

# CLINICAL STUDY PROTOCOL

## The Mi-ECMO Study

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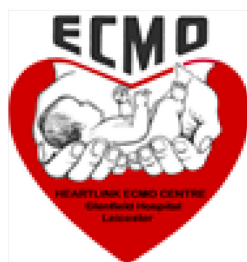
**A FEASIBILITY STUDY TO CONSIDER THE RELATIONSHIP BETWEEN MARKERS OF RED CELL DAMAGE, INFLAMMATION AND THE RECOVERY PROCESS OF NEWBORNS REQUIRING EXTRACORPOREAL MEMBRANE OXYGENATION (ECMO) FOR PERSISTENT PULMONARY HYPERTENSION OF THE NEWBORN (PPHN)**

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**Table of contents**

<b>Table of contents .....</b>	<b>4</b>
<b>Glossary / abbreviations .....</b>	<b>6</b>
<b>1. Study Synopsis.....</b>	<b>7</b>
<b>2. Lay Summary .....</b>	<b>10</b>
<b>3. Background .....</b>	<b>10</b>
3.1 Respiratory Failure in Newborns .....	10
3.2 Extracorporeal membrane oxygenation.....	10
3.3 ECMO, haemoglobin metabolism, endothelial injury and outcomes. ....	11
<b>4. Aims and Objectives.....</b>	<b>13</b>
<b>5. Plan of Investigation .....</b>	<b>14</b>
5.1 Study design .....	14
5.2 Study population .....	14
5.3 Primary and secondary endpoints.....	14
5.4 Clinical management of Study Patients.....	16
<b>6. Study Tests and Procedures.....</b>	<b>19</b>
6.1 Blood sampling .....	19
6.2 Urine sampling.....	20
6.3 Respiratory sampling .....	20
6.4 Sample storage .....	20
6.5 Blood and urine samples assays .....	21
6.6 MV Analyses .....	22
6.7 Bronchial aspirates analysis.....	22
6.8 Summary of trial specific tests and procedures .....	22
6.9 End of the trial.....	23
6.10 Data collection.....	24
6.11 Patient recruitment and consent .....	24
6.12 Planned recruitment rate and duration of follow-up.....	29
6.13 Likely rate of loss to follow-up, protocol violations and clinical data completeness.....	29
6.14 Expenses and benefits .....	30
<b>7. Statistical Considerations .....</b>	<b>31</b>
7.1 Sample Size .....	31
7.2 Measures taken to avoid bias.....	31
7.3 Plan of analysis .....	31
7.4 Criteria for stopping the study early .....	31
<b>8. Study Management.....</b>	<b>31</b>
8.1 Day-to-day management.....	31
8.2 Trial steering committee and data safety & monitoring committee .....	31
<b>9. Safety Reporting .....</b>	<b>32</b>
9.1 Definitions .....	32
9.2 Adverse event reporting.....	32
9.3 Expected adverse events .....	32
9.4 Period for recording adverse events .....	35
9.5 Methods of recording and assessing adverse events.....	35
<b>10. Ethical Considerations.....</b>	<b>36</b>
10.1 Review by a NHS Research Ethics Committee.....	36
10.2 Risks and anticipated benefits.....	36
10.3 Informing potential study participants of possible benefits and known risks .....	37
10.4 Obtaining informed consent from participants.....	37
<b>11. Research Governance.....</b>	<b>37</b>
11.1 Sponsor approval.....	37
11.2 NHS approval .....	37

11.3	Investigators' responsibilities .....	37
11.4	Monitoring by sponsor .....	38
11.5	Indemnity.....	38
11.6	Clinical Trial Authorisation.....	38
<b>12.</b>	<b>Data Protection and Patient Confidentiality.....</b>	<b>38</b>
12.1	Data protection.....	38
12.2	Data handling, storage and sharing.....	38
<b>13.</b>	<b>Dissemination of Findings .....</b>	<b>39</b>
<b>14.</b>	<b>References.....</b>	<b>40</b>
<b>15.</b>	<b>Protocol Signature Page .....</b>	<b>44</b>
<b>16.</b>	<b>Appendix 1 .....</b>	<b>45</b>

## Glossary / abbreviations

AE	Adverse event
AKI	Acute kidney injury
ARG1	Arginase 1
CD	Cluster of differentiation
CDH	Congenital diaphragmatic hernia
CO <sub>2</sub>	Carbon dioxide
CmH <sub>2</sub> O	Centimetre of water
CPB	Cardiopulmonary bypass
CRF	Case report form
CRRT	Continuous renal replacement therapy
CSCTT	Cardiac surgery clinical trials team
DMSC	Data monitoring and safety committee
ECMO	Extra-corporeal membrane oxygenation
EDTA	Ethylenediaminetetraacetic acid
FACS	Fluorescence activated cell sorting
FiO <sub>2</sub>	Fraction of inspired oxygen
GFR	Glomerular filtration rate
Hb	Haemoglobin
HFOV	High frequency oscillatory ventilation
HMOX1	Heme oxygenase 1
ICU	Intensive care unit
ICAM	Intercellular adhesion molecule
IL	Interleukin
INOS	Inducible nitric oxide synthase
KDIGO	The International Society of Nephrology: Kidney Diseases Improving Global Outcomes (KDIGO) definition of acute kidney injury (AKI)
MAS	Meconium aspiration syndrome
MABP	Mean arterial blood pressure
MCP	Monocyte chemoattractant protein
MI	Myocardial infarction
MP	Microparticles
MV	Microvesicles
NGAL	Neutrophil gelatinase associated lipocalin
NO	Nitric oxide
pCO <sub>2</sub>	Partial pressure of carbon dioxide
PEEP	Positive End Expiratory Pressure
PICU	Paediatric intensive care unit
PIL	Patient information leaflet
PPHN	Persistent pulmonary hypertension of the newborn
RDS	Respiratory distress syndrome
REC	Research ethics committee
RNA	Ribonucleic acid
SAE	Serious adverse event
SaO <sub>2</sub>	Oxygen saturation
SIRS	Systemic inflammatory response syndrome
SOP	Standard operating procedures
SSAR	Suspected serious adverse reaction
SUSAR	Suspected unexpected serious adverse reaction
TNF-α	Tumour necrosis factor - alpha
TSC	Trial steering committee

UHL	University Hospital Leicester
VAD	Ventricular assist device
VV-ECMO	Veno Venous-ECMO
VA-ECMO	Veno Arterial-ECMO

## 1. Study Synopsis

Title of Study	A feasibility study to consider the relationship between markers of red cell damage, inflammation and the recovery process of newborns requiring Extra-Corporeal Membrane Oxygenation (ECMO) for Persistent Pulmonary Hypertension of the Newborn (PPHN)
Acronym	<b>Markers of Inflammation and Lung recovery in ECMO patients for PPHN: the MI-ECMO study</b>
Name of Sponsor	University of Leicester
Study Hypothesis	<p>Our primary hypothesis is that damage to red blood cells by the exposure to the ECMO circuit will result in inflammatory responses that mitigate against successful weaning from Extra-Corporeal Membrane Oxygenation (ECMO) for Persistent Pulmonary Hypertension of the Newborn (PPHN).</p> <p>Our secondary hypothesis are:</p> <ol style="list-style-type: none"> <li>1. Damage to red cells will result in platelet, leukocyte and endothelial activation.</li> <li>2. Markers of platelet, endothelial and leukocyte activation are indicators of lung inflammation and injury severity and hence lung recovery.</li> <li>3. Markers of platelet, endothelial and leukocyte activation are indicators of kidney injury severity and hence acute kidney injury.</li> <li>4. The level of oxidative stress will correlate with type shifts in pulmonary macrophages, tissue iron deposition and organ injury.</li> <li>5. Ability to raise anti-oxidative response, measured by Heme Oxygenase-1 (HMOX 1) expression, will correlate with shorter intubation times and less severe kidney and lung injury.</li> <li>6. Granulocyte and platelets activation are secondary to rising redox potential and the levels of activation will correlate with longer intubation times and more severe organ injury.</li> <li>7. Markers of anti-oxidative response, platelet, endothelial and leukocyte activation, as well as oxidative stress levels have diagnostic and prognostic utility for the prediction of key clinical events including delayed time to recovery, acute kidney injury in paediatric patients undergoing Extra-Corporeal Membrane Oxygenation (ECMO) for Persistent Pulmonary Hypertension of the Newborn (PPHN).</li> </ol>
Study Objectives	<p>This is a pilot feasibility study that will establish the following:</p> <ol style="list-style-type: none"> <li>1. Recruitment rates and patient flows for 24 patients specified as the target population for the feasibility study</li> <li>2. Withdrawal rate, and completeness of follow-up and data collection in a paediatric population at high risk for death and major morbidity</li> <li>3. The proportions (categorical data) and variance (continuous data) for the primary and secondary outcomes of interest. These will be used to model the sample sizes and outcomes that may be used in a definitive study</li> </ol>

	4. Perceptions of family members whose children participate in the study as to the appropriateness of the screening and consent process (Appendix 1)
Study Design	Prospective, single-centre observational feasibility study
Setting	The study will be carried out at a regional paediatric ECMO centre in the UK, the University Hospitals of Leicester NHS Trust
Planned Sample Size	24 children undergoing ECMO will be enrolled
Recruitment Rate	This unit performs over 60 neonatal and paediatric ECMO per year, of which at least 40 are expected to be caused by PPHN in infants
Subject Selection Criteria	<p>Participant may enter study if ALL of the following apply:</p> <ol style="list-style-type: none"> <li>1. Patients with a diagnosis of PPHN</li> <li>2. Patients that require ECMO support as determined by the ECMO team</li> <li>3. Patients aged less than 30 days</li> <li>4. Emergency consent obtained within 12 hours from cannulation, and ultimately full consent</li> </ol> <p>Participant may not enter study if ANY of the following apply:</p> <ol style="list-style-type: none"> <li>1. PPHN is caused by a congenital heart pathology</li> <li>2. ECMO is required for a congenital heart disease</li> <li>3. Lack of consent</li> </ol>
Primary outcome of interest	Markers of platelet, leukocyte and endothelial cell activation in arterial blood at baseline (12 hours after ECMO commencement) and then at 24, 48 and 72 hours post commencement and at weaning (end of ECMO support: 24 hours after decannulation) or immediately prior to ECMO support being stopped in case of death or treatment withdrawal
Secondary outcomes of interest	<ol style="list-style-type: none"> <li>1. Serum haemoglobin levels.</li> <li>2. Data on recruitment, sampling, and compliance rates.</li> <li>3. Data on demographics, clinical characteristics and medications of study subjects.</li> <li>4. Time to wean from ECMO.</li> <li>5. Pulmonary inflammation as determined qualitatively by analysis of bronchial aspirates lysates</li> <li>6. Percentage of patients weaned from ECMO within 7 days</li> <li>7. Acute kidney injury as defined by the KDIGO definition (Table 2).</li> <li>8. Heart injury as determined by serum troponin levels.</li> <li>9. Renal inflammation as determined by urine NGAL concentrations.</li> <li>10. Allogenic red cell transfusion volume.</li> <li>11. Non red cell transfusion volume.</li> <li>12. Time to discharge from Glenfield Hospital ICU.</li> <li>13. Time to Glenfield Hospital discharge.</li> <li>14. Measures of haemoglobin metabolism, for example: serum hepcidin, transferrin saturation, serum ferritin, serum haptoglobin, plasma labile iron concentrations, oxidative stress, HMOX 1 expression in inflammatory cells from blood and bronchial aspirate.</li> <li>15. Levels of inflammatory cytokines in serum.</li> <li>16. MV levels and activation of leukocytes and platelets in circulation</li> <li>17. Other adverse events not specified above.</li> <li>18. A questionnaire will assess parents/family/guardians experience of the screening, consent and study procedures. (Appendix 1)</li> </ol>



Statistical Considerations	<p>A statistical analysis plan will be established prior to any analysis. Normally distributed data will be reported as means (standard deviations). Non normally distributed data will be presented as geometric means (95% confidence intervals). Dichotomous data will be presented as percentages. The analysis for the feasibility study will be descriptive, i.e. describing the relationship between continuous and dichotomous primary and secondary outcomes of interest to address the primary and secondary objectives above. These analyses will contribute to the design of a further prospective study that will consider the role of red cell derived inflammatory responses as early biomarkers of lung recovery and/or kidney injury in paediatric patients requiring ECMO for primary pulmonary hypertension of the newborn (PPHN).</p>
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## 2. Lay Summary

Respiratory failure in newborns is common and has high rates of death. Where conventional intensive care strategies have failed, newborn children are referred to treatment with Extra- Corporeal Membrane Oxygenation (ECMO) (1). This involves connecting children via large bore cannulas placed in their heart and major blood vessels to an artificial lung that adds oxygen to their blood and removes waste gases (carbon dioxide). Although this treatment saves lives (2, 3), it still has some limitations. In particular, severe complications like bleeding, or damage to the kidneys can occur. These complications can lead to death in some cases and long-term disability in others. Based on our ongoing research in adults and children undergoing cardiac surgery we have identified a new process that may underlie some of the complications observed in ECMO. We have noted that when transfused blood is infused in an ECMO circuit, this results in the accelerated release of substances from the donor cells that cause organ damage; at least in adults. There are treatments that can reverse this process. Before we explore whether these treatments should be used in newborn children on ECMO, we must first demonstrate that we can measure the complex inflammatory processes that occur in these critically ill children. We therefore propose to conduct a feasibility study to identify the practical issues and challenges that would need to be overcome in order to perform a successful trial in this high-risk population.

## 3. Background

### 3.1 Respiratory Failure in Newborns

Persistent Pulmonary Hypertension of the Newborn (PPHN) is a life-threatening condition that causes respiratory failure and death in neonates. PPHN is characterised by the absence of the normal postnatal fall in pulmonary vascular resistance, which leads to persisting right to left shunts across the foetal channels and resultant hypoxia and right ventricular dilatation. The condition primarily affects full term and late preterm babies with an incidence of 1–2/1000 live births (4). In many cases PPHN responds to treatments such as inhaled nitric oxide therapy or high frequency oscillatory ventilation (HFOV), however, in children refractory to those treatments, prior to the development of ECMO mortality was as high as 80% (5).

### 3.2 Extracorporeal membrane oxygenation

Randomised trials have shown that ECMO (6) can reduce mortality in children with respiratory failure refractory to conventional treatments, and ECMO has been shown to reduce mortality rates for neonates with PPHN (5) to as low as 10-20%. Despite this progress, challenges remain. ECMO is highly invasive, which in itself results in high levels of morbidity (7) including bleeding (cannulation site bleeding 7.6%, gastro-intestinal bleeding 1.6%, disseminated intravascular coagulation 2.8%), stroke (haemorrhagic 7% and ischaemic 7.4%), acute kidney injury 64% (8) and infection 6%. (9). In a study of neonates with primary PPHN, 74% developed a severe complication while on ECMO (10). This morbidity contributes to mortality, and to long-term health and developmental problems with long-term disability in 45% of survivors (11).

Aetiology of PPHN is also an important determinant of outcome. Meconium aspiration syndrome (MAS) is the most common cause of PPHN because it represents 42% affecting about 42-84/100.000 live-births; MAS is also the most common indication for ECMO (4). Idiopathic PPHN, a process that is poorly understood (4, 12, 13, 14), is the second PPHN cause and affects 27-54/ 100.000 live births (27% of all the PPHN), but is the most common in the near-term (>34 weeks gestation) and term newborns.

Congenital Diaphragmatic Hernia (CDH) complicates 1 of every 2000 to 4000 live births and represents the second most common ECMO indication (4). The Extracorporeal Life Support Registry (ELS) reports neonatal survival rates of 94% for MAS, 77% for Idiopathic PPHN, and 51% for CDH respectively (9). This compares to a mean survival in children treated with ECMO for cardiac failure of 43% overall (9), which reflects the reversible nature of PPHN in new-borns and the potential additional benefits of

refinements in care to the short and long-term outcomes in these children. Other factors that influence outcome are pre-ECMO status, and therapies. Risk factors for mortality include prematurity, a pre-ECMO pH <7.2, a pre-ECMO SaO<sub>2</sub> <65%, initiation of ECMO support at or beyond 5<sup>th</sup> day of life, and a duration of ECMO greater than 7 days (10).

Our understanding of how these clinical factors interact to determine which children recover or do not recover is poor, and there are no biochemical or laboratory measures that have been widely adopted as diagnostic or prognostic markers. Our ongoing research has identified important pathophysiological processes that are associated with organ failure and recovery in adults and children undergoing cardiac surgery with cardiopulmonary bypass. These processes, in particular the role of free haemoglobin within extracorporeal circuits have not been studied extensively in children treated with ECMO. We are proposing to conduct a study to explore the feasibility of measuring inflammatory processes in children in ECMO, thus enabling further studies that can provide insights into lung recovery during ECMO, provide diagnostic or prognostic markers that may refine treatment, or allow the transfer to children of developments and treatments for organ failure in adult patients. This has not been done previously.

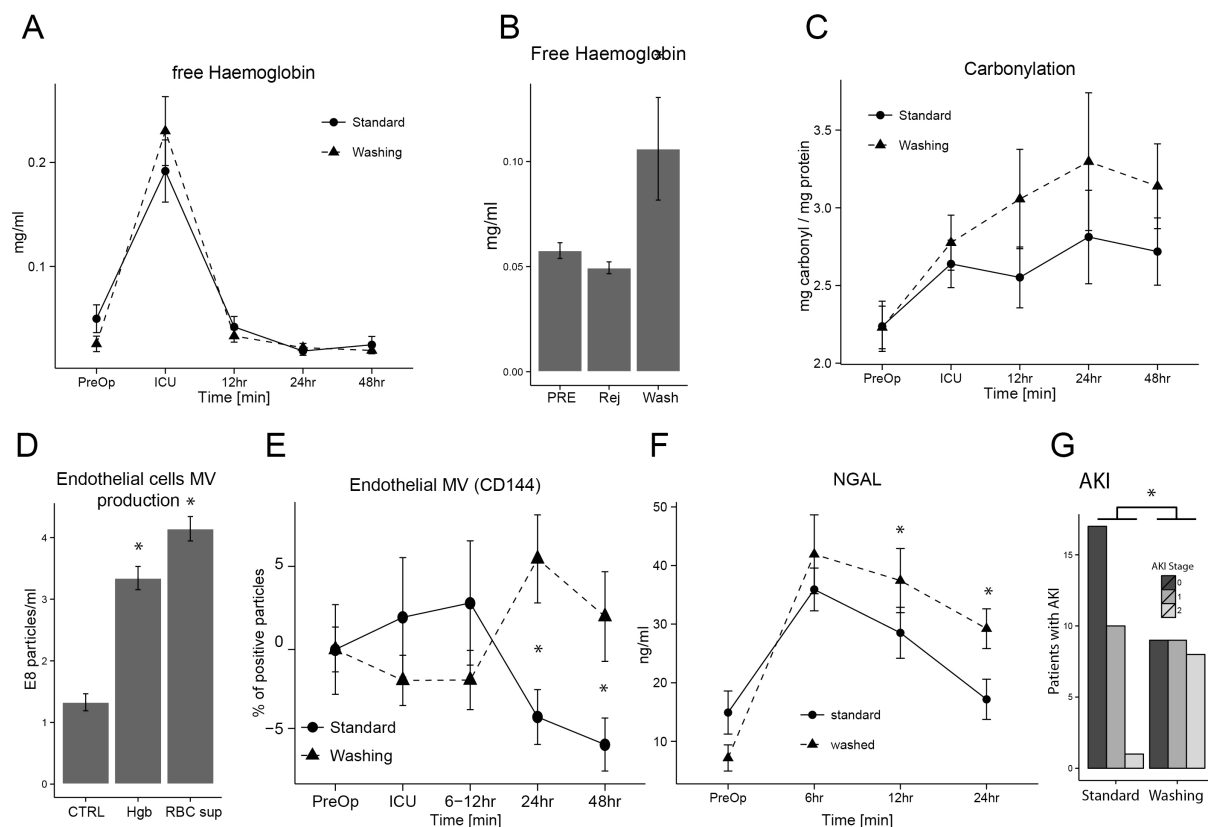
### **3.3 ECMO, haemoglobin metabolism, endothelial injury and outcomes.**

ECMO involves connecting the patients via large-bore cannulas placed in their heart and major blood vessels to an external device. During ECMO, blood is drained from the native vascular system, circulated outside the body by a mechanical pump, and reinfused into the circulation. While outside the body, the blood passes through an oxygenator and heat exchanger. In the oxygenator, haemoglobin becomes fully saturated with oxygen, while carbon dioxide (CO<sub>2</sub>) is removed. Oxygenation is determined by flow rate, where elimination of CO<sub>2</sub> can be controlled by adjusting the rate of countercurrent gas flow through the oxygenator.

In cases of Veno-Arterial ECMO the circuit augments perfusion pressures and cardiac output, whereas in Veno-Venous ECMO the circuit provides gas transfer only. Systemic anticoagulation is required to prevent circuit thrombosis attributable to the large non-endothelialized surface area that also elicits profound activation of inflammatory proteases in the blood along with leukocytes and platelets (15-19). These processes result in systemic inflammation, and this has parallels with the inflammatory response observed in patients undergoing cardiac surgery with cardiopulmonary bypass (20-24). Activation of inflammatory processes within the circuit coupled with the mechanical shear stress of the pump also leads to red cell damage and release of free haemoglobin into the plasma. This is considered a major problem in neonatal ECMO where severe haemolysis and subsequent rise in free haemoglobin are reported in over 10% of cases (25-30). Free haemoglobin is highly reactive and can oxidize multiple species including plasma proteins and membrane lipids causing inflammation, endothelial injury and organ dysfunction (31-38). In particular, free haemoglobin has been implicated in the progression of pulmonary hypertension (39-41), and this may be relevant to the recovery for neonates with PPHN.

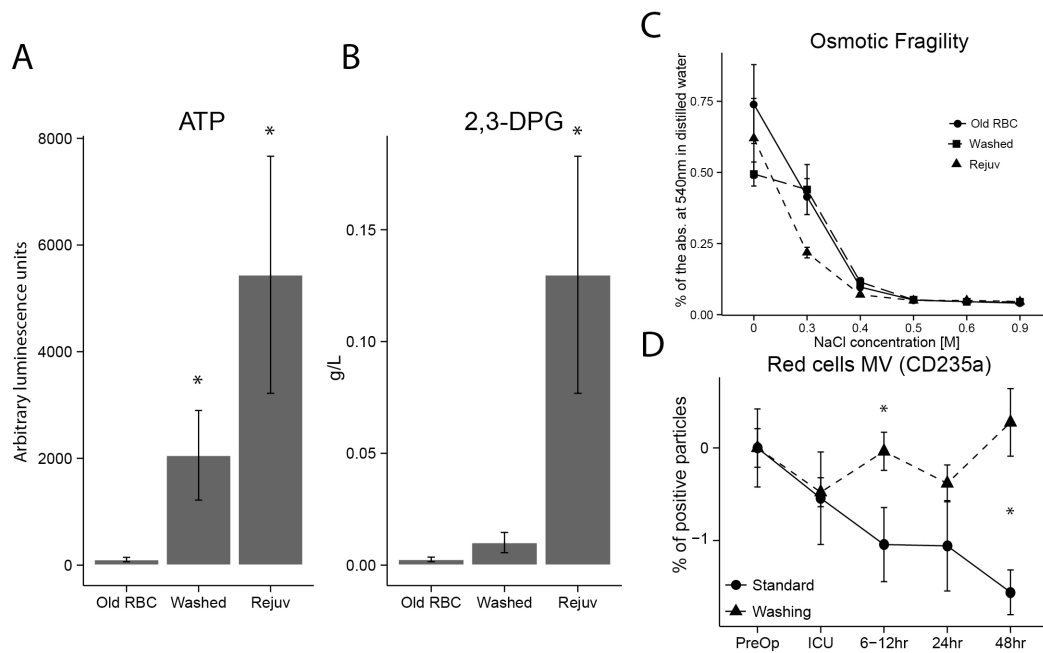
In the recent REDWASH study (42), we have characterised the effects of centrifugal pumps on red cells in adult patients undergoing cardiac surgery. We showed that patients receiving washed blood had increased free haemoglobin levels (fig. 1A), caused by exposure of stored donor red cells to a centrifugal pump (fig. 1B) during washing. This free haemoglobin was highly reactive and directly activated cultured endothelial cells in vitro. In trial patients higher free haemoglobin levels in patients that received washed blood resulted in oxidative stress (protein carbonylation), endothelial injury (CD144 positive microvesicles increased), renal inflammation (elevated NGAL) and acute kidney injury (fig 1C-G).

To prevent haemolysis, red cells can be treated with a rejuvenating solution (Rejuvesol), which restores energy levels (fig. 1A, 2A,B) and increases red cells resistance to physical stress (fig. 2C).



**Figure 1. Role of free haemoglobin in organ injury in REDWASH patients:** N=60 patients receiving a mean of 3 units of blood randomised to standard care or red cell washing using the Fresenius CATS device. Results showed that red cell washing increased circulating free haemoglobin levels (A), which was due to the haemolysis during washing (B). Free haemoglobin led to a mild oxidative stress, as indicated by elevated carbonyl modifications in plasma proteins (C). Our *in vitro* experiments indicate that free haemoglobin and oxidative stress lead to endothelial activation, as indicated by higher levels of MV production (D). Higher levels of endothelial MV (estimated with flow cytometry) are also observed in patients transfused with washed blood (E) and correlate with carbonylation levels. Endothelial dysfunction most likely affected kidney function as indicated by NGAL levels (F) and higher incidence of AKI (G) identified according to the KDIGO criteria.

Washing of stored red cells also led to higher ATP content by increasing RBC metabolic rate when exposed to increased shear stress in extracorporeal circuits. This results in red cell activation manifested by MV shedding (fig. 2D) and accelerated ATP depletion. The released MV could further increase oxidative stress by directly delivering haem to endothelial cells (Camus et al., 2015) or causing vaso-occlusions in kidney (Camus et al., 2012). Given that virtually all neonatal ECMO patients are transfused with stored red cells, the parallels we have observed between adults and children undergoing cardiac surgery in terms of inflammatory responses, and the results of the REDWASH trial, we now suggest that activation of red cells by the centrifugal pump and shear stress within the ECMO circuit may contribute to increased serum hemoglobin levels and inflammatory red cell microparticles release. We hypothesise that levels of free hemoglobin and red cell derived microparticles will be associated with endothelial activation, increased markers of pulmonary leucocyte activation and platelet activation, acute kidney injury and potentially failure to wean from ECMO (43-53).



**Figure 2. RBC biochemical properties and the role of RBC MV:** ATP (A), 2,3-DPG (B) and osmotic fragility (C) levels in 20 days old RBC, before and after washing or rejuvenation. (D) RBC MV production in REDWASH patients.

If our hypothesis is correct, this study will identify new processes involved in the pathogenesis of ECMO morbidity and potentially identify new biomarkers of recovery or delayed recovery. Finally, Rejuvesol is FDA licenced and may represent an effective therapy for the prevention of these processes. Before we can explore this in detail, we will first conduct a feasibility study to evaluate the practical challenges and key considerations for the successful completion of a research study that evaluates complex inflammatory pathways in critically ill children.

#### 4. Aims and Objectives

Our primary hypothesis is that damage to red blood cells by the exposure to the ECMO circuit will result in inflammatory responses that mitigate against successful weaning from extracorporeal membrane oxygenation (ECMO) for persistent pulmonary hypertension of the newborn (PPHN).

Our secondary hypotheses are:

1. Damage to red cells will result in platelet, leukocyte and endothelial activation.
2. Markers of platelet, endothelial and leukocyte activation are indicators of lung inflammation and injury severity and hence lung recovery.
3. Markers of platelet, endothelial and leukocyte activation are indicators of kidney injury severity and hence acute kidney injury.
4. The level of oxidative stress will correlate with type shifts in pulmonary macrophages, tissue iron deposition and organ injury.
5. Ability to raise anti-oxidative response, measured by Heme Oxygenase-1 (HMOX 1) expression, will correlate with shorter intubation times and less severe kidney and lung injury.
6. Granulocyte and platelets activation are secondary to rising redox potential and the levels of activation will correlate with longer intubation times and more severe organ injury.
7. Markers of anti-oxidative response, platelet, endothelial and leukocyte activation, as well as oxidative stress levels have diagnostic and prognostic utility for the prediction of key clinical events including delayed time to recovery, acute kidney injury in paediatric patients on ECMO for PPHN.

To assist with the design of a prospective observational study that will test these hypotheses we propose to undertake a feasibility study in 24 new-borns. The objectives of this feasibility study are:

1. Recruitment rates and patient flows for 24 patients specified as the target population for the feasibility study.
2. Withdrawal rate and completeness of follow-up and data collection in a paediatric population at high risk for death and major morbidity.
3. The proportions (categorical data) and variance (continuous data) for the primary and secondary outcomes of interest. These will be used to model the sample sizes and outcomes that may be used in a definitive study.
4. Perceptions of family members, whose children participate in the study as to the appropriateness of the screening and consent process. (Appendix 1)

## **5. Plan of Investigation**

### **5.1 Study design**

This is a prospective, single-centre observational feasibility study that will measure changes in free haemoglobin and markers of cellular activation, and their relationship to lung recovery or acute kidney injury in newborns with PPHN requiring ECMO support.

The initial feasibility study will enrol 24 patients and will assist with the design of a subsequent observational study that will test our hypotheses.

### **5.2 Study population**

The study will be conducted at a regional ECMO centre in the UK, the University Hospitals of Leicester NHS Trust. This unit performs over 60 neonatal and paediatric ECMO per year, of which at least 40 are expected to be performed for the treatment of PPHN in infants.

#### **5.2.1 Inclusion Criteria**

Participant may enter study if ALL of the following apply:

1. Patients with a diagnosis of PPHN
2. Patients that require ECMO support as determined by the ECMO team
3. Patients aged less than 30 days
4. Emergency consent obtained within 12 hours from cannulation, and ultimately full consent

#### **5.2.2 Exclusion criteria**

Participant may not enter study if ANY of the following apply:

1. PPHN is caused by a congenital heart pathology
2. ECMO is required for a congenital heart disease
3. Lack of consent

Participants will be 24 consecutive patients who match the study criteria and that are referred to Glenfield Hospital from different British centres according to the ECMO referral system. The Glenfield Hospital is a national paediatric referral centre and provides ECMO support across UK from phone consultation to onsite cannulation and transport on ECMO. Patient referred to Glenfield Hospital are transferred to the Paediatric Intensive Care Unit for further care.

### **5.3 Primary and secondary endpoints**

### 5.3.1 *Primary outcome*

Markers of platelet, leukocyte and endothelial cell activation in arterial blood at baseline (12 hours after ECMO commencement) and then at 24, 48 and 72 hours post commencement and at weaning (24 hours after decannulation) or immediately prior to ECMO support being stopped in case of death or treatment withdrawal.

### 5.3.2 *Secondary outcomes*

1. Serum haemoglobin levels.
2. Data on recruitment, sampling, and compliance rates.
3. Data on demographics, clinical characteristics and medications of study subjects.
4. Time to wean from ECMO.
5. Pulmonary inflammation as determined qualitatively by analysis of bronchial aspirates lysates.
6. Percentage of patients weaned from ECMO within 7 days
7. Acute kidney injury as defined by the KDIGO definition (Table 1).
8. Heart injury as determined by serum troponin levels.
9. Renal inflammation as determined by urine NGAL concentrations.
10. Allogenic red cell transfusion volume.
11. Non red cell transfusion volume.
12. Time to discharge from Glenfield Hospital ICU.
13. Time to Glenfield Hospital discharge.
14. Measures of haemoglobin metabolism, for example: serum hepcidin, serum haptoglobin, transferrin saturation, serum ferritin, plasma labile iron concentrations, oxidative stress, HMOX 1 expression in inflammatory cells from blood and bronchial aspirate.
15. Levels of inflammatory cytokines in serum.
16. MV levels and activation of leukocytes and platelets in circulation
17. Other adverse events not specified above.
18. A questionnaire will assess parents/ family/ guardians experience of the screening, consent and study procedures. (Appendix 1)

**Table 1.** KDIGO staging system for acute kidney injury (55), with modified Stage 3, as per the pRIFLE definition (56)

Stage	Serum creatinine (SCr)	Urine output
1	SCr $\geq$ 26.5 $\mu$ mol/L (0.3 mg/dL) increase <u>or</u> SCr $\geq$ 1.5- to 1.9-fold from baseline	<0.5 mL/kg/hr for > 6 consecutive hrs
2	SCr $\geq$ 2 to 2.9-fold from baseline	<0.5 mL/kg/ hr for > 12 hrs
3	SCr $\geq$ 3-fold from baseline <u>or</u> SCr of more than or equal to 353.6 $\mu$ mol/L (4 mg/dL) <u>or</u> renal replacement therapy irrespective of stage <u>or</u> , in patients <18 years, decrease in estimated creatinine clearance (eCrCl) to less than 35 mL/min/1.73 m <sup>2</sup> using the Schwartz equation: $eCrCl(\text{mL/min/1.73 m}^2) = (36.2 \times \text{Height in cm}) / \text{Creatinine in } \mu\text{mol/L}$	<0.3 mL/kg/ hr for > 24 hrs <u>or</u> anuria for > 12 hrs

The above criteria include both an absolute and a relative change in creatinine to accommodate variations related to age, gender, and body mass index and to reduce the need for a baseline creatinine but do require at least two creatinine values within 48 hours. Only one criterion (creatinine or urine output) has to be fulfilled to

qualify for a stage. Given the wide variation in indications and timing of initiation of renal replacement therapy (RRT), individuals who receive RRT are considered to have met the criteria for stage 3 irrespective of the stage they are in at the time of RRT. The modified stage 3 criteria for children reflect the observation that infants with small body mass may be unable to generate high serum creatinine values.

#### **5.4 Clinical management of Study Patients**

##### **5.4.1 Pre-ECMO care and ECMO indication**

Eligible patients receive standard care during the pre-ECMO period, according to the different respiratory failure aetiology and protocols from referral hospital. Patients requiring ECMO support are intubated and ventilated with conventional or High Frequency Oscillatory Ventilation (HFOV).

##### **5.4.2 The ECMO circuit and cannulation**

The ECMO circuit consists of vascular access cannulas, polyvinyl chloride tubing for blood drainage and reinfusion, a centrifuge pump, an appropriate sized oxygenator and a heat exchanger. Cannulation in neonates is always performed using an open surgical approach. VA (veno-arterial) or VV (veno-venous) cannulation is chosen based on the clinical status of the patient and the availability of the correct material. VA-ECMO is the first choice for patients who need both respiratory and hemodynamic support, while the VV-ECMO is preferred if only respiratory support is required. Currently, the indication for VA-ECMO in newborns is extended also to patients with good cardiac function since double lumen cannulas for VV-ECMO are not available for the smallest neck vessels. If the vessels size is adequate, VV-ECMO is chosen when the patient requires only a respiratory support. The ECMO consultant decides the best approach, according to the clinical status and the neck vessels diameter.

VA ECMO involves surgical cannulation of the right common carotid artery and internal jugular vein, with the tip of the venous cannula advanced into the right atrium and the arterial catheter positioned at the junction of the right common carotid artery and aortic arch. The right-sided vessels are used specifically for ease of advancement of the venous catheter into the right atrium.

Conversely, VV ECMO uses a single, double-lumen cannula that is advanced to the right atrium, where blood is drained and reinfused into the same chamber, thus only requiring cannulation of the right internal jugular vein while sparing the carotid artery.

##### **5.4.3 ECMO management**

Glenfield ECMO centre protocols will determine the conduct of ECMO support. In reference to VA-ECMO the flow is typically maintained at about 100-120 ml/kg/min to achieve normal saturation ( $\text{SaO}_2 > 95\%$ ), with a controlled  $\text{pCO}_2$  to achieve a normal pH. Venous saturation is typically kept greater than 60%. The target of saturation during VV ECMO is 85% with a venous saturation  $> 60\%$  and low lactates with targeted flow rates of 80 ml/kg/min. Sweep gas is usually maintained at a  $\text{FiO}_2$  100% and regulated on the  $\text{pCO}_2$  basis; the usual ECMO flow/sweep gas ratio is 1:1.

##### **5.4.4 Ventilator Management**

During ECMO, the patient is commonly ventilated in a “lung rest” setting. Rest setting is defined as respiratory rate of 10 b/min, PEEP (positive and expiratory pressure) of 10  $\text{cmH}_2\text{O}$  and pressure over PEEP 10  $\text{cmH}_2\text{O}$  with  $\text{FiO}_2 < 40\%$ . The clinician can decide to change this setting or to change in a HFOV modality according to clinical status.

##### **5.4.5 Haemodynamic support**



The haemodynamic management during VA and VV-ECMO is different. During VA-ECMO the patient receives haemodynamic support over the respiratory support, and the usual inotropes requirement is reduced or null. VV-ECMO provides only a respiratory support, in this case the clinician can use inotropic or vasoactive drug to maintain an adequate perfusion and cardiac output. In general practice targets are MABP > 40 mmHg, lactate < 2 mmol/l, central venous saturation > 60%.

#### 5.4.6 Anaesthesia induction

The standard Glenfield anaesthetic technique will be used where possible. This typically includes the administration of Ketamine 2 mg/kg for sedation and Atracurium 1 mg/kg as a neuromuscular blocking agent. The underlying sedation might be modified according to the requirements of the patient. Deviations from this protocol will be recorded.

#### 5.4.7 Fluid Management

Target urine output for neonates is > 0.5 ml/kg/h. This may be 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.

#### 5.4.8 Administration of non-RBC Blood Components

Children receiving blood transfusions frequently receive non RBC components that can affect inflammatory responses. These will be administered according to standard unit protocols, with the indication, volume and timing of their administration recorded.

#### 5.4.9 Red cell transfusion

The Glenfield ECMO red cell transfusion policy indicates transfusion with 10 ml/kg of allogenic red cells if the Hb < 13 g/dL. Red cells may be transfused outwith this protocol if indicated for bleeding.

#### 5.4.10 Concomitant Treatment

Patients may receive medications and/or other therapies to treat adverse events as deemed necessary by the investigator or the patient's physician. Concomitant medications and/or therapy that become necessary during the study and any changes in concomitant medication and/or therapy will be recorded on the CRFs. Details of concomitant medications and therapy will include generic drug name, dose, route, frequency, duration and indication.

#### 5.4.11 Weaning process

Clinician and patient clinical status guide ECMO weaning process. VA ECMO is usually weaned with "retrograde flow", in this case the flow in the cannula is inverted 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 leads to restart the ECMO support or to treatment withdrawal.

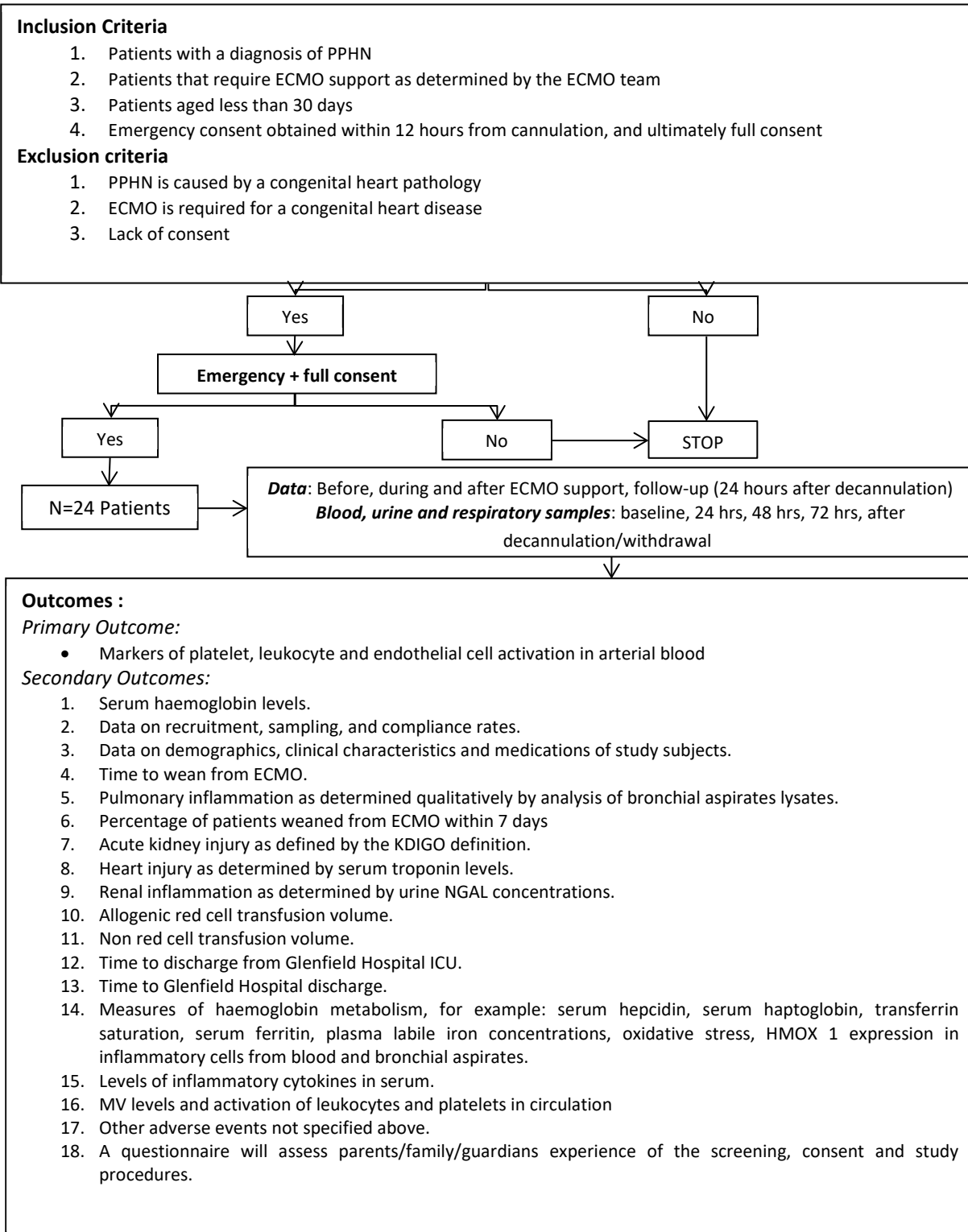
#### 5.4.12 Withdrawal criteria

The criteria for withdrawal of participants will be the following:

1. Parent/guardian's request
2. If the patient is found not to meet all the inclusion criteria

In the event of withdrawal, we will request that all patient's data and tissues collected until that time are retained for analysis. In case of denial, all data will be removed and tissue disposed of.

### Mi-ECMO study Patient Flow and outcomes



## 6. Study Tests and Procedures

### 6.1 Blood sampling

Blood samples will be collected from all participants in the trial at the following time points:

- During ECMO support: 12 hours, 24 hours, 48 hours, 72 hours after cannulation
- End of ECMO support: 24 hours after decannulation or immediately prior to ECMO support being stopped in case of death or treatment withdrawal

A total amount of 5 blood samples will be taken per patient.

The sample volumes to be taken from each patient will be determined by 4 factors:

1. **Patient's weight:** maximum safe volume (57) depends on body weight : patients will donate a maximum of 3 ml per sample or 15 ml in total over the whole study period, as in the p-Mivaki study (63).
2. **Minimum volumes required to perform the assays:** calculations are based on our existing studies in children, and are listed in Table 3.
3. **Minimum volume that can be collected in a sample tube:** blood will be collected in 3 different types of collection tubes (see Table 2) at each sampling time point from all patients. Although individual assays might require smaller volumes, it is not possible to collect accurately these smaller volumes in the available sample tubes. Therefore, in some cases, excess blood will be taken beyond what is required, and the design of the blood sampling study has anticipated this.
4. **Tests to be undertaken** (Table 2)  
We will only take blood required to complete the specified analysis (see below). We aim to remove a maximum of 15 ml in total over the 5 time points: 1.4 ml (Citrate) per time point for leukocyte, platelets and biochemical markers analyses, and 1.1 ml (clotting) per time point for troponin, and cytokine analyses and 0.5 ml (Hirudin) for MP analysis.

**Table 2. Blood Sample Distribution**

Test	Tube	Volume Required [ml]	Storage	Tubes
<b>Creatinine, troponin, MAGPIX tests,</b>	clotting	0.6 (serum)	-80C	
<b>Total clotting sample</b>		0.6 (serum)		<b>1x 1.1 ml</b>
<b>Leukocyte, platelets (flow), full blood count</b>	citrate	0.4 (blood)	Hot test	
<b>Free Hgb, hepcidin, carbonylation, iron</b>	citrate	0.5 (plasma)	-80C	
<b>Total citrated sample</b>				<b>1x 1.4 ml</b>
<b>MV analysis</b>	hirudin	0.5	-80C	
<b>Total hirudin sample</b>				<b>1x 0.5ml</b>
<b>Total/time-point</b>				<b>3.0ml</b>
<b>TOTAL</b>				<b>15ml</b>

Blood samples will be taken by medical or nurse staff responsible for the care of participants through an arterial line, which is routinely placed before or during ECMO support. The authorised ECMO fellow/trial coordinator will 'track' each patient and prompt staff to take the samples required at the designated times, and will be responsible for the secure storage and transfer of samples until they are

analysed in the central laboratory. Serum and plasma samples will be batched and transferred for storage in the -80°C freezer located in the Cardiovascular Research Centre based at Glenfield Hospital until analysis. They will be labelled and identified by the trial acronym, patient's study ID, initials, encrypted date of birth and the time point of the sample.

## **6.2 Urine sampling**

Urine samples will be collected from all participants in the trial at the following time points:

- Baseline: 12 hours after cannulation
- During ECMO support: 24 hours, 48 hours, 72 hours after cannulation
- End of ECMO support: 24 hours after decannulation, or immediately prior to ECMO support being stopped in case of death or treatment withdrawal

All available urine at the time-point will be collected for each patient. Urine sample collection will occur at the same time of blood sample collection. Urine samples for measuring renal function and injury will be taken through an in situ urinary catheter that is usually positioned before or soon after ECMO commencement.

Samples for the research will be taken over a 24-hour period. The start and stop times of the collection period will be recorded and the total urinary output will be measured.

Urine samples will be taken through an in situ urinary catheter by the authorised ECMO fellow/trial coordinator at the predefined time points. Urine samples will be batched and transferred for storage in the -80° freezer located in the Cardiovascular Research Centre based at Glenfield Hospital until analysis. They will be labelled and identified by the trial acronym, patient's study ID, initials, encrypted date of birth, sample type and the time point of the sample.

## **6.3 Respiratory sampling**

Respiratory samples will be collected from all participants in the trial through a respiratory tract suction procedure at the following time points:

- Baseline: 12 hours after cannulation
- During ECMO support: 24 hours, 48 hours, 72 hours after cannulation
- End of ECMO support: 24 hours after decannulation or immediately prior to ECMO support being stopped in case of death or treatment withdrawal

A total amount of 5 respiratory samples will be taken per patient.

Each intubated patient routinely receives a regular suction to evacuate the secretion from respiratory tract. The routine suction procedure is regulated by a hospital protocol and completed by the bed space nurse as required by clinical purposes. The collected sputum would be normally wasted. We will collect part of the sputum routinely aspirated and no extra procedure will be performed in order to complete the sample collection for the study. After the suction is completed, the fluid will be collected for the analysis.

The authorised ECMO fellow/trial coordinator will 'track' each patient and prompt staff to take the samples required at the designated times, and will be responsible for the secure storage and transfer of samples until they are analysed in the central laboratory. Sputum samples will be transferred to the lab and analysis will be performed. The remaining cells will be lysed as described below and stored at -80°C in a freezer located in the Cardiovascular Research Centre based at Glenfield Hospital until analysis. Samples will be labelled and identified by the trial acronym, patient's study ID, initials, encrypted date of birth, sample type and the time point of the sample.

## **6.4 Sample storage**

The urine, blood and respiratory samples will be transferred to the academic laboratory of Glenfield Hospital where they will be stored until analysed. After the samples have been analysed, they may be

stored with patient's permission for further REC and R&D approved researches. The patient/parents/guardians will be informed through the patient information leaflet about the possible storage of the samples for future research and they will be asked to sign a consent form if they agree. We may share anonymised tissue or data with external collaborators, with patient permission. Otherwise, the samples will be disposed in accordance with the Human Tissue Authority's Code of Practice and the institution's standard operating procedure for clinical wastage.

In the event that the neonate does not survive before obtaining full consent, parents/legal representatives will be approached to obtain permission to retain the samples. If permission is not obtained, the samples will be destroyed and this will be fully documented in the medical notes.

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Detailed description of the sample collection, storage and transfer procedures will be provided in the study laboratory manual.

### **6.5     *Blood and urine samples assays***

Serum creatinine will be measured using fluorometric creatinine kit (Abcam, UK) at 12, 24, 48, 72 hours after cannulation, and 24 hours after decannulation or immediately prior to ECMO support being stopped in case of death or treatment withdrawal. Estimated creatinine clearance will also be used to define Stage 3 AKI, using the Schwartz equation (table 2).

Cytokines and chemokines important in monocyte/macrophage differentiation and renal injury, e.g. IL-6, -8, TNF $\alpha$ , MCP-1 and MIP-1, as well as markers of endothelial injury (circulating ICAM or E-selectin) may be measured from serum on the Luminex MAGPIX Analyser (Oosterhout, NL).

Troponin will be measured in serum using Troponin I ELISA kit (ENZO Life Sciences, Exeter, UK). Hepcidin (key regulator of iron in circulation) and carbonylation levels reflecting oxidative stress will be measured in citrated plasma using commercially available ELISA kits from DRG International Inc. (Springfield, USA) and Abcam (Cambridge, UK).

Total iron and non-transferrin-bound iron (NTBI) levels will be measured as previously described (Gosriwatana et al., 1999). Iron is detected by measuring absorbance at 525 nm in colorimetric reaction with bathophenanthroline disulfonic acid disodium salt hydrate (BPT). For NTBI samples will be treated with iron chelator (nitrilotriacetic acid, NTA), ultrafiltered and analysed with BPT.

Oxidative stress will be measured in plasma using commercially available carbonylation kit (R&D Systems, Minneapolis MN).

HMOX-1, iNOS and Arg-1 expression will be estimated in cells from bronchial aspirates by Western blotting with specific antibodies.

Platelets and leukocyte activation, as well as platelets—leukocyte aggregates will be determined with specific antibodies (CD41, PAC-1 and CD62P for platelets, CD64, CD163 and CD11a for leukocytes and CD14, CD16, CD41 for the aggregates) and analysed by flow cytometry. Full blood count will be measured.

Urine biomarkers of renal oxidative stress; NGAL (ELISA Bioporto Diagnostics A/S Denmark) and/will be measured on the DS2 automated ELISA platform (Dynex, through Alere, UK).

## 6.6 MV Analyses

Circulating blood will be anticoagulated with hirudin at the defined time points (Section 6.1). MV will be isolated by centrifugation (1,500 g, 15 min ×2 at 4°C). MV size will be evaluated using the Nanosight. Mean size and particle concentration values will be calculated using Nanoparticle Tracking Analysis software using the manufacturer's instructions. Antigen expression on MV, indicative of the cell of origin will be determined using flow cytometry (Beckman Coulter MCL-XK or a Cyan ADP 9 colour instrument) as previously described [39, 40].

MV will be identified with Annexin V-APC and subpopulations will be identified by co-staining with antibodies against cellular marker proteins. A suggested range of markers is listed in table 3.

**Table 3.**

Probe	Target molecule	Target cells
<b>Annexin V</b>	Phosphatidylserine	Microvesicles, apoptotic cells
<b>CD235-FITC</b>	Glycophorin A	Red blood cells
<b>CD41-PE</b>	Integrin $\alpha$ -IIb	Platelets
<b>CD144-PE</b>	VE-cadherin	Adherens junctions
<b>CD162-PE</b>	PSGL-1	Adhesion molecule
<b>CD29-PE</b>	Integrin- $\beta$ 1	Platelets
<b>CD142-FITC</b>	Tissue factor	Coagulation
<b>CD 62-PE</b>	E-selectin	Endothelium
<b>CD284-Ax488</b>	TLR-4	Monocytes , granulocytes
<b>Phalloidin-FITC</b>	F-actin	Cell fragmentation

## 6.7 Bronchial aspirates analysis

Bronchial aspirates (BA) analysis is a valuable tool to assess the degree of lung injury. Analysis of immune cells will allow us to extrapolate the results obtained from blood analysis and conclude on events leading to injury and more importantly to recovery from lung injury and consequently shorter ECMO time.

BA samples will be spun and the pelleted cells washed with PBS. Iron deposition will be assessed using ferrocyanide method where BA smears are stained with Prussian Blue and analysed by light microscopy.

Remaining cells will be lysed and frozen at -80C for WB analysis with HMOX-1 antibodies to estimate oxidative stress response and iNOS and Arginase (Arg-1) antibodies to assess macrophage/T-cells M1/Th1 and M2/Th2 responses in lungs.

## 6.8 Summary of trial specific tests and procedures

	Baseline (12h after ECMO start)	Day 1 (24 hrs)	Day 2 (48h)	Day 3 (72h +/- 12 hrs)	24h after decannulation/
--	---------------------------------	----------------	-------------	------------------------	--------------------------

	+/- 6 hrs)	+/- 12 hrs)	+/- 12 hrs)		prior to ECMO stop for death or withdrawal (+/- 12 hrs)
Eligibility	✓				
Written emergency consent	✓				
Trial Registration	✓				
Clinical outcomes	✓	✓	✓	✓	✓
Serious adverse event monitoring	✓	✓	✓	✓	✓
Bloods: Full blood count	✓	✓	✓	✓	✓
Bloods: Cytokines, total iron, non-transferrin-bound iron	✓	✓	✓	✓	✓
Bloods: Platelets and leukocyte activation	✓	✓	✓	✓	✓
Bloods: MV analysis	✓	✓	✓	✓	✓
Respiratory Sample: ferrocyanide, oxidative stress	✓	✓	✓	✓	✓
Urine sample & volume: NGAL	✓	✓	✓	✓	✓
Serious adverse event monitoring	✓	✓	✓	✓	✓
Questionnaire on perception of family members					✓

† Indicates samples taken as part of normal care

## 6.9 End of the trial

For an individual participant, the end of the trial is defined as a successful weaning from ECMO, or death. We consider finished the follow-up after discharge from UHL. The participant's parents /guardians are free to withdraw from the study at any time for any reason. In the event of withdrawal, the primary reason must be recorded in the subject's medical record and on the withdrawal form in the CRF. Any comments (spontaneous or elicited) or complaints made by the patient or clinical care team, together with the reason for termination, date and time of withdrawal must be recorded in the CRF and source documents.

Patients will be considered lost to follow-up only if there is insufficient information to determine the patient's status at end of trial, whether because of transfer to another unit, no contact has been re-established following 3 documented attempts by the research team.

The definition of the end of the trial as a whole is the date when all participants have completed the follow-up or have been lost to follow-up.

### **6.10 Data collection**

After cannulation, the authorised ECMO fellow/trial coordinator will collect data on pre-printed forms to characterise the patients who are approached about the trial (e.g. eligibility criteria, key demographic data, willingness to participate and, if not willing, main reason for refusal). These data will be entered promptly in the master subject tracker in order for the trial registration number to be generated for a study participant.

Other pre-ECMO data likely to influence the speed of post cannulation recovery, procedure details (cannulation, details of the ECMO circuit), morbidity, duration of ICU and hospital stay and other non-biochemical secondary outcomes will also be collected prospectively on pre-printed forms, including dates and times of relevant outcomes.

These data will be entered into a database immediately after the end of follow-up. In the event that a patient has been withdrawn from the study, we will continue to analyse any data already collected, unless the patients expresses a wish for their samples and any associated data to be destroyed.

Source data will be collected prospectively on designated CRF's. In addition, the patient's medical notes will be a source for additional clinical data, including blood and urine results performed in the clinical setting. University laboratory databases will be the source for other trial specific biochemical markers analysed in the blood and urine.

Protocol adherence will be evaluated through the monitoring of protocol violations defined as any change, deviation, or departure from the study design or procedures of research project that is not approved by the Sponsor and Ethics Committee prior to its initiation or implementation.

Protocol violations will be categorized as major or minor violations, according to the following definitions:

A major protocol violation:

- a serious and/or continuous failure on the part of the study team to comply with the protocol, standard operating procedures, good clinical practice (GCP), trial regulations.
- has significant negative impact to subject safety. It may or may not result in actual harm to the subjects.
- Significantly damages the completeness, accuracy and reliability of the data collected for the study.

A minor protocol violation/deviation/non-compliance:

- Does NOT represent a serious or continuous failure on the part of the study team to comply with the protocol, standard operating procedures, GCP, trial regulations.
- Does NOT significantly impact subject safety or substantially alter potential risks to subjects
- Does NOT result in actual harm
- Does NOT significantly damage the completeness, accuracy and reliability of the data collected for the study

Clinical protocol non-adherence will be defined as the failure to obtain the specified clinical data, blood volume or urine samples required for analysis at the required time. Protocol violation concerning the time of sample collection will be defined as a deviation of more than 6 hours from the planned collection time for the baseline data, and 12 hours for the remaining time points. Laboratory protocol non-adherence will be defined as the failure to complete laboratory essay as described in the protocol.

### **6.11 Patient recruitment and consent**

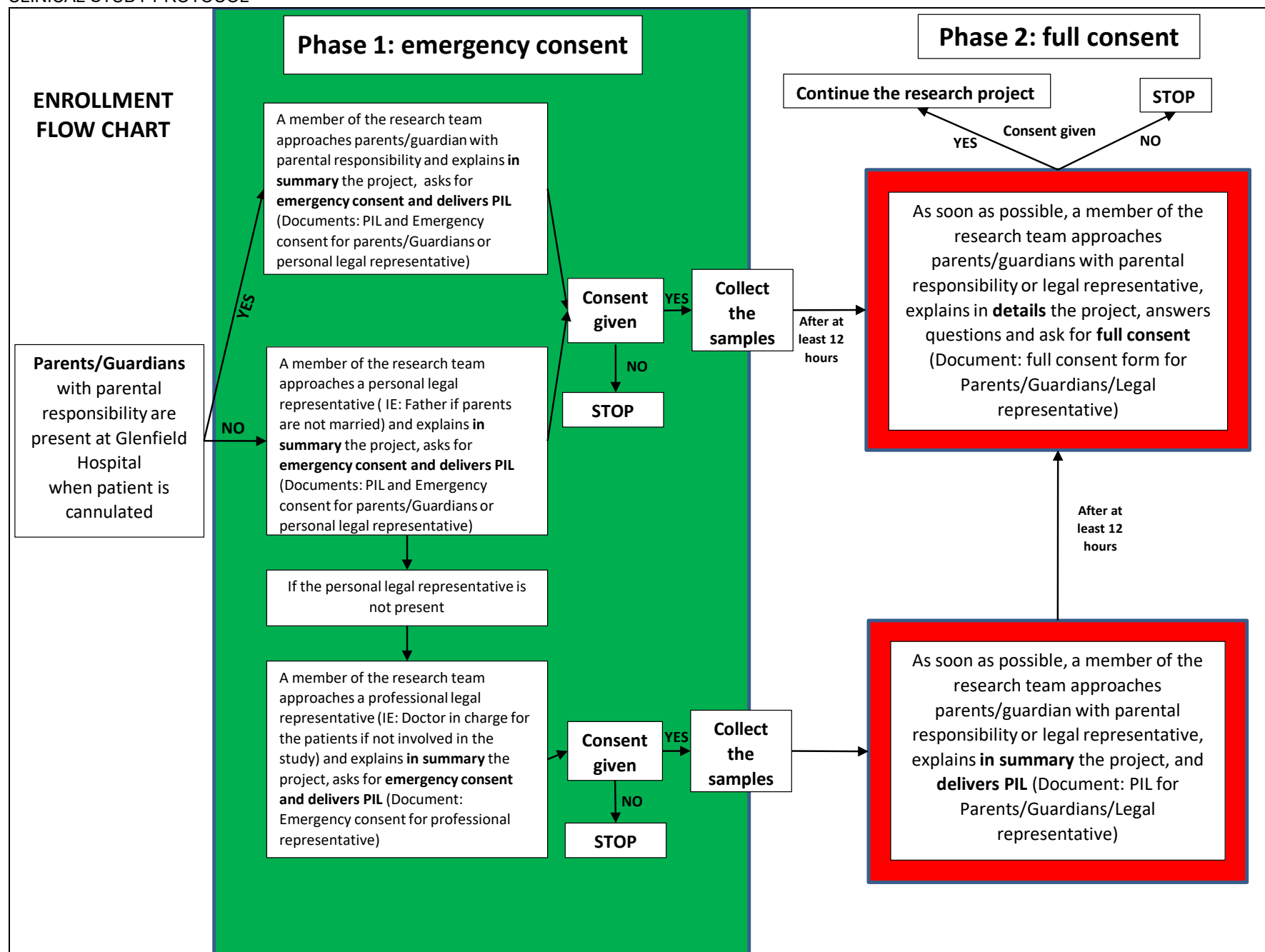


Patients who meet the inclusion criteria and have been referred for ECMO at the Glenfield Hospital will be considered eligible for the study. Parents/guardians will be required to provide full written informed consent. Patient's willingness to participate cannot be assessed since all the participants are younger than 1 month, therefore, they will be considered as unable to provide consent or assent. According to the Children Act and the Children Act (Northern Ireland) Order, parents and those with parental responsibility are allowed to consent to medical treatment or research on their behalf. Consent of only one parent is required. Patients' parents/guardians are expected to read and understand the parent/guardian information leaflet and (with the assistance of a member of the clinical team) understand the purposes and consequences of the study to the patient at a level suitable for them.

Patients can be formally enrolled only if emergency consent (59, 60) is obtained within 12h from ECMO cannulation. Details of all patients approached for the trial and reason(s) for non-participation (e.g. reason for being ineligible or patient refusal) will be documented.

Since the Glenfield Hospital is a national paediatric referral centre collecting patients from different parts of UK, we expect that a percentage of parents/guardians will not reach the Hospital within the first hours from cannulation and will not be promptly available onsite. Moreover, we expect that in rare cases the mother will not reach the hospital for days after ECMO start due to delivery's complications or health reasons. On the other hand, it is important to establish baseline values in order to test our hypothesis. For that reason, the first sample has to be collected within 12 hours from the cannulation. The Regulations prescribe a hierarchy for determining who should be approached to give informed consent on behalf of a minor prior to their inclusion in the trial as shown in Table 4. The provisions for informed consent by a legal representative only apply if due to the emergency nature of the trial, no person with parental responsibility can be contacted prior to the proposed inclusion of the minor.

For the reasons discussed above, the recruitment strategy will follow the scheme described in the following flowchart.



<b>Table 4</b>		
<b>Person who may give the consent</b>	<b>Definition</b>	<b>Commentary</b>
Parent	A parent or person with parental responsibility	Should always be approached if available
Personal legal representative	A person not connected with the conduct of the trial who is: (a) suitable to act as the legal representative by virtue of their relationship with the minor, and (b) available and willing to do so	May be approached if no person with parental responsibility can be contacted prior to the proposed inclusion of the minor, by reason of the emergency nature of the trial
Professional legal representative	A person not connected with the conduct of the trial who is: (a) the doctor primarily responsible for the medical treatment of the minor, or (b) a person nominated by the relevant health care provider (e.g. an acute NHS Trust or Health Board).	May be approached if no person suitable to act as a personal legal representative is available. Informed consent must be given before the minor is entered into the trial

#### 6.11.1 Parents/guardians with parental responsibility available within 12 hours from cannulation

If parents/guardians with parental responsibility are available at patient admission to the Glenfield Hospital, an authorised member of the research team will approach them in order to explain the study and ask for emergency consent.

Parents/guardians with parental responsibility will be provided with an emergency parent/guardian/personal legal representative information leaflet at the time of their arrival at the Glenfield Hospital. At the same time, an authorised member of the research team will explain in summary the purpose of the study in person and will provide answers to any questions that might be raised. Due to the urgency of the situation, if parents/guardians are willing to take part in the study, they will be asked to provide a written emergency consent.

After parents/guardians' emergency consent, the samples will be collected following the protocol and the parents/guardians will be provided with a complete version of the parent/guardian/personal legal representative information leaflet. They should therefore have at least 12 hours to read and consider the information and to ask questions before being asked to decide whether they are willing to continue the study. An authorised member of the research team will approach again parents/guardians and will explain in details the research project, answer the questions and ask for full consent (59, 60).

In the event that a child withdraws, we will request that all patient's data and tissues collected until that time are retained for analysis. If this request is denied, all data will be removed and tissues disposed of. All the leaflets and forms will be approved by the local Research Ethics Committee (REC).

**6.11.2 Parents/guardians with parental responsibility not available within 12 hours from cannulation**

If parents/guardians with parental responsibility are not available at patient admission to the Glenfield Hospital, an authorised member of the research team will approach a personal legal representative (defined as in Table 4) in order to explain the study and ask for emergency consent.

As personal legal representative will be considered the following persons:

- Putative father
- Grandparents

The personal legal representative will be provided with an emergency parent/guardian/personal legal information leaflet at the time of his/her arrival at the Glenfield Hospital. At the same time, an authorised member of the research team will explain in summary the purpose of the study in person and will provide answers to any questions that might be raised. Due to the urgency of the situation, if the personal legal representative is willing to take part in the study, he/she will be asked to provide a written emergency consent.

After the personal legal representative's consent, the samples will be collected following the protocol and the personal legal representative will be provided with a complete version of the parent/guardian/personal legal representative information leaflet. He/she should therefore have at least 12 hours to read and consider the information and to ask questions before being asked to decide whether he/she is willing to continue the study. An authorised member of the research team will approach again the personal legal representative and will explain in details the research project, answer the questions and ask for full consent (59, 60).

In the event that a child withdraws, we will request that all patient's data and tissues collected until that time are retained for analysis. If this request is denied, all data will be removed and tissue disposed of. All the leaflets and forms will be approved by the local Research Ethics Committee (REC).

**6.11.3 Neither parents/guardians with parental responsibility nor personal legal representative available within 12 hours from cannulation**

If parents/guardians with parental responsibility and a personal legal representative are not available at patient admission to the Glenfield Hospital, an authorised member of the research team will approach a professional legal representative (defined as in Table 4) in order to explain the study and ask for emergency consent. The professional legal representative have to be a Consultant in Paediatric Intensive Care and/or ECMO not connected with the conduct of the trial. Before the start of the recruitment phase, the study will be explained to the whole medical staff working in the Paediatric Intensive Care Unit and in the ECMO team through several meetings. This will allow them to understand the details of the study in advance, consider all the information and ask questions.

The professional legal representative will be provided with an emergency professional legal representative information leaflet at the time of the child's arrival at the Glenfield Hospital. At the same time, an authorised member of the research team will explain in summary the purpose of the study in person and will provide answers to any questions that might be raised. Due to the urgency of the situation, if the professional legal representative is willing to take part in the study, he/she will be asked to provide a written emergency consent. After the professional legal representative's consent, the samples will be collected following the protocol.

As soon as possible, a member of the research team will approach parents/guardians with parental responsibility or a personal legal representative, explain in summary the project, and provide them with a complete version of the parent/guardian/personal legal representative information leaflet. They should therefore have at least 12 hours to read and consider the information and to ask questions before being asked to decide whether he/she is willing to continue the study. An authorised member of the research team will approach again the parents/guardians with parental responsibility or a personal legal representative and will explain in details the research project, answer the questions and ask for full consent (59-60).

In the event that a child withdraws, we will request that all patient's data and tissues collected until that time are retained for analysis. If this request is denied, all data will be removed and tissue disposed of. All the leaflets and forms will be approved by the local Research Ethics Committee (REC).

#### 6.11.4 *Satisfaction questionnaire*

Perceptions of family members whose children participate in the study as to the appropriateness of the screening and consent process will be assessed by means of a 11 items questionnaire (Appendix 1) adapted from previous studies (61, 62). The questionnaire will be administered at the end of the study prior to hospital discharge.

### 6.12 *Planned recruitment rate and duration of follow-up*

On the basis of historical data we estimate that there will be a target population of 40-60 eligible patients over 12 months at Glenfield Hospital. We plan to recruit, 24 of all eligible patients. In a previous observational clinico-experimental study undertaken by this group [The p-MiVAKI study (63)], that also required serial blood sampling in children undergoing cardiac surgery, an 84.37% consent rate was achieved.

Participants will be followed up until discharge from UHL.

### 6.13 *Likely rate of loss to follow-up, protocol violations and clinical data completeness*

Until discharge from hospital, the likely losses to follow-up will be due to death or participant withdrawing, or more commonly incomplete collection of the samples. In a previous observational clinico-experimental study undertaken by this group [The p-MiVAKI study (63)], that also required serial blood sampling in children, clinical data showed a completeness of 84.09% as described in Table 5.

<b>Table 5 – Data collection</b>	
<b>Variable</b>	<b>Completeness (%)</b>
Overall	84.09
Clinical variables	94.61
Clinical laboratory data	77.5
Categorical variables	100

Total sample collection reached a percentage of 93.75% (Table 6-7). Only in one case, parents refused consent for one sample donation because of children discomfort. All patients concluded the study and no major protocol violations were recorded.

**Table 6 – Blood sample collection**

	<b>Patients (n)</b>	<b>Expected samples (n)</b>	<b>Collected samples (n)</b>	<b>Collected samples (%)</b>
<b>Patient's weight: 2-5 Kg</b>	6	18	18	100%

<b>Patient's weight: 5-20 Kg</b>	14	42	41	95.2%
<b>Patient's weight: &gt; 20 Kg</b>	4	12	9	75%
<b>Total</b>	<b>24</b>	<b>72</b>	<b>68</b>	<b>94.4%</b>

**Table 7 – Urine sample collection**

	<b>Patients (n)</b>	<b>Expected samples (n)</b>	<b>Collected samples (n)</b>	<b>Collected samples (%)</b>
<b>Patient's weight: 2-5 Kg</b>	6	18	18	100%
<b>Patient's weight: 5-20 Kg</b>	14	42	40	97.9%
<b>Patient's weight: &gt; 20 Kg</b>	4	12	9	75%
<b>Total</b>	<b>24</b>	<b>72</b>	<b>67</b>	<b>93.1 %</b>

**6.14 Expenses and benefits**

Participants will not receive payment for taking part in the trial. As they will not be attending as outpatients above and beyond their normal care there will be no additional reimbursement for travel or other expenses.

## **7. Statistical Considerations**

### **7.1 Sample Size**

This is a feasibility study and no formal power calculation has been performed. Our sample size is based on our observations from the 24 patients in the p-MiVAKI study (63), where we have demonstrated significant differences in markers of platelet, leukocyte and endothelial activation between patients who subsequently did or did not develop AKI, and between those that did or did not receive transfusion.

### **7.2 Measures taken to avoid bias**

All necessary steps will be taken to reduce the risk of bias. Detection bias will be minimised by blinding of laboratory staff analysing cytokines, biomarkers and inflammatory processes. Specifically, urine, serum and sputum samples obtained exclusively for the trial will be identified only by a trial acronym, patients' study ID, initials and date of birth and the time at which the sample was taken, ensuring that laboratory staff performing analyses are blinded. Detection bias for the clinical outcomes will also be minimised by the use of objective outcome criteria. Other samples, that are also obtained as part of routine care will be analysed routinely in NHS laboratories by personnel who are unaware that the participant is in a trial. Clinical decision about patient care will be made by clinical staff on the basis of existing institutional protocols and will not be affected by enrolment in the study.

### **7.3 Plan of analysis**

A statistical analysis plan will be drafted prior to analysis. Normally distributed data will be presented as means (standard deviation). Non normally distributed data will be presented as geometric means (95% confidence intervals). Dichotomous data will be presented as percentages. This data will be used to quantify the number of patients required to design a further prospective observational analysis.

### **7.4 Criteria for stopping the study early**

The study may be terminated early if the results of another study supersedes the necessity for completion of this study.

## **8. Study Management**

The study will be managed by the Cardiac Surgery Clinical Trials Team (CSCTT) at the University of Leicester. The CSCTT will prepare trial documentation and data collection forms, register patients and carry out trial procedures; the CSCTT will also develop and maintain the study database, check data quality as the trial progresses, and carry out trial analyses in collaboration the statistician.

### **8.1 Day-to-day management**

The trial will be managed by a Clinical Trial Manager, supported by a Clinical Trial Coordinator from the Cardiac Surgery Clinical Trials Team at the University of Leicester. An authorised ECMO fellow/coordinator will be responsible for managing the trial on a day-to-day basis. They will be responsible for identifying potential trial participants, seeking informed patient consent, liaising with blood and laboratory services, collecting trial data and ensuring the trial protocol is adhered to.

### **8.2 Trial steering committee and data safety & monitoring committee**

The trial will be monitored by the Cardiovascular Surgery Research Group Steering Committee. As this is an observational study, no Data Monitoring and Safety Committee (DMSC) will be convened.

The Trial Steering Committee will provide overall supervision of the trial and ensure that the local, national and international research framework is adhered to. The Trial Steering Committee will agree trial amendments, as necessary, and provide relevant guidance on study design and conduct to participating investigators. Membership includes members of the Trial executive, principal investigators, independent experts, a service user representative and a representative of the study Sponsor.

## **9. Safety Reporting**

### **9.1 Definitions**

As defined by the ICH Guidelines for Good Clinical Practice (CPMP/ICH/135/95) the following definitions will be used:

#### Adverse Event

Any untoward medical occurrence in a patient or clinical trial subject, who has been administered a clinical intervention, procedure, treatment or medicinal product and which does not necessarily have a causal relationship with this medical intervention. An adverse event can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the intervention, whether or not related to the medical intervention.

#### Serious Adverse Event

Any untoward medical occurrence, which

- results in death
- is life threatening
- requires inpatient hospitalisation or prolongation of existing hospitalisation
- results in persistent or significant disability or incapacity
- consist of a congenital anomaly or birth defect.
- other situations, such as significant medical events, which may not be immediately life threatening or result in death or hospitalisation, but which may jeopardise the subject or may require intervention to prevent one of the other serious outcomes listed in the definition above, should also be considered.

### **9.2 Adverse event reporting**

Adverse events will be recorded and reported in accordance with the University of Leicester's and University Hospitals Leicester NHS Trust's policies for reporting Research Related Adverse Events.

In intensive care management and especially in ECMO practice, adverse events are not unexpected and are not infrequent. The research team will only notify deaths and 'unexpected' non-fatal SAEs to the Trial Sponsor (University of Leicester Research Support Office). Unexpected events are those not listed in the trial protocol or on the case report forms. The sponsor will inform the research team which SAEs should be reported to the REC.

### **9.3 Expected adverse events**

The following adverse events are 'expected':



Cardiovascular:

- Cardiac arrest
- Myocardial stunning
- Cardiac arrhythmias and arrhythmias requiring temporary or permanent pacing
- Myocardial infarction including:
  - New Q's in 2 contiguous leads
  - Raised Troponin I >0.5µg/l
  - New ST depression >2mm in 2 leads
- Cardiac tamponade
- Pericardial effusion
- Perforation of heart or vessels
- Cardiopulmonary resuscitation including cardiac defibrillation/DC shock
- Chest opening
- External/internal cardiac massage
- Low cardiac output
- Haemodynamic support, including use of:
  - Any inotropes
  - Pulmonary artery catheter
  - Any vasodilators

Pulmonary:

- Prolonged invasive ventilation
- Tracheostomy
- Bronchomalacia
- Tracheomalacia
- Subglottic stenosis
- Initiation of mask continuous positive airway pressure ventilation after weaning from ventilation
- Acute Respiratory Distress Syndrome
- Pneumothorax or effusion requiring drainage

Thromboembolic complications, including:

- Deep vein thrombosis
- Pulmonary embolism
- Clotting of the ECMO circuit
- Stroke
- Arterial thrombosis
- Limb ischemia

Hemorrhagic complications, including:

- Disseminated intravascular coagulation
- Haemothorax
- Cannulation site bleeding
- Gastrointestinal bleeding
- Brain haemorrhage
- Haemolysis
- Airways bleeding

Renal complications, including:

- Acute kidney injury

- Haemofiltration/dialysis

Infective complications, including:

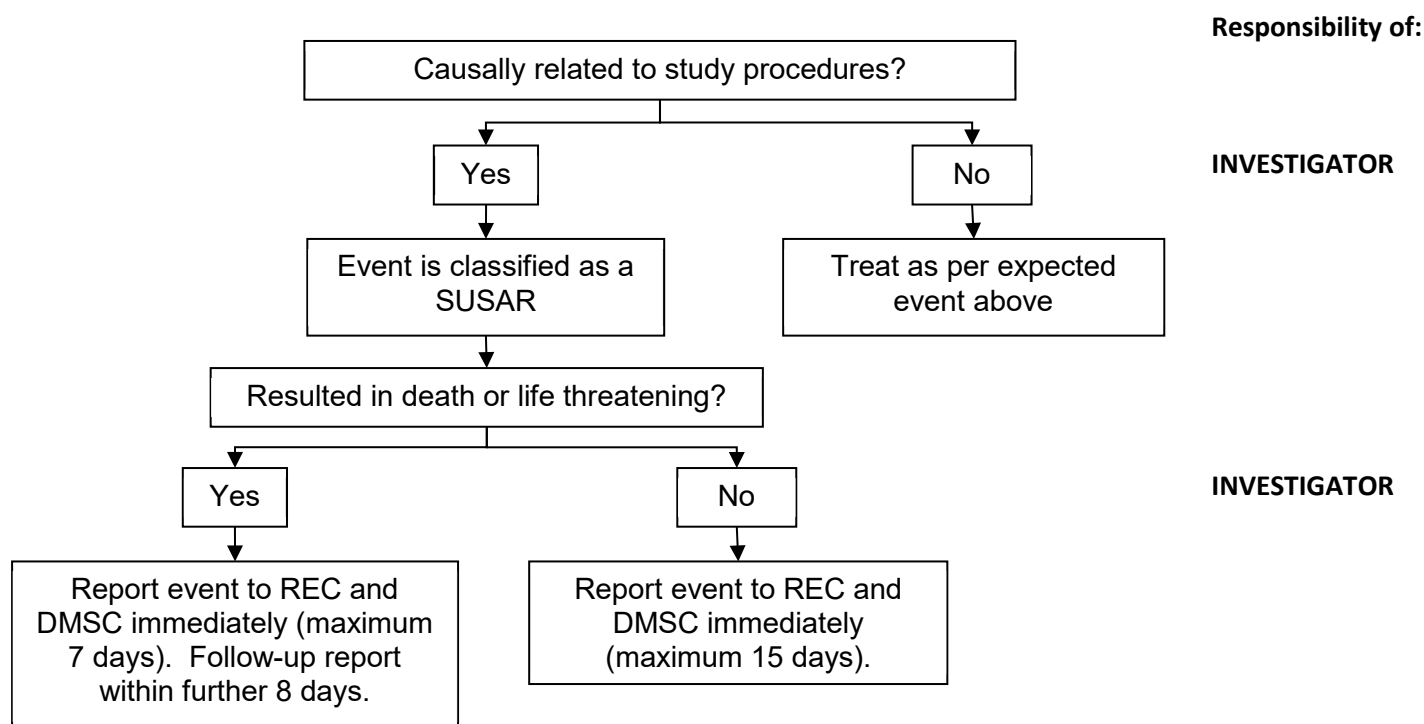
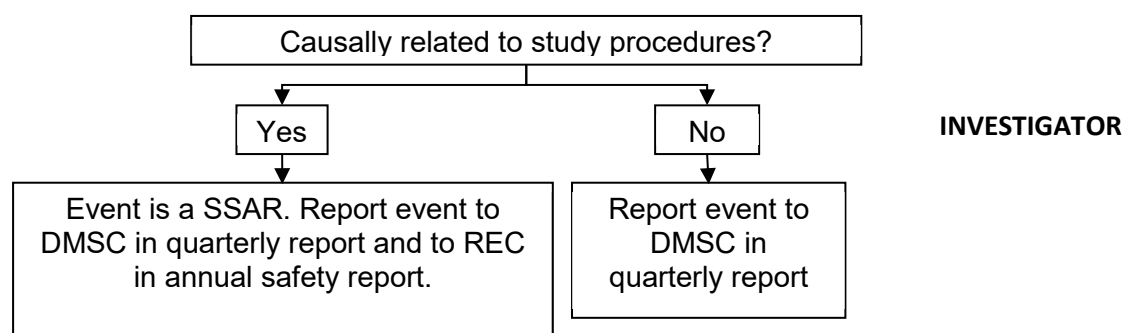
- Sepsis (defined as antibiotic treatment for suspected infection, and the presence of SIRS<sup>1</sup> within 24 hours prior to start of antibiotic treatment) - SIRS will be defined as  $\geq 2$  of the following conditions: temperature  $>38^{\circ}\text{C}$  or  $<36^{\circ}\text{C}$ ; heart rate  $>90$  beats/minute; respiratory rate  $>20$  breaths/min or  $\text{PaCO}_2 < 32$  mm Hg or  $<4.3$  kPa; white cells count  $>12,000/\text{mm}^3$  or  $<4,000/\text{mm}^3$ .
- Septic shock
- Wound infection
- Respiratory infection
- Ventilator associated pneumonia
- Urinary tract infection
- Catheter related bloodstream infection

GI complications, including:

- Pancreatitis (amylase  $>1500\text{iu}$ )
- NEC (necrotizing enterocolitis )
- Other (e.g. laparotomy)

Neurological complications

- Irreversible neurological deficit
- Seizures
- Peripheral nerve damage

**Figure 3: Serious adverse event reporting flow chart****(a) Unexpected serious adverse events – i.e. those not listed in study protocol****(b) Expected serious adverse events – i.e. those listed in study protocol****9.4 Period for recording adverse events**

Data on adverse events will be collected from ECMO commencement to UHL discharge.

**9.5 Methods of recording and assessing adverse events**

Prior to study registration, a detailed medical history and physical examination, including laboratory tests, will be taken for baseline reference. At all subsequent days of hospital admission and during the follow up, the occurrence of AEs will be recorded in the appropriate section of the CRF. Among these, all SAEs must also be recorded in the SAE report form and will be notified to the Trial Sponsor (University of Leicester Research Support Office) in case of death or 'unexpected' non-fatal SAEs.

The following aspects of AEs will be captured in the CRF:

- Description of event in medical term
- Date of onset
- Date of recovery, if applicable
- Intensity of the event:
  - Mild: Awareness of event, but easily tolerated
  - Moderate: Discomfort enough to interfere with usual daily activity
  - Severe: Inability to carry out usual daily activity
  - Life-threatening: Life-threatening or disabling
  - Fatal: Death related to the AE
- Causal relationship to the study procedures as assessed by the Investigator. The decisive factor in documentation is the temporal relationship between the AE and study intervention. The following judgements of causality are to be used:
  - Not related: The event is related to an aetiology other than study procedures
  - Remote: The event is unlikely to be related to the study procedures based on existing knowledge and likely to be related to other factors.
  - Possibly: Reasonable possibility that the AE was caused by the study procedures and there is a plausible mechanism of action, but there may also be an alternative aetiology such as the participant's clinical status or underlying disease.
  - Definite: There is an association between the event and the administration of study procedures, a plausible mechanism for the event to be related to the study product and causes other than study product have been ruled out.
- Action taken: None, Concomitant medication or therapy or other

## **10. Ethical Considerations**

### **10.1 Review by a NHS Research Ethics Committee**

Ethics review of the protocol for the trial and other trial related essential documents (e.g. patient information leaflet, consent form) will be carried out by a UK NHS Research Ethics Committee (REC). Any amendments to these documents, after a favourable opinion from the REC has been given, will be submitted to the REC for approval prior to implementation.

### **10.2 Risks and anticipated benefits**

The response of neonates to ECMO is still poorly understood in terms of inflammatory signalling and interaction between the body and the machine. However, the haematological response to the circuit in low weight patients is considered to play an important role in the recovery process and in morbidity and mortality. There is a clear need for the development of novel interventions that reduce the severity of inflammatory organ injury during ECMO support. This study may lead to further research that will help future ECMO patients however there are no direct benefits to the recruited patients from taking part.

As this is a non-interventional study, the potential risks from participating in the study are those risks associated with the trial procedures. Specifically the withdrawal of significant volumes of blood from children subject to an ECMO where circulating volume is smaller than in adults is our chief concern.

To minimise this risk, we have taken steps to reduce the volume of blood removed from the children during and after the extracorporeal support. The volumes and tests to be undertaken are determined by patients weight and the minimum volumes of blood required to complete the assays. The detailed consideration of these factors is described in detail in Section 6.1 and Table 2. Our experience with a previous study in paediatric cardiac surgery [The p-MiVAKI study (63)], has shown that these volumes

are safe. We therefore do not anticipate that any child will be exposed to increased risk by participation in the study.

No further invasive procedure will be carried out only for research purposes. Since the airway suctioning is performed routinely for clinical purpose and in the interest of the patient, no ethical issues can be raised.

In summary, the study investigations have a low risk profile, but there are no direct benefits to be gained from trial participation other than helping the researchers in gaining a better understanding about the inflammatory processes that are involved in neonatal ECMO.

### **10.3    *Informing potential study participants of possible benefits and known risks***

Information about benefits and risks of participation will be described in a Patient Information Leaflet. This information sheet will be part of our application for research ethics approval to the REC.

### **10.4    *Obtaining informed consent from participants***

All participants will be required to give written informed consent to participate in the study. The consent process, including the information about the trial given to patients in advance of recruitment, has been described previously. Members of the clinical research team will be responsible for the consent process.

## **11.      Research Governance**

This clinical trial will be sponsored by the University of Leicester and will be conducted in accordance with:

- International Conference for Harmonisation of Good Clinical Practice (ICH GCP) guidelines
- Research Governance Framework for Health and Social Care, the European Union Directive 2001/20/EC on clinical trials
- Data Protection Act 1998.
- Human Tissue Act 2004
- Protection of Children Act, 1999

### **11.1    *Sponsor approval***

Any amendments to the trial documents must be approved by the sponsor prior to submission to the REC and/or implementation.

### **11.2    *NHS approval***

Approval from University Hospitals Leicester NHS Trust will be obtained prior to the start of the trial.

Any amendments to the trial documents approved the REC will be submitted to the Trust for information or approval as required.

### **11.3    *Investigators' responsibilities***

The investigators will be required to ensure that the local research approvals have been obtained and that any contractual agreements required have been signed off by all parties before recruiting any participants. Investigators will be required to ensure compliance to the protocol and study manual and with completion of the CRFs. Investigators will be required to allow access to study documentation or source data on request for monitoring visits and audits performed by the Sponsor or any regulatory authorities.

Investigators will be required to read, acknowledge and inform their trial team of any amendments to the trial documents approved the REC that they receive and ensure that the changes are complied with.

#### **11.4    *Monitoring by sponsor***

The study will be monitored and audited in accordance with the University of Leicester policy which is consistent with the Research Governance Framework. All trial related documents will be made available on request for monitoring and audit by University of Leicester, the relevant REC and for inspection by the Human Tissue Authority (HTA) or other licensing bodies.

#### **11.5    *Indemnity***

University of Leicester insurance will cover potential legal liability of the sponsor from harm to participants arising from the management and design of the research. NHS indemnity will cover potential legal liability of investigators/collaborators arising from harm to participants in the conduct of the research.

#### **11.6    *Clinical Trial Authorisation***

This is not a randomised trial of an Investigational Medicinal Product and as such a Clinical Trial Authorisation from the MHRA is not required.

### **12.    *Data Protection and Patient Confidentiality***

#### **12.1    *Data protection***

Data will be collected and retained in accordance with the Data Protection Act 1998.

#### **12.2    *Data handling, storage and sharing***

##### **12.2.1    Data handling**

Data will be entered onto a purpose designed database and data validation and cleaning will be carried out throughout the trial. Standard operating procedures (SOPs) for database use, data validation and data cleaning will be available and regularly maintained.

##### **12.2.2    Data storage**

All study documentation will be retained in a secure location during the conduct of the study. All the personal data will be stored for 3 years and all the anonymised data will be stored for 15 years after the end of the study, when all paper records will be destroyed by confidential means. In compliance with the MRC Policy on Data Preservation, the pseudonymised dataset, a separate secure electronic 'key' with a unique patient identifier, and relevant 'meta'-data about the trial will be retained in

electronic form indefinitely because of the potential for the raw data to be used subsequently for secondary research.

#### *12.2.3 Data sharing*

Data will not be made available for sharing until after publication of the main results of the study. Thereafter, anonymised individual patient data will be made available for secondary research, conditional on assurance from the secondary researcher that the proposed use of the data is compliant with the MRC Policy on Data Preservation and Sharing regarding scientific quality, ethical requirements and value for money. A minimum requirement with respect to scientific quality will be a publicly available pre-specified protocol describing the purpose, methods and analysis of the secondary research, e.g. a protocol for a Cochrane systematic review. The second file containing patient identifiers would be made available for record linkage or a similar purpose, subject to confirmation that the secondary research protocol has been approved by a UK REC or other similar, approved ethics review body.

### **13. Dissemination of Findings**

The findings will be disseminated by usual academic channels, i.e. presentation at international meetings, as well as by peer-reviewed publications and through patient organisations and newsletters to patients, where available.

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**15. Protocol Signature Page**

<b>Study Title:</b>	
<b>Study Identifier:</b>	
<b>Protocol Version:</b>	
<b>Details of Sponsor:</b>	University of Leicester Research Support Office University Road Leicester, LE1 7RH  Tel: 0116 252 2759 Fax: 0116 252 2028 Email: uolsponsor@le.ac.uk
<b>Chief Investigator:</b> (Specify Name, Institution, Address)	Professor Gavin Murphy Cardiovascular Sciences University of Leicester Glenfield Hospital Clinical Sciences Wing Groby Road Leicester, LE3 9QP

**Chief Investigator Agreement:**

I, the undersigned, have reviewed this protocol and agree to conduct the study in accordance with Good Clinical Practice, the ethical principles set forth in the Declaration of Helsinki, The Medicines for Human Use Regulations and the applicable Local Research Governance Policies.

Printed Name:

Signature:

Date:

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**Sponsor's Representative Agreement:**

I, the undersigned, have reviewed this protocol and agree to conduct the study in accordance with Good Clinical Practice, the ethical principles set forth in the Declaration of Helsinki, The Medicines for Human Use Regulations and the applicable Local Research Governance Policies.

Printed Name:

Signature:

Date:

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\_\_\_\_\_

\_\_\_\_\_

**16. Appendix 1****Mi-ECMO QUESTIONNAIRE**

When you signed the consent form to allow your baby to participate in the study, how well did you understand the following aspects of the study? *If you didn't understand the item at all, please circle 1. If you understood it very well, please circle 5. If you understood it somewhat, please circle a number between 1 and 5*

	<div style="display: flex; justify-content: space-between; align-items: center;"> <span>I didn't understand this at all</span> <span>→</span> <span>I understood this very well</span> </div>				
The fact that your baby's treatment involves research	1	2	3	4	5
What the researchers are trying to find out in the study	1	2	3	4	5
How long your baby will be in the study	1	2	3	4	5
The procedures your baby will undergo	1	2	3	4	5
The possible disadvantages of participating in the study	1	2	3	4	5
How participation in this study may benefit future babies	1	2	3	4	5
The effect of the study on the confidentiality of your baby's medical records	1	2	3	4	5
Whom you should contact if you have questions or concerns about the study	1	2	3	4	5
The fact that participation in the study is voluntary	1	2	3	4	5
The fact that you can withdraw your baby from the study any moment	1	2	3	4	5
Overall, how well did you understand the study when you signed the consent form?	1	2	3	4	5