STATISTICAL ANALYSIS PLAN

Mi-ECMO Study

NCT02940327

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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Glossary / abbreviations

AE	Adverse event
AKI	Acute kidney injury
ARG1	Arginase 1
CD	Cluster of differentiation
CDH	Congenital diaphragmatic hernia
CO_2	Carbon dioxide
CmH_2O	Centimetre of water
CPB	Cardiopulmonary bypass
CRF	Case report form
CRRT	Continuous renal replacement therapy
CSCTT	Cardiac surgery clinical trials team
ECMO	Extra-corporeal membrane oxygenation
EDTA	Ethylenediaminetetraacetic acid
FACS	Fluorescence activated cell sorting
FiO_2	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_2	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
SaO_2	Oxygen saturation
SIRS	^{Sy}stemic inflammatory response syndrome
SOP	Standard operating procedures
TNF-α	Tumour necrosis factor – alpha

1. Introduction

The Mi-ECMO study is a pilot 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).

This Statistical Analysis Plan (SAP) describes the analysis and reporting for the Mi-ECMO study. It outlines the planned analysis to be performed on the data. Exploratory post-hoc or unplanned analysis not necessarily identified in this SAP may be performed as required.

1.1 Aims and Objectives

Hypothesis

The 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 ECMO for PPHN.

The secondary hypothesis 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. 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.
5. Markers of platelet, endothelial and leukocyte activation have diagnostic and prognostic utility for the prediction of key clinical events including delayed time to recovery, acute kidney injury in paediatric patients undergoing ECMO for PPHN.

Objectives

The Mi-ECMO study was designed to be a feasibility study, and the results will be used to assist with the design of a prospective observational study that will test the above hypotheses. The objectives of the study are:

1. To determine the recruitment rates and patient flows for 24 patients recruited for the feasibility study.
2. To determine the withdrawal rate and the completeness of follow-up and data collection in a paediatric population at high risk for death and major morbidity.
3. To collect data on primary and secondary outcomes of interest, which may be used to model the sample sizes and outcomes in future study.
4. To collect data on perception of family members, whose children participate in the study, as to their understanding of the screening and consent process (Appendix 1).

Changes in hypothesis

Due to the lack of ethnical approval, this study is not conducting analysis of bronchial aspirates, and measuring markers of oxidative stress and MV levels. The following secondary hypothesis which were stated in the study protocol have not been included in this SAP:

1. The level of oxidative stress will correlate with type shifts in pulmonary macrophages, tissue iron deposition and organ injury.
2. Ability to raise anti-oxidative response, measured by Heme Oxigenase-1 (HMOX 1) expression, will correlate with shorter intubation times and less severe kidney and lung injury.
3. Markers of anti-oxidative response and 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 ECMO for PPHN.

Correspondingly, all related secondary outcomes would not be included in this SAP.

1.2 Study Design

The Mi-ECMO study is a prospective, single-centre observational feasibility study.

1.3 Sample Size

The Mi-ECMO study enrolled 24 patients. There was not a formal power calculation for this feasibility study. The sample size was based on the observations from a similar study, p-MiVAKI which had recruited 24 paediatric patients and was able to demonstrate differences in the primary outcomes, markers of platelets, leukocyte and endothelial activation, between those who did and did not develop AKI, and between those did and did not receive transfusion.

1.4 Study population

The study was conducted at Glenfield Hospital, a regional ECMO centre in the UK, the University Hospitals of Leicester NHS Trust. 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. 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.

Participants of this study were consecutive patients referred to the Glenfield Hospital according to the ECMO referral system who match the following study criteria.

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

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

1.5 Schedule of Major Assessments

	Baseline (12h after ECMO start +/- 6 hrs)	**Day 1 (24 hrs +/- 12 hrs)**	**Day 2 (48h +/- 12 hrs)**	**Day 3 (72h +/- 12 hrs)**	**24h after decannulation/ 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	✓	✓	✓	✓	✓
Questionnaire on perception of family members					✓

2. Endpoints

A list of variables to be evaluated and reported in the statistical analysis is provided in the Appendix.

2.1 *Primary outcomes*

Markers of cellular activation including 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.

Markers of cellular activation include CD41, PAC-1 and CD62P for platelets, CD64 and CD163 for leukocytes, CD14, CD16, and CD41 for platelet-leukocyte aggregates, as well as circulating ICAM and E-selectin for endothelial injury.

2.2 *Secondary outcomes*

Clinical outcomes

1. Serum haemoglobin levels.
2. Time to wean from ECMO.
3. Percentage of patients weaned from ECMO within 7 days
4. AKI as defined by the KDIGO pediatric definition (Table 1)
5. AKI as defined based on the neonatal modification (Table 2)
6. Heart injury as determined by serum troponin levels.
7. Renal inflammation as determined by urine NGAL concentrations.
8. Allogenic red cell transfusion volume.
9. Non red cell transfusion volume.
10. Time to discharge from Glenfield Hospital ICU.
11. Time to Glenfield Hospital discharge.
12. Measures of haemoglobin metabolism e.g. serum hepcidin, serum haptoglobin, transferrin saturation, serum ferritin, plasma labile iron concentrations.
13. Inflammatory cytokines in serum, measured by IL-6, IL-8, TNFα, MCP-1 and MIP-1.
14. Other adverse events not specified above.

Feasibility outcomes

15. Recruitment, sampling rates, and Withdrawal rates
16. Protocol adherence including clinical and laboratory protocol adherence
17. Data completeness
18. Experience of parents/ family/ guardians in relation to the screening, consent and study procedures based on a questionnaire

Table 1. Pediatric KDIGO-staging system for acute kidney injury, as per reference [1], with modified Stage 3 as per the pRIFLE definition [2].

Stage	Serum creatinine (SCr) criteria	Urine output criteria
1	SCr ≥ 26 µmol/L increase or SCr ≥1.5- to 2-fold from baseline	<0.5 ml/kg/hr for > 6 consecutive hrs
2	SCr ≥ 2 to 3-fold from baseline	<0.5 ml/kg/hr for > 12 hr
3	SCr ≥3-fold from baseline or SCr ≥ 354 µmol/L or Renal replacement therapy irrespective of stage or *Decrease in estimated Creatinine Clearance (eCrCl) to less than 35 mL/min/1.73 m^2 using the Schwartz equation: eCrCl(mL/min/1.73 m2) = (36.2 × Height in cm) / Creatinine in µmol/L	<0.3 ml/kg/hr for > 24 hr or anuria for 12 hr

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. 200% to 300% increase = 2- to 3-fold increase. Given 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.

Table 2. Neonatal acute kidney injury definition – modification from KDIGO pediatric AKI definition [3].

Stage	Serum creatinine (SCr) criteria Neonatal modification: 2015-2016	Urine output criteria Neonatal modification 2016
1	SCr ≥ 26 µmol/L increase or SCr ≥1.5- to 2-fold from baseline	≤1 ml/kg/hr for 24 hr
2	SCr ≥ 2 to 3-fold from baseline	≤0.5 ml/kg/hr for 24 hr
3	SCr ≥3-fold from baseline or SCr ≥ 221 µmol/L or Renal replacement therapy irrespective of stage	≤0.3 ml/kg/hr for 24 hr

2.3 *Target conditions*

1. Prolonged ECMO defined as patients remained on ECMO at day 7 (duration of ECMO >168 hours)
2. AKI as defined by the KDIGO stage 1, 2 or 3 using the paediatric AKI definition (Table 1).
3. AKI as defined by the KDIGO stage 1, 2 or 3 using the serum creatinine criteria for the neonatal AKI definition (Table 2).

3. Analysis Sets/Populations

3.1 *Full analysis set population*

The full analysis set population comprises all recruited patients with full consent. This population comprises 24 patients. All analyses of clinical outcomes will be carried out on the full analysis set population.

3.2 *Safety population*

The safety population comprises all recruited patients with either emergency consent or full consent even those withdrew from the study. This population will be used for safety analyses.

3.3 *Protocol deviations*

Potential deviations from the protocol and their categorisation into major/minor are to be discussed by the trial team. In general, protocol deviations would be considered as major if they could potentially lead to biased estimations of primary or secondary endpoints (e.g. entered into the study in error). Time window violations are generally not considered as major protocol deviations.

4. Statistical Methodology

4.1 *Timing of Analysis and Interim Analysis*

Analysis are to be performed when the final dataset with all laboratory data are ready. No interim analyse have been planned before the end of the recruitment phase.

4.2 *Disposition of Patients*

Patient disposition will be presented with respect to recruitment rates, withdrawal rates, as well as protocol adherence including clinical and laboratory protocol adherence.

Data completeness will also be summarized.

4.3 *Descriptive Analysis*

Descriptive analysis will be presented for demographic and baseline characteristics, as well as the primary and secondary outcomes, and targeted conditions described in Section 2. For categorical variables, numbers and percentages will be presented. For continuous variables, mean and standard deviation or median with lower and upper quartiles, as appropriate, will be presented.

4.4 *Statistical Methods*

Mixed effects model with patient as random effect will be used to analyse outcomes measured at multiple time points. Logarithm transformation will be considered for skewed variables. Linear graphs showing the variation of the mean measurements over time will be displayed.

Exploratory analysis are to be carried out using mixed effects models to examine serial makers of cellular activation and their relationship with:

- Duration on ECMO

- Transfusion volume
- Free cell haemoglobins
- NGAL concentrations
- Serum troponin levels
- Inflammatory cytokines

4.5 *Subgroup analysis*

Exploratory analysis may be carried out to assess the relationship of cellular activation with the targeted subgroups:

- Patients on ECMO within 7 days vs 7+ days
- Patients with and without AKI

4.6 *Multiple Comparisons*

There will be no adjustments for multiple comparisons, as this is a feasibility study to gather data on the primary and secondary outcomes for future study, and to explore the relationship between these outcomes to address the objectives described above.

4.7 *Handling of Missing Data*

Lab measurements below the limit of quantification will be imputed as half the limit of quantification. Measurements above the limit of quantification will be imputed as the upper limit. No other missing values will be imputed.

5. Safety Analysis

5.1 *Adverse events reporting*

Data on adverse events are collected from ECMO commencement till hospital discharge. Adverse events shall be reported with the Safety Population.

5.2 *Expected adverse events*

The following adverse events are 'expected'.

- Suspected MI
- Cardiac arrest
- Heart or vessels perforation / chest opening
- Arrhythmias
- Pericardial effusion / cardiac tamponade
- Thromboembolic complications
- Haemodynamic support
- Pulmonary complications
- Renal complications
- Gastro-intestinal complications
- Neurological complications
- Bleeding
- Wound infection / infective complications (sepsis, septic shock etc)

Refer to the protocol for a full list of the expected adverse events.

6. References

1. KDIGO Clinical Practice Guideline for Acute Kidney Injury. Kidney Int Suppl, 2 (2012), pp. 122–123

2. Akcan-Arikan A, Zappitelli M, Loftis LL, Washburn KK, Jefferson LS, Goldstein SL. Modified RIFLE criteria in critically ill children with acute kidney injury. Kidney Int. 2007 May;71(10):1028-35. Epub 2007 Mar 28.

3. Zappitelli M, Ambalavanan N, Askenazi DJ, Moxey-Mims MM, Kimmel PL, Star RA, Abitbol CL, Brophy PD, Hidalgo G, Hanna M, Morgan CM, Raju TNK, Ray P, Reyes-Bou Z, Roushdi A, Goldstein SL. Developing a neonatal acute kidney injury research definition: a report from the NIDDK neonatal AKI workshop. Pediatr Res. 2017 Oct;82(4):569-573.

Appendix List of variables for reporting

Type	Variable	Unit/Reference	Time points
Demographic, Characteristics at birth	Gestational age	week	
	Preterm	Y/N	
	Gender	M/F	
	Ethnicity	Caucasian /Other	
	Weight at birth	kg	
	Weight	kg	
	APGAR score at birth	0 - 10	
Clinical characteristics			
Heart Rhythm	Sinus Rhythm	Sinus rhythm/AF /Heart block /Paced/Other	
Diagnosis	Diagnosis	Idiopathic PHN /MAS/CDH/ pneumonia/RDS/PH/others	
Cardiac function	LV function	mild / moderate / severe impairment	
	RV function	mild / moderate / severe impairment	
	Shunt	None/Right to left/Left to right /Bidirectional	
	Shunt function	Restrictive / not restrictive	
Mechanical ventilation	Mechanical ventilation	Y/N	
	Modality		
Cannulation	Haemodynamic instability	Y/N	prior to ECMO
	Blood priming	Y/N	
	Clear priming	Y/N	
ECMO Parameters	MAP	mmHg	Pre-ECMO (MAP only); t=0, 12h, 24h, 48h, 72h after start of ECMO and before discontinuation
	FiO2 on ECMO	%	
	FiO2 on ventilator	%	
	ACT	sec	
	Pump flow	ml/min	
	Sweep gas	ml/min	
	RPM	revolutions/min	
Blood saving	Transexamic acid	Y/N	
	Cell Saver (autologous)	Y/N	
Arrhythmia	Arrhythmia		Placement, removal ECMO
	Cardioversion	Y/N	
	Defibrillation	Y/N	

Type	Variable	Unit/Reference	Time points
Blood gas	pH		
	pO2	kPa	
	pCO2	kPa	
	SaO2	%	Pre-ECMP (PH, Lactate, PCO2, HCO3); t=0, 12h, 24h, 48h, 72h after start of ECMO and before discontinuation
	Hb	g/L	
	Glu	mmol/L	
	Lactate	mmol/L	
	HCO3	mmol/L	
	Hct	%	
	Na	mmol/L	
	K	mmol/L	
	Ca	mmol/L	
PICU	Lactates	mmol/L	t=0, 12, 24, 48 and 72 hrs post PICU admission & 24hrs after ECMO stop
	PaO2/FiO2		
	Total bilirubin	umol/L	
	Fibrinogen	g/L	
	Urea	mmol/L	
	PIM2 score	Total Criteria Point Count	PICU admission
	PMODS score	Total Criteria Point Count	PICU admission & 24h after ECMO stop
Medication	Inotrope Score	Total Criteria Point Count	
	Vasoactive-Inotrope score	Total Criteria Point Count	
Laboratory test			
Platelets, leukocyte activation	CD41, PAC-1, CD62P, CD64, CD163, CD14, CD16, CD41		t=12h, 24h, 48h, 72h ECMO, and 24h post ECMO and discharge
Granulocyte	CD11b		
Endothelial	ICAM, E-selectin		
Cytokines	IL-6, IL-8, TNFα, MCP-1, MIP-1		
Iron	Total iron, non-transferrin-bound iron		
Free Hb	Free cell Haemogloblins		
Routine lab tests	Creatinine	umol/L	Pre-ECMO, t=12h, 24h, 48h, 72h ECMO, and 24h post ECMO and discharge
	Adjusted Calcium	mmol/L	
	Alkaline Phosphatase	iu/L	
	ALT	iu/L	
	Total Bilirubin	umol/L	
	Amylase	iu/L	
	CRP	mg/L	
	Haemoglobin	g/L	
	Haematocrit	L/L	
	Platelet count	x10^9/L	
	INR		
	APTT ratio		
	Fibrinogen	g/L	

Type	Variable	Unit/Reference	Time points
Outcome	Total hours on ECMO	hours	
	Alive at discharge	Y/N	
	Died	Y/N	
	Chest open	Y/N	
	Patient weaned from ECMO?	Y/N	
	Reintubation	Y/N	
	Fluid balance	ml	12h, 24hr
	Bleeding cannulation site	ml	12h, 24hr
	Urine output		
	Any RBC transfused?	Y/N	Pre, during & post
	Any non RBC transfused?	Y/N	ECMO
	Blood products transfused	No of units	
	Volume transfused	ml	
	ICU length of stay	days	
	Hospital length of stay	days	

	Item	Score
Questionnaire	The fact that your baby's treatment involves research	1 - 5
	What the researchers are trying to find out in the study	1 - 5
	How long your baby will be in the study	1 - 5
	The procedures your baby will undergo	1 - 5
	The possible disadvantages of participating in the study	1 - 5
	How participation in this study may benefit future babies	1 - 5
	The effect of the study on the confidentiality of your baby's medical records	1 - 5
	Whom you should contact if you have questions or concerns about the study	1 - 5
	The fact that participation in the study is voluntary	1 - 5
	The fact that you can withdraw your baby from the study any moment	1 - 5
	Overall, how well did you understand the study when you signed the consent form?	1 - 5