Biomarkers of Inflammation and Lung recovery in ECMO patients with Persistent Pulmonary Hypertension of the Newborn (PPHN): A feasibility study

Paolo Pais[1], MD, Simon Robinson[2], MD, Gavin Majitha-Beet[3], BSc, Attilio Lotto[1,4], MD FRCS (CTh), Tracy Kumar[1], BSc, Claire Westrope[2], MD, Nikol Sullo[1,7], PhD, Bryony Eagle Hemming[1], BSc, Lathishia Joel-David[1], BSc, Maria JnTala[3], MSc, Claudio Corazzari[1,5], MD, Lorenzo Grazioli[2,6], MD, Dawn Smallwood[1,8], PhD, Gavin J Murphy[1], MD FRCS (CTh), Florence Y Lai[1], MPhil, and Marcin J Woźniak[1], PhD.

1. Leicester NIHR Biomedical Research Unit and Department of Cardiovascular Sciences, University of Leicester, Glenfield Hospital, Leicester LE3 9QP, UK.

2. Paediatric Intensive Care, University Hospitals Leicester NHS Trust, Glenfield Hospital, Leicester LE3 9QP, UK

3. Clinical Perfusion, University Hospitals Leicester NHS Trust, Glenfield Hospital, Leicester LE3 9QP, UK.

4. Department of Congenital Cardiac Surgery, Alder Hey Children's Hospital, Eaton Road, Liverpool L12 2AP, UK

5. Cardiac Surgery Unit, Insubria University, Varese, Italy.

6. ASST Papa Giovanni XXIII, 24127 Bergamo, Italy

7. University of Nottingham, Royal Derby Hospital, Derby, DE22 3DT

8. School of Allied Health Sciences, De Montfort University, Leicester LE1 9BH

Address for correspondence: Marcin J Woźniak, Leicester NIHR Biomedical Research Unit and Department of cardiovascular Sciences, University of Leicester, Glenfield Hospital, Leicester LE3 9QP, Email mw299@leicester.ac.uk. Telephone +44116 2583028

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Abstract

Objective: Extra-corporeal membrane oxygenation (ECMO) is a treatment for Persistent Pulmonary Hypertension of the Newborn (PPHN) with high mortality. Hypothesis: the ECMO circuit results in inflammatory responses that mitigate against successful weaning.

Design: Single centre prospective observational feasibility study.

Setting: Paediatric Intensive Care Unit

Patients: Twenty-four neonates requiring ECMO support for PPHN.

Interventions: None

Measurements and Main Results: The reference outcome was death or more than 7 days of ECMO support. Other outcomes inceluded serial measures of plasma-free haemoglobin and markers of its metabolism, leucocyte, platelet and endothelial activation, and biomarkers of inflammation. Of 24 participants recruited between February 2016 and June 2017, 10 died or required prolonged ECMO support. These patients were sicker at baseline with higher levels of plasma-free haemoglobin within 12 hours of cannulation (geometric mean ratio 1.92, 95% confidence intervals 1.00-3.67, p=0.050) but not thereafter, versus those requiring <7 days ECMO. Serum haptoglobin concentrations were significantly elevated in both groups. Patients who died or required prolonged ECMO support demonstrated elevated levels of platelet-leucocyte aggregation, but decreased concentrations of mediators of the inflammatory response: interleukin-8, C-reactive protein and Tumour Necrosis Factor α.

Conclusion: Clinical status at baseline and not levels of plasma-free haemoglobin or the systemic inflammatory response may determine the requirement for prolonged ECMO support in neonates.

Introduction

Persistent Pulmonary Hypertension of the Newborn (PPHN) complicates 1–2 per 1000 live births, primarily affecting full term and late preterm babies.[1] Some neonates with PPHN and severe respiratory failure require Extracorporeal Membrane Oxygenation (ECMO) support. This reduces mortality rates from >80% to 10-20%.[2] However, ECMO remains an invasive form of life support. Some 74% of neonates with PPHN develop complications while on ECMO with cardiovascular and renal complications amongst the most common.[3] The ECMO circuit causes haemolysis and systemic inflammation along with leukocyte and platelet activation that can lead to organ injury and prolonged cannulation times.[4-7] Severe haemolysis and rise in plasma-free haemoglobin (PFH) have been reported in over 10% of neonatal ECMO cases.[8-11] PFH is highly reactive and can oxidize multiple species including proteins and membrane lipids causing inflammation, endothelial injury and organ dysfunction.[12-17] Elevated PFH may also contribute to persistent pulmonary hypertension.[18-21] These observations led us to hypothesise that damage to red blood cells by the exposure to the ECMO circuit results in inflammatory responses that mitigate against successful weaning from ECMO. As a first step to testing this hypothesis, we evaluated the feasibility of measuring PFH and its metabolism, cellular activation processes, and inflammation, in neonates on ECMO support.

Materials and Methods

Study Population

The Markers of Inflammation and Lung recovery in ECMO patients for Persistent Pulmonary Hypertension of the Newborn (PPHN): MI-ECMO study, was a prospective, single-centre observational feasibility study. The study was reviewed by the 'North East – York' Research Ethics Committee on 11th December 2015, and granted a favourable ethical opinion on 23rd December 2015. Neonates (<30 days of age) having a diagnosis of PPHN and requiring ECMO support were eligible for inclusion. We excluded neonates with PPHN caused by a congenital heart pathology or those requiring ECMO for a congenital heart disease. We recruited consecutive patients referred to the Glenfield Hospital (a regional ECMO centre in the UK) according to the prevalent ECMO referral system who met the inclusion/exclusion criteria. Emergency assents were obtained from patients' parents/legal guardians within 12 hours of cannulation and full consents were obtained within 24 hours. Study withdrawal occurred at the parent/guardian's request or if the patient was found not to meet all the inclusion criteria. In the event of withdrawal, we requested that all patient's data and tissues collected until that time were retained for analysis. The study had ethical approval (REC reference 15/NE/0398). The trial protocol was registered (NCT02940327) and is attached as a digital supplement. The corresponding author (MJW) attests to the validity of the data, its analysis, and interpretations, on behalf of all the co-authors.

Outcome measures

Outcomes of interest included markers of haemoglobin and iron metabolism (plasma free haemoglobin, bilirubin, haptoglobin, ferritin, transferrin, total and catalytic iron), markers of platelet, leukocyte and endothelial cell activation (please see Research procedures below) and markers of the systemic inflammatory and acute phase response (interleukin (IL-) 1β, IL-6, IL-8, IL-18, Tumour Necrosis Factor α (TNFα), Monocyte chemoattractant protein 1 (MCP1), C-reactive protein (CRP), fibrinogen) were measured in arterial blood at four time points after the initiation of ECMO - at 12, 24, 48 and 72 hours and at weaning 24 hours after

decannulation or immediately prior to termination of ECMO support in case of death or treatment withdrawal.

Research Procedures

PFH levels were estimated by measuring absorbance at 415, 450 and 700 nm of citrated plasma samples using EnSpire spectrophotometer (PerkinElmer, Waltham, USA) and calculated as described in Fairbanks et al.[22] Total iron was measured in citrated plasma samples using Iron Assay Kit (Abcam, Cambrige, UK). Catalytic iron was estimated using bleomycin method, as described in Burkit et al.[23] Platelet and leukocyte activation, and leukocyte-platelet interactions were measured using flow cytometry (CyAn ADP, Beckman Coulter, Pasadena, USA) in citrated blood with specific fluorescently-labelled antibodies: PAC-1 (against activated GPIIb/IIIa, BD Biosciences, Abingdon, Oxford, UK), CD41 (Affymetrix, Santa Clara, USA) and CD62P (Abcam, Cambridge, UK) for platelets; CD64, CD163, CD11b (Affymetrix) for leukocytes; and CD14, CD16, CD41 (Affymetrix) for leukocyte-platelet interactions. Cytokines (IL-1β, 6, 8, 18, TNFa) and soluble ICAM1, as a marker of endothelial activation, were measured in citrated plasma using MAGPIX platform (Luminex Corporation, Austin, USA) and magnetic Luminex assays (BioTechne, Abingdon, UK). Troponin (Enzo Biochem, Farmingdale, USA), Haptoglobin (Abcam), ferritin and transferrin (ThermoFisher Scientific, Waltham, USA), were measured in plasma using ELISA DS2 Dynex platform (Worthing, UK). Levels of bilirubin, alkaline phosphatase, pH, pO2, pCO2, SaO2 and lactate were collected from routine hospital diagnostic procedures.

Clinical procedures

Pre-ECMO care and ECMO indication: Eligible patients received standard care during the pre-ECMO period, according to the different respiratory failure aetiology and protocols from referral hospitals. The data on the respiratory protocols at the referring hospitals were not collected.

The ECMO circuit and cannulation: The ECMO circuit consisted of vascular access cannulas (Biomedicus, Medtronic, Dublin, Ireland), polyvinyl chloride tubing for blood extracorporeal circulation (Leicester Neonatal circuit, Chalice Medical, Worksop, UK), a pump console (2nd generation Centrimag, Thoratec Corporation, Pleassanton, USA), a centrifugal pump (Centrimag, Thoratec Corporation) and head (PediVAS Blood pump., Thoratec Corporation) and an oxygenator (Paragon infant PMP oxygenator, Chalice Medical). Cannulation was always performed using an open surgical approach. VA (veno-arterial) or VV (veno-venous) cannulation was chosen based on the clinical status of the patient and the availability of the correct material by the attending clinician. VA ECMO involved surgical cannulation of the right common carotid artery and internal jugular vein, with the tip of the venous cannula 10fg Biomedicus (Medtronic, Minneapolis, USA) advanced into the right atrium and the arterial catheter positioned at the junction of the right common carotid artery and aortic arch. VV ECMO involved insertion of a 13F (OriGen Biomedical, Austin, USA) arterial / venous cannula advanced to the right atrium via the right internal jugular vein.

ECMO management: Established Glenfield ECMO centre protocols were used. In VA-ECMO flows were typically maintained at about 100-120 mL kg^{-1} min^{-1} to achieve normal saturation (SvO2 > 65%), with a controlled pCO_2 to achieve a normal pH. The target of arterial blood oxygen saturation during VV ECMO was >85% with a venous oxygen saturation > 60% and low lactates with targeted flow rates of 80 mL kg^{-1} min^{-1}. Sweep gas was usually maintained at a FiO_2 100% and regulated on the pCO_2 basis; the usual starting ECMO flow:sweep gas ratio was 1:1.

Ventilator Management: During ECMO, patients were commonly ventilated in a “lung rest” setting defined as a respiratory rate of 10 breaths $minute^{-1}$, PEEP (positive end expiratory pressure) of 10 cmH_20 and pressure over PEEP 10 cmH_20 with FiO_2 40% for VA ECMO and 30% for VV ECMO. These parameters were changed according to clinical status.

Haemodynamic support: During VA-ECMO patients received haemodynamic support in addition to the respiratory support, and the usual inotropes requirement was reduced or null. During VV-ECMO inotropic or vasoactive support was used to maintain an adequate perfusion and cardiac output defined by general practice targets: Mean Arterial Blood Pressure (MABP) > 40 mmHg, lactate < 2 mmol L^{-1}, central venous saturation > 60% with evidence of end organ perfusion e.g. urine output.

Anaesthesia induction: The anaesthetic technique used a standard unit protocol that while titrated to effect, is typically Ketamine 2 mg kg^{-1} for sedation and Atracurium 1 mg kg^{-1} as a neuromuscular blocking agent. Underlying sedation was modified according to the requirements of the patient and deviations from the protocol were recorded. Details of concomitant medications and therapy including generic drug name, dose, route, frequency, duration and indication were recorded.

Fluid Management: Target urine output was > 0.5 mL kg^{-1} h^{-1}. This was maintained using fluid boluses or diuretics at the discretion of the attending clinician. In some circumstances the clinician can choose CRRT (continuous renal replacement therapy) according the local protocols and fluid balance.

Transfusion: The standard unit protocol is transfusion with 10 mL kg^{-1} of allogenic red cells if the heamoglobin< 13g dL^{-1} and SvO_2 < 65% or in the presence of bleeding. Non red cell components were administered according to standard unit protocols, with the indication, volume and timing of their administration recorded.

Weaning: Clinician and patient clinical status guided ECMO weaning. VA ECMO was typically with “retrograde flow”, in this case the flow in the cannula is reversed inside the system using the ECMO as a brake and not as an engine. With this kind of procedure, the observational weaning time is prolonged with less clotting risk for the circuit. In VV ECMO, the support blood flow is maintained but the sweep gas is unplugged, the patient is fully ventilated to assess the lung function. Failure to wean the patient results in restarting ECMO support or treatment withdrawal.

Statistical analysis

Continuous data are presented as the mean (standard deviation, SD) or median (interquartile range, IQR) as appropriate. Categorical data are presented with frequency and percentage. Duration on ECMO was recorded in terms of hours and converted to days by dividing by 24. Patients were classified as prolonged stay if they stayed on ECMO for at least 7 days or if they did not survive to discharge (patients who died cannulated or after weaning). Patients not experiencing a prolonged stay were surviving patients who stayed on ECMO for less than 7 days. Using the linear mixed effects model with patients as random effect, we compared the trajectory of outcome measures between those experiencing a prolonged stay on ECMO and those not. Separate models were built for each of the outcome measures. All models included the main effects of group (prolonged stay or not) and time (5 time points) together with the group—time interaction term. A significant interaction term indicates that the two groups exhibit different trajectories of the outcome or the effect of groups depends on the time points. Models with a significant group effect but insignificant interaction indicates the effect of groups are similar over the time points. Interaction or group effects were considered significant if p-value < 0.05. For laboratory measurements and ECMO and blood gas parameters where data prior to ECMO or at start of ECMO were measured, the models would be adjusted for these baseline values. Outcome data analysed on a logarithmic scale would be transformed back to the original scale after the analysis and results reported as geometric mean. A p-value of <0.05 was considered statistical significance. No adjustment for p-values has been made for multiple comparisons as this was a feasibility study. All analyses were performed with SAS version 9.4 (SAS Institute Inc., Cary, NC, USA).

Results

Study cohort

Between February 2016 and June 2017, we screened 52 neonates referred to the Glenfield Hospital for ECMO support, of which 24 with PPHN were eligible. Informed full consent was obtained from parents in every case. **Figure 1** and **Supplemental Table 1** show participant flow and completeness of data for individual endpoints. The reference outcome (7+ days on ECMO) occurred in 10 (41.7%) participants, two of which died while cannulated and 1 who died 6.3 days after weaning. A further 14 neonates with PPHN (58.3%) stayed on ECMO for <7 days and survived to discharge (<7 days). There was no difference between the two groups with respect to age (median 2 days), gestational age (median 40 weeks), male: female ratio, or birth weight (median 3.2kg) (**Table 1**). The aetiology of PPHN differed significantly between the two groups; congenital diaphragmatic hernia accounted for 8/10 (80%) in the 7+ days group and meconium aspiration syndrome (MAS) accounted for 12/14 (85%) of the <7 days group (**Table 1**). This was reflected by differences in ventilation strategies at baseline; 10/10 (100%) in the 7+ days group underwent HFOV whereas 7/14 (50%) of the <7 days group underwent SIMV (**Table 1**).

Baseline Clinical Status

A greater proportion of participants in the 7+ days group were haemodynamically unstable prior to cannulation; n=7/10 (70%) versus n=5/14 (36%, **Table 2)**. Arterial pCO_2 and serum lactate concentrations were higher in the 7+ days group, and arterial SaO2 was lower. None of these changes reached conventional statistical significance ($P<0.05$). Baseline values for PIM2 (mortality risk) or PMODS (organ dysfunction) scores at ICU admission were not significantly different between the two groups. At baseline participants in the <7 days group were less acidotic; mean PH 7.3, SD 0.08 versus 7.3, SD 0.12, $p_{Baseline}=0.018$, but had significantly higher levels of CRP(median 39 mg L^{-1}, IQR 11-18 versus 6 mg L^{-1}, IQR 5-15, $p_{Baseline}=0.019$) and alkaline phosphatase (median 198 iu L^{-1}, IQR 113 – 613, $p_{Baseline}=0.03$ vs 107 iu L^{-1}, IQR 66 – 131) relative to the 7+ days group.

ECMO Course

There was no difference between the groups with respect to use of VA (n=21) versus VV ECMO (n=3), choice of prime (blood/crystalloid), pump flows, or sweep (**Tables 1 & 2**). Participants in the <7 days group spent a median of 80 hours (IQR 59-91), and patients in the 7+ days group spent a median of 292 (186-360) hours on ECMO. Mean arterial blood pressure was similar between the groups for the duration of ECMO support (**Supplemental Table**). SaO2 and PaO2 were higher (all participants received FiO2 1.0, **Supplemental Figure 1A – 2B, Supplemental Table**) and PaCO2 values were lower in the <7 days group (**Supplemental Figure 1C, Supplemental Table**). There was no difference between the groups for haemoglobin levels and haematocrit, both decreased after cannulation and remained unchanged until discharge (**Supplemental Table**). Serum lactate concentration was higher (not significantly) in the 7+ days group prior to cannulation. The difference was not sustained during ECMO and the concentrations gradually dropped during the course of ECMO ($p_{Time} < 0.001$). The 7+ days group experienced a greater number of adverse events **(Table 3)**; median 4 (IQR 3-5) versus median 2 (IQR 0-2), p=0.001, received a larger volume of allogenic red cells, and clotting of the ECMO circuit (6 (60%) versus 6 (25%), p=0.002, **Table 3**). Patients in both groups had their PaO2/FiO2 ratio improved post weaning to 208 (SD 86) for 7+ days group and 212 (SD 69) for <7 days group and the difference between the two groups was not statistically significant (p=0.944; **Supplemental Table**).

Inflammation

Haemoglobin metabolism There was a significant interaction between group and time for PFH concentrations ($p_{group*time}$=0.02). Neonates requiring >7 days ECMO had higher levels at 12 hours post cannulation (p_{12hr}=0.05) but not thereafter (**Supplemental Figure 1D, Supplemental Table**). The >7 day group also had higher levels of serum bilirubin at 48 hours (p_{48hr}=0.03, **Supplemental Figure 1E, Supplemental Table**). There was no significant difference between groups with respect to plasma haptoglobin (**Supplemental Figure 1F, Supplemental Table**) although levels were high in both groups and gradually

increased during the ECMO course (p_{Time}<0.001). There was no difference between the groups for catalytic iron, ferritin, transferrin and total iron concentrations (**Supplemental Table**).

Platelet, leucocyte and endothelial activation. Participants in the 7+ days group showed higher levels of platelet-granulocyte and platelet-monocyte aggregates (CD16/CD41 and CD14/CD41, respectively; p_{group}=0.001, **Supplemental Figure 1G – H, Supplemental Table**), peaking at 24 hr post cannulation in the 7+ days group (p<0.001) but not thereafter. For levels of platelet-monocyte aggregates there was also a significant interaction between group and time ($p_{group*time}$=0.028, **Supplemental Table**). Monocyte activation was also higher in the +7 days group (p_{group}=0.018), with the greatest difference at 48 hours (p_{48hr} = 0.02; **Supplemental Figure 1I, Supplemental Table**). There were no differences between groups for platelets activation measured with P-selectin/CD62P antibodies, CD11b (total leukocyte activation) and ICAM-1 (canonical endothelial cell activation, **Supplemental Table**).

***Inflammation biomarkers*.** There was a significant interaction between groups and time for the pro-inflammatory cytokines IL-8 ($p_{group*time}$=0.003, **Figure 2A, Supplemental Table**) and MCP-1 ($p_{group*time}$=0.013, **Figure 2B, Supplemental Table**) as well as the acute phase response biomarkers CRP ($p_{group*time}$<0.001, **Figure 2C and Supplemental Table**) and IL-1b ($p_{group*time}$<0.001, **Figure 2D, Supplemental Table**). For all these markers, participants in the <7 days group had significantly higher plasma concentrations of systemic inflammatory response and acute phase response biomarkers during the first 12 to 48 hours post cannulation versus those in the 7+ days group. TNFα and IL-1β levels were higher in the <7 days (p_{group}=0.018 and 0.013, respectively) with the test for interaction between group and time insignificant for TNFα (**Figure 2E, Supplemental Table**). There was no difference between the groups for IL-6, IL-8 and MCP-1, and Fibrinogen (**Supplemental Table**).

Discussion

In this single centre observational feasibility study we observed that neonates with PPHN who required prolonged ECMO support or who died (7+ days group) were more likely to have: 1. Presentation with congenital diaphragmatic hernia. 2. To be sicker at baseline with more severe hypoxia and hypercapnoea during ECMO. 3. A higher number of adverse events. 4. Higher PFH concentrations at 12 hours post cannulation but not thereafter and higher bilirubin concentrations at 48 hours without group differences in other markers of haemoglobin and iron metabolism. 5. Increased platelet (platelet-leukocyte aggregates[24]) and monocyte activation but no differences in canonical endothelial activation. 6. In an apparent paradox, lower plasma concentrations of biomarkers of the systemic inflammatory response (IL-8, MCP-1) and acute phase response (CRP, TNFα, IL-1β) within 48 hours of cannulation. These findings did not support our primary hypothesis that the release of PFH by the ECMO circuit drives inflammatory responses that can prolong ECMO support.

The study has several strengths. It is the first study to our knowledge that has systematically phenotyped cellular and platelet activation, cytokine responses, and haemoglobin metabolism in a cohort of neonates with PPHN requiring ECMO. The study results are counter to our initial hypotheses but these are supported by the rigorousness of our analytical methods, the high level of completeness of data, and the consistency of our results. A limitation of the study is that we were not able to measure our biomarkers prior to ECMO cannulation. This is because many neonates are placed on ECMO at their referring centre by a retrieval team and prior to assent or consent by legal guardians for sample collection. Stored samples collected at the time of cannulation were not suitable for the inflammation assays used in the study and a window of 12 hours post cannulation was therefore selected for the collection of baseline bloods for these assays. It is possible that the changes measured at this time will have altered dramatically from the pre-cannulation values, although this potential source of bias will have been present in both patient groups. The observational nature of the study means that we cannot demonstrate a causal

relationship between the processes that we have measured and clinical outcomes. The small sample size is also a limitation. We cannot conclude that these findings would be replicated in a larger cohort. Nonetheless, our findings were remarkably consistent and highly statistically significant across measures of linked processes, indicating adequate power to detect differences in markers of inflammation even in this limited sample.

The results have clinical application. First, outcomes in participants of the Mi-ECMO study were significantly associated with patient status before cannulation. Earlier use of ECMO support may be indicated in patients with congenital diaphragmatic hernia who were sicker at presentation relative to other groups. Second, patients with higher levels of systemic inflammation and acute phase inflammation where associated with shorter duration of ECMO support. This contrasts findings in adults requiring ECMO support[25,26] where increased levels of pro-inflammatory cytokines[27] are predictive of mortality, but is in agreement with the p-MIVAKI study where very different inflammatory responses were observed in neonates versus older children undergoing cardiac surgery with CPB.[28] This may reflect different pathologies at baseline; MAS is primarily an inflammatory condition, whereas CDH is associated with lung hypoplasia and impaired chest wall dynamics, rather than a pathological response to ECMO.[7] Alternatively, the results may represent the failure of the sicker patients in the 7+ days group to generate an inflammatory response. Third, the cytokine levels observed in the <7 days group were relatively low and were not associated with significant differences in downstream effectors of tissue inflammation IL-6 and IL-18, or measures of myocardial or renal injury between groups. These changes may reflect an inflammatory response that promotes repair rather than a dysregulated response that results in organ injury.[29] Finally, the lower levels of inflammatory biomarkers are apparently contradictory to the higher PFH measured in the 7+ days group with levels comparable to those (0.3 mg mL^{-1}) associated with organ injury in other paediatric ECMO cohorts.[30] In mitigation, the difference between the groups was transient. In addition, the levels of haptoglobin in both groups were high, and comparable to levels observed in neonates with

sepsis or other inflammatory insults.[31] Neonates exposed to inflammatory stress undergo a 'haptoglobin switch' characterised by significant increases (20-30 fold) in protein expression as part of the acute phase response.[32] These changes may precondition neonates against plasma free haem mediated tissue damage. A greater transfusion volume was observed in the 7+ day group. The importance of this finding is unclear; the absolute difference in the volume of red cells transfused was low and this may simply represent the effects of a prolonged stay in a critical care environment on circulating red cell mass in the 7+ day group. There was also no detectable difference in markers of haemolysis after 48 hours suggestive of consumption by the circuit. A final observation is the increased circulating levels of platelet-leukocyte aggregates, a sensitive measure of platelet activation [24] observed in the 7+ days group. PFH is known to activate platelets leading to adverse clinical outcomes,[10,33,34] however whether these processes are linked in the current study is unproven.

The study design dichotomising the analysis into two groups may have reduced the number of significantly associating biomarkers. That provides scope for future post hoc analyses. Also, in a larger study, it would be interesting to analyse the outcomes of patients with CDH and those with adverse events during ECMO.

Conclusions Neonates requiring ECMO support for PPHN attributable to congenital diaphragmatic hernia, with higher levels of acidosis, PFH, platelet and leucocyte aggregation, and lower levels of systemic and acute phase inflammation are likely to require prolonged ECMO support. These findings highlight a knowledge gap with respect to the mechanisms underlying failure to wean in this vulnerable population.

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Table and Figure legends

Table 1. Demographics and diagnosis. Data is expressed as n (%) for binary variables, and mean(SD) or median (Q1-Q3) where appropriate for continuous variables. # n=1 from 7+ days group missing; **ECMO**: Extra-Corporeal Membrane Oxygenation; **PPHN**: Persistent Pulmonary Hypertension of the Newborn; **RV** – right ventricular; **LV** – left ventricular; SIMV – synchronised intermitent mechanical ventilation; **PSV** - Pressure support ventilation; **HFOV** - High frequency oscillatory ventilation

Table 2. Baseline clinical characteristics. Data expressed as n(%) for binary variables, and mean(SD) or median (Q1-Q3) where appropriate for continuous variables. **PIM2**: Paediatric Index of Mortality v2; **PMODS**: Paediatric Multiple Organ Dysfunction Score (range from 0 to 20); **FiO_2** - Fraction of inspired oxygen; **PiO_2** – partial pressure of oxygen; **pCO_2** (partial pressure of carbon dioxide); **SaO_2** – oxygen saturation; **MAP** - mean arterial pressure; **ACT** - activated clotting time.

Table 3. Clinical outcomes. Data is expressed as n(%) for binary outcomes, and mean(SD) or median (Q1-Q3) where appropriate for continuous outcomes. **RBC** – red blood cells; **FFP** – fresh frozen plasma; **SVT/AF** - Supraventricular tachycardia/Atrial fibrillation.

Figure 1. CONSORT Diagram showing patient flow through the study.

Figure 2. Inflammation markers Geometric mean and data points for **A** – IL-8, **B** – MCP-1, **C** – CRP, **D** – IL-1β, **E** – TNFα) over time in patients who died or required prolonged stay (7+ days) vs those requiring less than 7 days (<7 days). p_{group} refers to the p-value for the effect of group (7+ vs <7 days). $p_{group*time}$ refers to the p-value for the interaction between group and time.

Supplemental Table: The table shows the mean (arithmetic or geometric for variables not requiring or requiring log transformation, respectively) and standard deviation (**SD**) for 7+days and <7 days groups. For ***ECMO course*** and ***ECMO parameters*** variables, ***p-value***

column shows the p-values from the linear mixed models with effects of group, and time and interaction of group*time adjusted for baseline. For variables in ***plasma free haemoglobin, cellular activation*** and ***Inflammation biomarkers***, ***p-value*** shows the p-values from the linear mixed models with effects of group, and time and interaction group*time; and ***P_timept*** shows the p-value for the effect of groups at each measured time point. ***FValue*** – F-statistics with corresponding degrees of freedom (***NumDF*** and ***DenDF***).

Supplemental Figure 1. Oxygenation, iron metabolism and cellular activation

Estimated mean and data points for **A** – SaO2, **B** – PO2, **C** – pCO2, geometric mean and data points for **D** – Plasma Free Haemoglobin, **E** – Bilirubin, **F** – Haptoglobin, platelet (**A** – platelet-granulocyte aggregates CD16/CD41, **B** – platelet-monocyte aggregates) and monocyte activation biomarkers (**C** – CD14/CD41) over time in arterial blood of patients who died or required prolonged stay (7+ days) vs those requiring less than 7 days (<7 days). All participants received 100% FiO2. p_{group} refers to the p-value for the effect of group (7+ vs <7 days). $p_{group*time}$ refers to the p-value for the interaction between group and time.

Figure 1

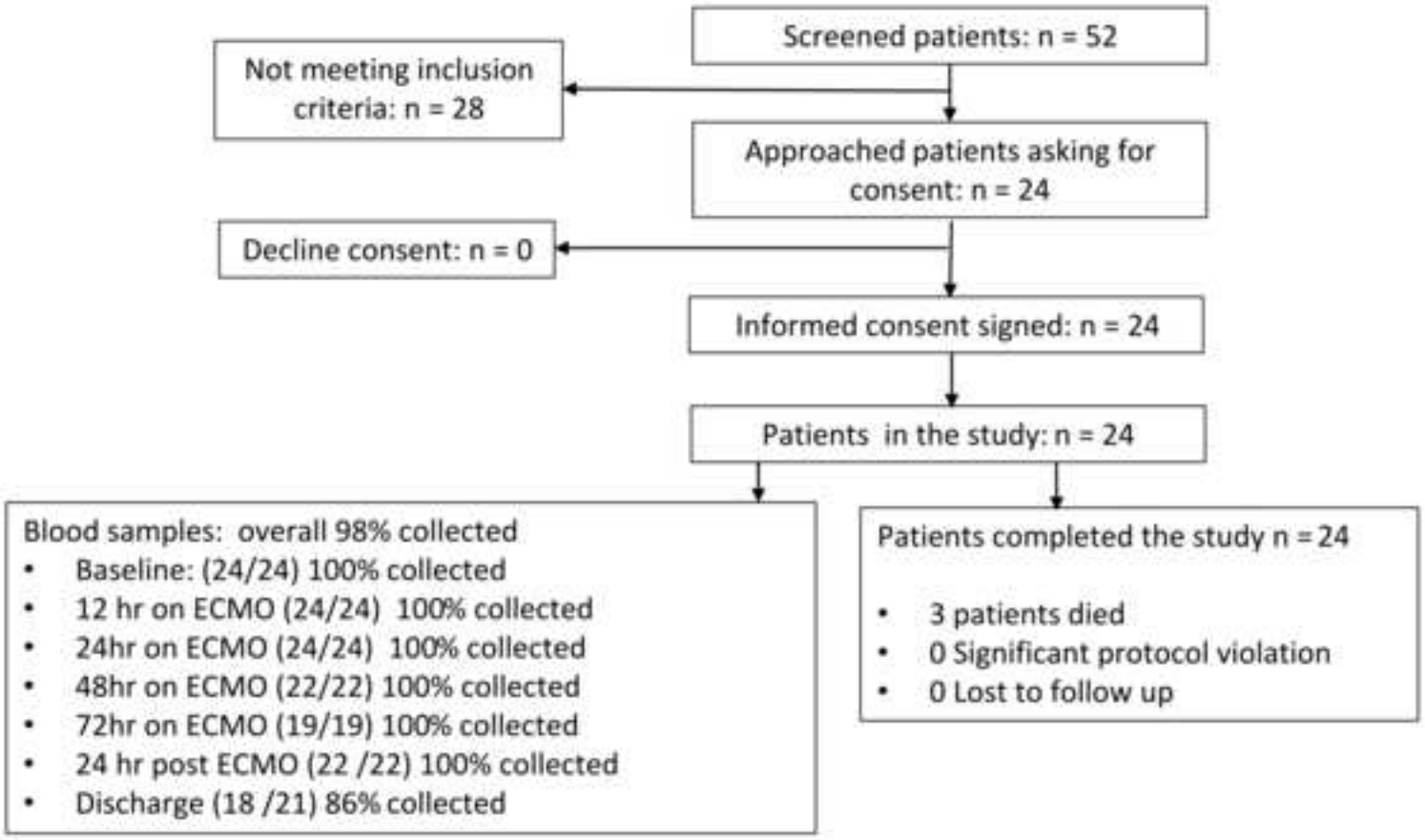

Figure 2

Click here to access/download;Figure;fig3.tif

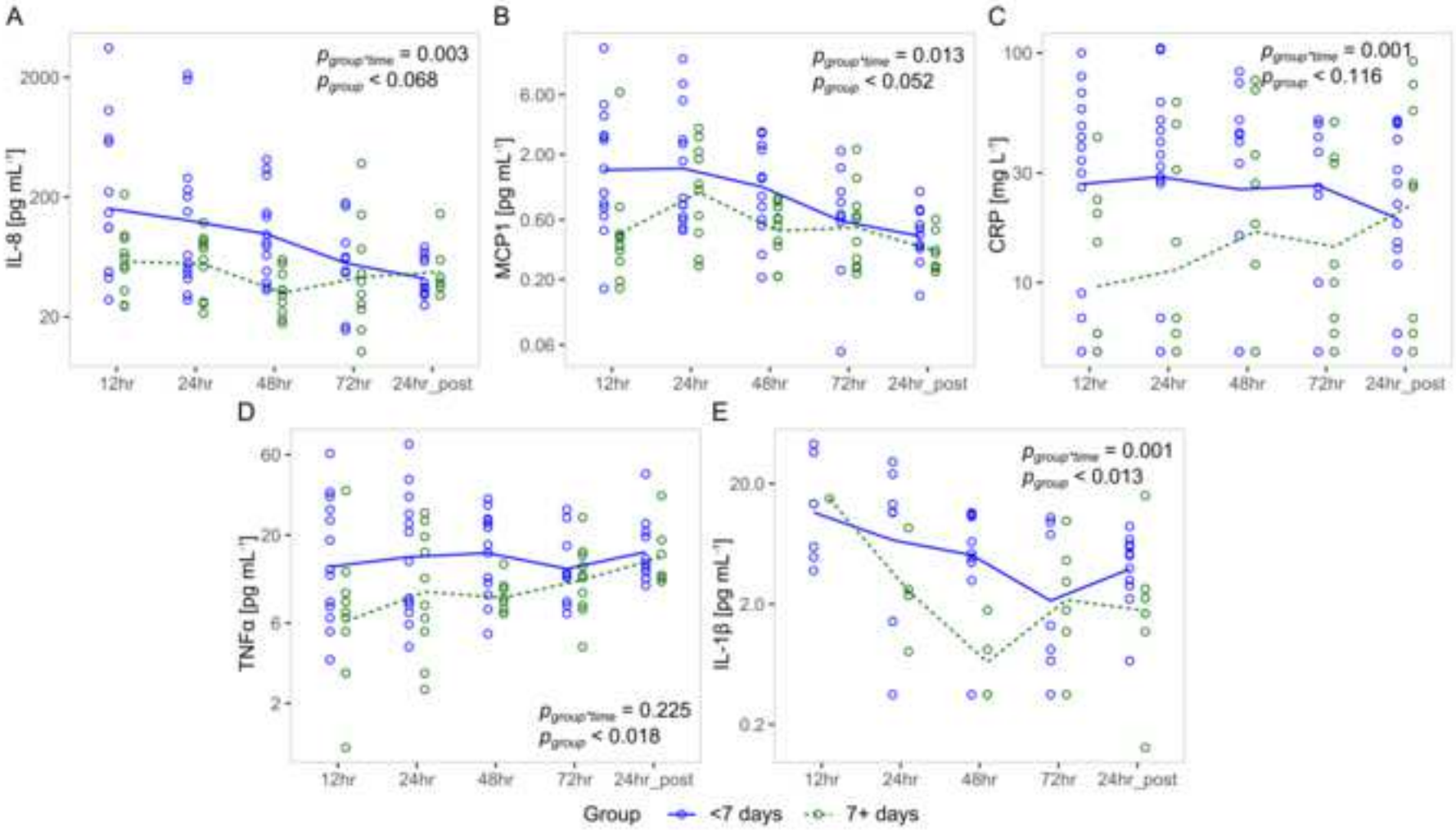

Table 1

Table 1. Demographics and diagnosis. Data is expressed as n (%) for binary variables, and mean(SD) or median (Q1-Q3) where appropriate for continuous variables. [#] n=1 from 7+ days group missing; **ECMO**: Extra-Corporeal Membrane Oxygenation; **PPHN**: Persistent Pulmonary Hypertension of the Newborn; **RV** – right ventricular; **LV** – left ventricular; SIMV – synchronised intermitent mechanical ventilation; **PSV** - Pressure support ventilation; **HFOV** - High frequency oscillatory ventilation

Parameter	overall (n = 24)	ECMO 7+ days (n = 10)	ECMO <7days (n = 14)	p-value
Age (day) on ECMO	2 (2 - 4)	2 (2 - 3)	2 (2 - 4)	0.890
Gestational age (week)	40.6 (2.6)	40.2 (3.3)	40.9 (2.1)	0.508
Sex - male	13 (54.2%)	5 (50.0%)	8 (57.1%)	0.999
Ethnicity - White British	24 (100%)	10 (100%)	14 (100%)	--
Weight at birth (kg)	3.2 (2.9 - 3.5)	3.1 (2.8 - 3.3)	3.2 (3.1 - 4.1)	0.102
Diagnosis				
Idiopathic PPHN	1 (4.2%)	0 (0.0%)	1 (7.1%)	0.999
Meconium Aspiration Syndrome	12 (50.0%)	0 (0.0%)	12 (85.7%)	<0.001
Congenital Diaphragmatic Hernia	9 (37.5%)	8 (80.0%)	1 (7.1%)	0.001
Pneumonia/ Respiratory Distress Syndrome	4 (16.7%)	1 (10.0%)	3 (21.4%)	0.615
other diagnosis	4 (16.7%)	1 (10.0%)	3 (21.4%)	0.615
Cardiac function				
LV - moderate/severe impairment	2 (8.3%)	1 (10.0%)	1 (7.1%)	0.999
RV - moderate/severe impairment	3 (12.5%)	1 (10.0%)	2 (14.3%)	0.999
Shunt[#]				
Left to right	8 (34.8%)	2 (22.2%)	6 (42.9%)	0.333
Right to left	6 (26.1%)	3 (33.3%)	3 (21.4%)	
Bidirectional	5 (21.7%)	1 (11.1%)	4 (28.6%)	
None	4 (17.4%)	3 (33.3%)	1 (7.1%)	
Mechanical ventilation	24 (100.0%)	10 (100.0%)	14 (100.0%)	--
Modality				
SIMV	7 (29.2%)	0 (0.0%)	7 (50.0%)	0.006
PSV	1 (4.2%)	0 (0.0%)	1 (7.1%)	
HFOV	16 (66.7%)	10 (100.0%)	6 (42.9%)	
Cannulation data				
Site – VV	3 (12.5%)	1 (10.0%)	2 (14.3%)	0.999
Blood priming	2 (8.3%)	2 (20.0%)	0 (0.0%)	0.163
Clear priming	14 (58.3%)	5 (50.0%)	9 (64.3%)	0.679

Table 2

Table 2. Baseline clinical characteristics. Data expressed as n(%) for binary variables, and mean(SD) or median (Q1-Q3) where appropriate for continuous variables. **PIM2**: Paediatric Index of Mortality v2; **PMODS**: Paediatric Multiple Organ Dysfunction Score (range from 0 to 20); **FiO_2** - Fraction of inspired oxygen; **PiO_2** – partial pressure of oxygen; **pCO_2** (partial pressure of carbon dioxide); **SaO_2** – oxygen saturation; **MAP** - mean arterial pressure; **ACT** - activated clotting time.

Parameter	n	Overall (n=24)	ECMO 7+ days (n = 10)	ECMO <7days (n = 14)	p-value
ICU admission					
PIM2 risk	24	0.39 (0.21 - 0.55)	0.37 (0.24 - 0.43)	0.43 (0.20 - 0.56)	0.623
Haemodynamic instability prior to ECMO		12 (50.0%)	7 (70.0%)	5 (35.7%)	0.214
PMODS PICU admission	23	8 (7 - 9)	9 (7 - 11)	7 (6 - 9)	0.122
Lactates (mmol L^{-1})	24	4.0 (3.4)	5.3 (4.6)	3.1 (2.0)	0.191
PaO2/FiO2 ratio	24	49.8 (25.4)	46.6 (27.7)	52.1 (24.5)	0.618
Total bilirubin (umol L^{-1})	24	51 (29 - 70)	51 (34 - 59)	51 (17 - 70)	0.526
Fibrinogen (g L^{-1})	23	1.8 (1.4 - 2.9)	1.5 (1.1 - 2.0)	2.5 (1.7 - 2.9)	0.085
Urea (mmol L^{-1})	24	4.8 (1.6)	5.3 (1.4)	4.5 (1.6)	0.197
Start of ECMO parameters					
MAP(mmHg)	23	48 (44 - 61)	59 (40 - 75)	46 (45 - 50)	0.289
FiO2 on ECMO (%)	23	100 (100 - 100)	100 (100 - 100)	100 (100 - 100)	
ACT (sec)	17	304.3 (62.9)	320.3 (36.4)	293.1 (76.2)	0.398
Pump flow (ml min^{-1})	22	362.3 (83.5)	358.0 (59.6)	365.8 (101.9)	0.833
Sweep gas (ml min^{-1})	22	300 (200 - 300)	250 (200 - 300)	300 (225 - 310)	0.244
RMP (revolutions min^{-1})	22	2,786 (361)	2,840 (423)	2,742 (312)	0.537
Start of ECMO - arterial blood gas					
pH	24	7.3 (0.12)	7.2 (0.14)	7.3 (0.08)	0.018
PiO2 (kPa)	24	6.1 (1.2)	6.0 (1.3)	6.2 (1.2)	0.710
pCO2 (kPa)	24	6.4 (1.7)	7.2 (2.1)	5.8 (0.9)	0.078
SaO2 (%)	23	84.3 (7.4)	80.9 (5.1)	86.9 (8.0)	0.050
Glu (mmol L^{-1})	24	7.9 (3.2)	8.0 (2.8)	7.9 (3.5)	0.925
HCO_3 (mmol L^{-1})	24	20.8 (3.8)	21.1 (4.4)	20.5 (3.4)	0.700
Biochemistry					
C-reactive protein (mg L^{-1})	24	16 (5 - 47)	6 (5 - 15)	39 (11 - 48)	0.019
Platelet count (x10^9 L^{-1})	24	152.6 (83.1)	139.9 (99.1)	161.6 (72.1)	0.539
Creatinine (umol L^{-1})	24	63 (44 - 71)	53 (43 - 66)	66 (51 - 73)	0.358
Adjusted Calcium (mmol L^{-1})	23	2.24 (0.17)	2.19 (0.16)	2.27 (0.19)	0.326
Alkaline Phosphatase (iu L^{-1})	23	131 (75 - 207)	107 (66 - 131)	198 (113 - 613)	0.030
Haemoglobin (g L^{-1})	24	148.0 (37.5)	151.5 (33.0)	145.4 (41.5)	0.705
Haematocrit (L L^{-1})	21	0.44 (0.12)	0.45 (0.11)	0.43 (0.14)	0.725
International Normalised Ratio	23	1.8 (0.5)	1.7 (0.4)	1.8 (0.6)	0.661
Activated partial thromboplastin time	22	1.9 (1.2 - 6)	1.8 (1.5 - 6)	1.9 (1.2 - 5.6)	0.357

Table 3. Clinical outcomes. Data is expressed as n(%) for binary outcomes, and mean(SD) or median (Q1-Q3) where appropriate for continuous outcomes. **RBC** – red blood cells; **FFP** – fresh frozen plasma; **SVT/AF** - Supraventricular tachycardia/Atrial fibrillation.

Clinical outcomes	overall (n = 24)	ECMO 7+ days (n = 10)	ECMO <7days (n = 14)	p-value
Transfusion				
RBC transfusion	24 (100.0%)	10 (100.0%)	14 (100.0%)	--
RBC transfusion volume	289.6 (241.0)	479.2 (268.2)	154.1 (74.6)	0.004
any non-RBC transfusion	20 (83.3%)	10 (100.0%)	10 (71.4%)	0.114
FFP transfusion	16 (66.7%)	7 (70.0%)	9 (64.3%)	0.999
Platelet transfusion	19 (79.2%)	10 (100.0%)	9 (64.3%)	0.053
Cryoprecipitate transfusion	13 (54.2%)	8 (80.0%)	5 (35.7%)	0.047
Complications				
SVT/AF requiring treatment	1 (4.2%)	1 (10.0%)	0 (0.0%)	0.417
ECMO circuit clotting	6 (25.0%)	6 (60.0%)	0 (0.0%)	0.002
Inotrope	20 (83.3%)	10 (100.0%)	10 (71.4%)	0.114
Vasopressor	17 (70.8%)	9 (90.0%)	8 (57.1%)	0.172
Tracheostomy	1 (4.2%)	1 (10.0%)	0 (0%)	0.417
Pneumothorax or effusion requiring drainage	5 (20.8%)	5 (50.0%)	0 (0%)	0.006
Acute kidney injury	3 (12.5%)	3 (30.0%)	0 (0%)	0.059
Peptic Ulcer / GI Bleed / Perforation	2 (8.3%)	2 (20.0%)	0 (0%)	0.163
Transient ischaemic attack	1 (4.2%)	1 (10.0%)	0 (0%)	0.417
Excess bleeding (400ml hr^{-1} for 1 hour or 200ml hr^{-1} for 4 hours))	1 (4.2%)	1 (10.0%)	0 (0%)	0.417
Wound requiring treatment	1 (4.2%)	1 (10.0%)	0 (0%)	0.417
Residual anatomical abnormalities requiring surgery	1 (4.2%)	1 (10.0%)	0 (0%)	0.417
Catheter related bloods stream infection	1 (4.2%)	1 (10.0%)	0 (0%)	0.417
Any complications	20 (83.3%)	10 (100.0%)	10 (71.4%)	0.114
Number of complications	2 (1.5 - 4)	4 (3 - 5)	2 (0 - 2)	0.001
Outcome				
Chest open	1 (4.2%)	1 (10.0%)	0 (0.0%)	0.417
Survival to discharge	21 (87.5%)	7 (70.0%)	14 (100.0%)	0.059
Duration on ECMO (hours)	101 (76 - 242)	292 (186-360)	80 (59 - 91)	<0.001

Supplemental Figure 1