaded, dilated right heart include a shift in the interventricular septum and compression of the left ventricle, both resulting in decreased LV filling and hence cardiac LV output (LVO). In addition, the high rate of iNO non responders, particularly in infants with congenital diaphragmatic hernia, has prompted the neonatal community to institute additional therapies. However, there is no consensus on the choice of therapeutic interventions in addition to iNO.

This study was designed to assess the feasibility of recruiting infants in accordance to our protocol; we therefore chose a short term primary clinical outcome of time on iNO to facilitate a short duration of enrolment and outcome assessment. Unfortunately, following four years of recruitment in three neonatal centres, the study was terminated due to futility following the enrolment of only nine infants, all of whom came from one centre (The Rotunda Hospital, Dublin, Ireland). Our ability to draw any robust conclusions is therefore limited. The recruitment challenges of this study align with increasing trends with the enrolment of critically ill term or preterm infants with haemodynamic instability into an interventional trial involving a vasoactive agent. For example, recent studies including an RCT of hydrocortisone for neonatal hypotension, the HIP trial of Dopamine for hypotension, and the Bosentan trial for aPH in newborn infants (FUTURE-4), all terminated early due to poor recruitment [13–15]. A current pilot RCT of Milrinone in Congenital Diaphragmatic Hernia (CDH)



**Fig. 2 Haemodynamic and Echocardiography parameters.** Data are presented as means (diamonds) and one standard error (whiskers). No statistical analysis was performed.

(clinicaltrials.gov / NCT02951130) is actively recruiting infants to assess the feasibility of the protocol, safety and efficacy of the medication in improving oxygenation. The study group aim to enrol 66 infants over a five year period, likely acknowledging the difficulty in enrolling infants into such trials. However, since CDH is usually diagnosed antenatally, preparation for consent and enrolment can be planned in advance thereby potentially improving recruitment.

The reasons for the poor recruitment are multifactorial. In our experience, the incidence of severe acute pulmonary hypertension not accompanying other important morbidities including neonatal encephalopathy or Down syndrome seems to be declining. In addition, the incidence of incomplete or failed response to iNO has fallen in our unit secondary to earlier recognition of aPH, and improvements in antenatal and early neonatal care. The mandated early recruitment of infants with a condition that only manifests following delivery adds additional challenges. Approaching parents for consent shortly after discussing potentially devastating news about the health and wellbeing of their baby is a delicate process given the time sensitivity of the need for early enrolment.

Conducting the trial presented its own challenges. The requirement for echocardiography assessment prior to enrolment coupled with the small number of study investigators capable of performing the scans means that enrolment was limited to daytime working hours. In addition, the lack of 24-hour availability of pharmacy staff to prepare the study drug in a blinded fashion also curtailed enrolment. Finally, the COVID-19 pandemic led to further challenges in recruitment due the necessary diversion of staff and recourses.

We did observe some interesting difference in the haemodynamics in infants between the two groups: surrogate markers of pulmonary vascular resistance including PAATi, LV EI and PDA systolic velocity all showed a favourable trend toward lower PVR following 24 h of milrinone infusion. This was also accompanied by a rise in RV and LV strain. The rate of adverse effects in the two groups was similar with a high rate of hypotension and the use of inotropes likely reflecting the unstable nature of the condition rather than being a side effect of the medication. The low cardiac output state resulting from reduced LV preload can lead to a fall in blood pressure in infants with aPH necessitating the use of vasoactive inotropes such as dopamine and adrenaline. Animal data suggest that these inotropes raise systemic and pulmonary vascular resistance and may further contribute to RV compromise in the setting of aPH [30, 31]. Several studies have demonstrated the association of a low cardiac output in the setting of aPH with morbidity and mortality [32–34].

Cyclic nucleotide phosphodiesterases (PDE) are a family of enzymes that hydrolyse the phosphodiester bond in cAMP and cGMP thereby promoting pulmonary vascular constriction. PDE3 has a predominant hydrolysing effect on cAMP. The selective inhibition by milrinone of PDE3 lead to a rise in the levels of cAMP leading to relaxation of vascular smooth muscle, enhanced myocardial contractility (inotropy) and improved myocardial relaxation (lusitropy) [6, 7]. In the newborn lamb, intravenous milrinone augments the action of prostaglandins (PGI<sub>2</sub>) on pulmonary vasculature by significantly shortening the onset and prolonging the duration and degree of pulmonary vasodilation produced by PGI<sub>2</sub> [35, 36]. The use of milrinone is established in neonates and children following cardiac surgery for the

prevention of low cardiac output syndrome and the treatment of pulmonary hypertension [37, 38].

In conclusion, we demonstrated the conducting an interventional trial of a vasoactive agent in the setting of acute pulmonary hypertension in term infants is challenging using our current approach. The apparent fall in the incidence of severe pulmonary hypertension, and the difficulty in approaching parents during a critical treatment window have led to very slow recruitment. Future endeavours aimed at assessing the impact of vasoactive medications in clinically important neonatal cardiovascular conditions should consider alternative methods of investigation. Those should include cluster randomisation by centre to facilitate comparisons, the use of well-designed registries, or the implementation of Standardised Clinical Assessment and Management Plans (SCAMPs). Clinical trialists should engage with hemodynamic physiologists to determine strategies to enhance patient recruitment and trial conductance, taking into account the increased recognition of the variable pathophysiological underpinnings of the clinical phenotype of pulmonary hypertension.

## DATA AVAILABILITY

The datasets generated during and/or analysed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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### AUTHOR CONTRIBUTIONS

All authors made substantial contributions the conception and/or design of the work; AEK, AS, NB, CB performed recruitment and data acquisition, AEK, PM and ED performed the data analysis, and interpretation of data for the work; AEK drafted the first draft of the manuscript, the remainder of the authors revised it critically for important intellectual content; All authors provided final approval of the version published; All authors agree to be accountable for all aspects of the work. In addition, OLF provided managerial and administrative support, ORF provided cardiac expertise, and BC provided pharmaceutical expertise.

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### COMPETING INTERESTS

The authors declare no competing interests.

### ADDITIONAL INFORMATION

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