Statistical Analysis Plan

DRAFT

MiLe-1

The prophylactic effect of Levosimendan in reducing acute kidney injury postoperatively in pediatric patients undergoing corrective heart surgery

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1 STUDY DETAILS

1.1 Study Objectives

The aim of the study is to assess the ability of Levosimendan to reduce the postoperative acute kidney injury in pediatric patients undergoing surgery for congenital heart disease (CHDs).

The primary objective of this study is to investigate the preventive effect of Levosimendan on postoperative acute kidney injury in pediatric patients undergoing surgery for their CHDs. Creatinine at the second postoperative day will be the primary endpoint.

1.2 Study Design

This is a parallel-group, prospective, randomized, double-blinded controlled study in pediatric patients in need for surgery due to their CHD.

A total of 70 infants will be included in the study and undergo open heart surgery according to routine procedures at the two participating hospitals. Patients will be randomly assigned to receive either Levosimendan (test group) or Milrinone (control group). Serum creatinine, the common marker of acute kidney injury, will be measured daily according to the clinical routines. The length of treatment (Levosimendan or Milrinone) is 26 hours. The treatment will be started during the operation at the same time-point at which Milrinone infusion routinely is started. Blood samples will be obtained at five occasions, during 24 hours perioperatively. Patients will be followed for at least 48 hours after termination of the treatment, including hemodynamic monitoring, blood gases, serum creatinine. Furthermore, there is a follow-up for possible adverse events/serious adverse events for 28 days from the surgery (phone call to parents and/or to the local hospital).

Figure 1 ***Study design***

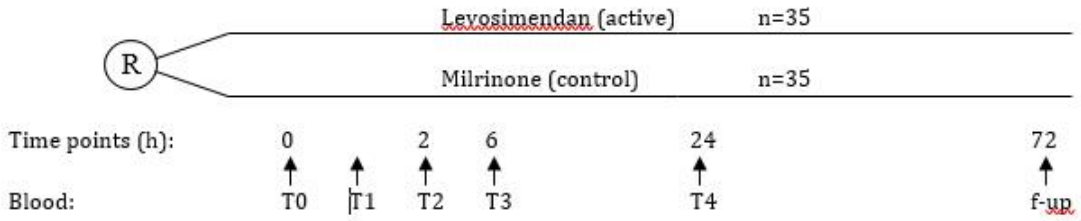

Figure 2 ***Study flow chart***

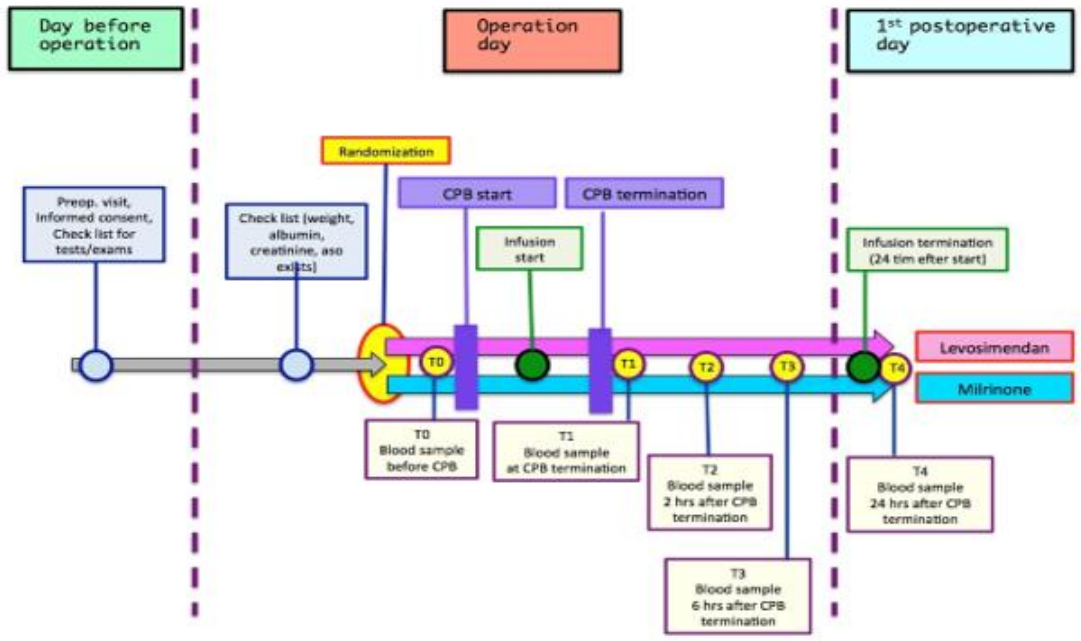

Table 1 ***Study activities***

	Pre-op	Surgery	Surgery	Post-op	Post-op	Post-op	Follow-up	Follow-up	Follow-up
		T0	T1	T2	T3	T4		T5	
		Baseline (Before CPB)	Directly after CPB	2hours after CPB	6hours after CPB	24 hours after CPB	Postop days 1 - 4	96 hours after CPB	28 days
Informed consent	X								
Demography	X								

Weight, height	X								
Medical history	X								
Physical examination	X								
Inclusion/exclusion criteria	X								
Randomization	X								
Surgery		X							
Levosimendan/ Milrinone		X	X	X	X	X			
Pulse,Blood pressure, CVP, blood gases		X	X	X	X	X			
Blood for laboratory screen		X							
Serum Hb, WBC, CRP Albumin, Creatinine, Urea, CystatinC		X					X		
Volume status, diuresis, weight							X		
Pulse, BP							X		
Study specific blood		X	X	X	X	X		X	
Continuous ECG monitoring		X	X	X	X	X			
Continuous hemodynamic and respiratory monitoring		X	X	X	X	X			
Serious Adverse event and Adverse event recording		X	X	X	X	X	X	X	X

1.3 Treatment Groups

Active: Levosimendan 2.5 mg/mL solution, diluted to 0.05 mg/mL i.v.

Control: Milrinone i.v. standard of care

1.4 Sample Size

The number of patients needed for the study is based on a 20% reduction in serum creatinine in the Levosimendan group compared with the Milrinone group in the day after stopping the infusion of Levosimendan/Milrinone (second postoperative day). The power of study is set on 80 %.

The study will contain two groups; Levosimendan (n=35) and Milrinone n=35). Patients in the Levosimendan group will receive Levosimendan as inotrope agent and patients in the

Milrinone group will receive Milrinone as inotrope agent. Patients will randomly be assigned to either group. The caregivers (physicians, nurses and nurse-assistants) and the investigators will be blinded for the study drug. The same infusion rate and total volume of Levosimendan and Milrinone will be applied to both groups.

2 STUDY DATA

Analyses regarding inflammatory and brain injury biomarkers, pharmacokinetics and echo-cardiography will not be presented in this analysis plan. Separate analysis plans will be written for these data.

3 STUDY POPULATIONS

Infants, between the age of 1 to 12 months, with one of following congenital heart diagnoses, in need of total corrective and elective heart surgery: 1) Non-restrictive VSD, Tetralogy of Fallot, acyanotic AVSD.

3.1 Definition of Study Populations

3.1.1 Intent-to-Treat Population (Full Analysis Set)

All randomized subjects with at least one creatinine measurement at follow-up (24h, 48h, 72h or 96h) will be included in the Intent-to-Treat (ITT) population.

3.1.2 Per-Protocol Population

All ITT subjects with no major protocol violations which can affect the creatinine values will be included in the Per Protocol (PP) population. The final decisions regarding the PP population will be taken at the Clean File meeting before the database lock.

3.1.3 Safety Population

All enrolled subjects who received study drug will be included in the safety population.

4 STUDY VARIABLES

4.1 Baseline Variables

4.1.1 Screening

- Indication (Non-restrictive VSD (corrective surgery)/Complete AVSD (bi ventricular repair)/Tetralogy of Fallot/Non-restrictive VSD with RVOT obstruction and/or PS)
- Gestational age weeks
- Gestational age days
- Gender (Male/Female)
- Age at (weeks)
- Mother's biological nationality
- Father's biological nationality

4.1.2 Preoperative Data

- Pre_Weight (kg)
- Height (cm)
- Surface/area (m^2)
- Diseases and/or congenital anomalies (free text coded into 0/1 variables; Mb Down, None, Other)
- Furosemide (Yes/No)
- Aldosterone (Yes/No)
- Betablocker (Yes/No)
- ACE-inhibitor (Yes/No)
- Digitalis (Yes/No)
- FS (%)
- ROSS score (Calculated value in the CRF)

4.1.3 Preop samples taken in OR from art and cvc line

- Hb (g/L)
- Hct
- Leucocytes (x10^9/L)
- Trombocytes (x10^9/L)
- CRP (mg/L)
- Na (mmol/L)
- K (mmol/L)
- Albumin (g/L)
- Creatinine(µmol/L)
- eGFR (Calculated value in CRF)
- Cystatin C (mg/L)
- Urea (mmol/L)
- NT-Pro-BNP (nanog/L)
- p H
- p C O 2 (kPa)
- p O 2 (kPa)
- ABEc (mmol/L)
- cHCO3 (mmol/L)
- s O 2 (%)
- cLac (mmol/L)

- cGlu (mmol/L)
- Ca i (mmol/L)
- Central venous sO2 (%)

4.2 Concomitant treatments and medications

4.2.1 Treatments and medication in OR

- Weaning of CPB: Only include the box “extra bolus study drug”
- Inotropic score (calculated value in CRF)
- Other medication (OR only):
    - Betablocker (YES/NO),
    - Furosemide (YES/NO),
    - Cortisone (YES/NO),
    - Cyklokapron (YES/NO),
    - Fibrinogen (YES/NO),
    - Other medication (YES/NO)
- Fluid balance (OR only):
    - Albumin (YES/NO),
    - Plasma (YES/NO) ,
    - RBC (YES/NO) ,
    - Thrombocytes(YES/NO) ,
    - Diuresis (in ml)

4.2.2 Treatments and medication in ICU

Concomitant medications will be presented per day (Medications in the ICU on the operative day (Status and medication_1), postop day 1 (Status and medication_2), postop day 2 (Status and medication_3), postop day 3 (Status and medication_4), postop day 4 (Status and medication_5)) and postop day 1+2 and at any time.

- Albumin infusion (Yes (>0 ml)/No(0 ml))
- Trombocyte transfusion (Yes (>0 ml)/No(0 ml))
- RBC (Yes (>0 ml)/No(0 ml), RBS in CRF miss spelling)
- Plasma (Yes (>0 ml)/No(0 ml))
- Betablocker (Yes/No)
- Cordarone (Yes/No)
- Furosemide (Yes/No)
- Furosemide (mg)
- Ethacrynic acid (Yes/No)

- Ethacrynic acid (mg) (Reomax in CRF)
- hANP (Yes/No)
- Spironolactone (Yes/No)
- Spironolactone (mg)
- Methylprednisolone (Yes/No)
- Hydrocortisone (Yes/No)
- Betametason (Yes/No)
- Cortisone: Methylprednisolone, Hydrocortisone or Betametason (Yes/No)
- NO (Yes/No)
- Other medication (free text)
- Dialysis (Yes/No)
- NSAID (Yes/No, Search text Other specify field, Comments field, Extra comments and Important information:info_other_comments for NSAID, Ibuprofen, Brufen) (listing only)
- Vancomycin (Yes/No, Search text field Other specify field, Comments field, Extra comments and Important information:info_other_comments for Vancomycin, Vancocin) (listing only)
- Use of contrast (Yes/No from Important information) (listing only)
- Study drug in the PICU (Study drug in CRF):
    - Study drug according to protocol (YES/NO),
    - Milrinone started (YES/NO)

4.3 Surgery variables

Name of anesthesiologist

4.4 Efficacy Variables/Endpoints

4.4.1 Primary endpoint

$AKI_{(48\ hours)}$ is defined as 50% increase in corrected creatinine within 48 hours. $AKI_{(48\ hours)}$ is calculated using screening creatinine (uncorrected) compared to 24 and 48 hours corrected creatinine.

Corrected creatinine [(creatinine (µmol/ml)/1000 x [1 + (fluid balance $(L)_{(+24\ hours)}$ / (weight (kg) x 0.6))] only calculated for day 1-3. For example for day 1 the Fluid balance measured at day 2 will be used. If negative values in fluid balances, fluid balance is set to 0.

4.4.2 Secondary endpoints post-op

Post-op will be measured at 5-10 min before CBP (T0), 5-10 min after CBP (T1), 2h after weaning from CPB (T2), 6h after weaning from CPB (T3), 8-12 hours post CBP-weaning, 24h after weaning from CPB (T4), 4 days after weaning from CPB (T5) (all variables are not

present at all timepoints). Changes from T0 will be calculated for all variables where applicable.

Hemodynamic parameters measured at T0-T4 (not T5) and additionally at 8-12 hours post CPB-weaning

- Heart rate (BPM)
- Heart rhythm (SR/NR/Other)
- Pacing (Yes/No) (Missing on T0)
- Mean BP M A P (mmHg)
- CVP (mmHg)
- Central venous saturation (%)
- NIRS brain (%)
- NIRS kidney (%)
- Ventilated (Yes/No)

Arterial blood gas test

- p H
- s O 2 (%)
- cLac (mmol/L)
- Hb (g/L)
- Hct

Rate of vasoactive drugs

- Inotropic score
- 2 ml blood sample in ice taken (YES/NO)

NGAL

- NGAL plasma

T5

- T5 taken at postoperative day nr (2-7)

4.4.3 Secondary endpoints Follow up postop days 1-4

Fluid balance

- Fluid balance ((ml)
- Urine (ml)
- Weight (g)
- %FO (fluid balance (ml)/$1000_{(+24\ hours)}$ /preop weight (kg)) x 100. For example for day 1 the Fluid balance measured at day 2 will be used. If a negative fluid balance set %FO to 0.
- Cumulative %FO (day 1+2, day 1+2+3, day 1+2+3+4)

- Peritoneal Dialysis day 1 and day 2 from Status and medication_1 and Status and medication_2

Morning lab results (Postop day 1 is under hemodynamics_1, etc.)

- Hb (g/L)
- Hct
- Leucocytes (x10^9/L)
- Trombocytes (x10^9/L)
- C R P (mg/L)
- Albumin (g/L)
- Creatinine (µmol/L)
- Corrected creatinine [(creatinine (µmol/L) x [1 + (fluid balance (L)$_{(+24\ hours)}$ / (weight (kg) x 0.6))] only calculated for day 1-3. For example for day 1 the Fluid balance measured at day 2 will be used. If negative values in fluid balances, fluid balance is set to 0.
- Corrected creatinine (age differentiated), same as above but (in the formula) using 0.75 for infants younger than 5 months and 0.6 for infants/adults older than 5 months.
- AKI$_{(72\ hours)}$ and AKI$_{(96\ hours)}$ in same fashion as in primary endpoint
- AKI stage at 48 (24 and 48), 72 and 96 hours.
    - Stage 0 (no AKI, corrected creatinine up to 1.49 times screening creatinine)
    - Stage 1 (corrected creatinine 1.5-1.99 times screening creatinine)
    - Stage 2 (corrected creatinine 2-2.99 times screening creatinine)
    - Stage 3 (corrected creatinine 3 (or more) times screening creatinine or need of dialysis)
- eGFR Creatinine (calculated value from CRF)
- Cystatin C (mg/L)
- eGFR Cystatin C, calculated using formula 91 x Cystatin C (mg/L)$^{-1.213}$
- Urea (mmol/L) Use value in database (no recalculation is needed), in CRF the unit stands as µmol/L but it is actually mmol/L.
- Combined eGFR (mL/min per 1,73 m^2)
    - 39.1 x [height (m)/Serum creatinine (mg/dl)]$^{0.516}$ x [1.8/ Cystatin C (mg/L)]$^{0.294}$ x [30/Urea (mg/dl)]$^{0.169}$ x [1.099]^{male} x [height (m)/1.4]$^{0.188}$
    - Conversion formula Creatinine (mg/dl) = Creatinine (µmol/L)/88.42
    - Conversion formula Urea (mg/dl) = Urea (mmol/L)/0.3571

Morning Hemodynamics (Postop day 1 is under Test results and hemodynamics_1 etc)

- Heart rate (BPM)
- Rythm (Sinus rhythm/Nodal rythm/Other)

- Pacing (Yes/No)
- M A P (mmHg)
- Ventilated (Yes/No)

Rate of vasoactive drugs at 06:00 am

Inotropic scoreBlood gas at 6 am (Postop day 1 is under Test results and hemodynamics_1 etc)

- p H
- s O 2 %
- pO2 (kPa)
- Hb (g/L)
- Hct
- cLac (mmol/L)
- CV saturation (%)

Cardiopulmonary bypass and use of cortisone in OR

- CPB time (minutes) (taken from CRF page “Drugs and treatment; cardiopulmonary bypass”)
- Crossclamp time (minutes)
- Lowest temperature CPB (degrees celcius)
- Use of cortisone in OR (Yes/No)

Other parameters

- length of mechanical ventilation postoperatively (hours) (collected outside of CRF, external file)
- RACHS-Score 1 (VSD = 2, Fallot = 2, AVSD = 3) (collected outcome crf page)
- Comprehensive Aristotle Score (collected outcome crf page)
- Length of stay PICU (days) (change Finland subjects by minus 1 day)
- Length of hospital stay (days) (change Finland subjects by minus 1 day)
- Re-operation within 96 hours (Yes/No from Important information) (listing only)
- Re-operation (Yes/No from Important information) (listing only)

Other relevant biomarkers may be analysed at a later occasion and will not be specified here.

4.5 Safety Variables

4.5.1 Adverse Events

Adverse Event within 28 days from surgery. AEs are collected in the following 5 categories at CRF page Adverse Events:

- Heart rate > 200/min at these time points: 2, 6, 10-12 hours after CPB-weaning, first postop morning at 6 am, 24 hours after CPB-weaning

- Arrytmias at same time points as for heart rate >200/minInotropic score > 20 at same time points as for heart rate >200/minThrombocytes < 50 10^9/L on the first postoperative morning
- Hypokalemia < 3.5 mmol/L at same time points as for heart rate >200/min

4.5.2 Serious Adverse Events

Serious Adverse Event within 28 days from surgery. SAEs are collected in the following 6 categories:

- Death
- Immediately life-threatening
- In-patient hospitalisation or prolongation of existing hospitalisation
- Persistent or significant disability or incapacity
- Congenital abnormality or birth defect
- Important medical event that may jeopardise the subject or may require medical intervention to prevent one of the outcomes listed above.

5 STATISTICAL METHODOLOGY

5.1 General Methodology

All baseline, efficacy and safety variables will be summarized by treatment group. Continuous variables will be presented with mean, standard deviation (SD), Median, minimum, maximum and number. Categorical variables will be presented as number and percentage (%).

All tests will be two-tailed and conducted at 0.05 significance level. All analyses will be performed by using SAS® v9.4 (Cary, NC).

5.2 Patient Disposition and Data Sets Analyzed

The number of subjects included in each of the ITT, PP and safety populations will be summarized for each treatment group and overall. The number and percentage of subjects randomized and treated will be presented. Subjects who completed the study and subjects who withdrew from study prematurely will also be presented with a breakdown of the reasons for withdrawal by treatment group for the ITT, PP and safety populations.

5.3 Protocol Violations/Deviations

Major protocol deviations are those that are considered to have an effect on the analysis. A list of potential major protocol deviations will be generated programmatically from the data captured before the clean file meeting. The clinical monitors of the study will review the list and the finalisation of the major protocol deviations will be done at the clean file meeting.

The number of patients with major protocol deviations will be summarized per treatment group.

Major protocol deviations

1) Inadequate informed consent
2) Inclusion or exclusion criteria are not met
3) Unreported serious adverse events
4) Improper randomization
5) Improper breaking of the blind
6) Baseline serum Creatinine missing
7) Serum Creatinine postoperative day 1 and 2 are both missing (primary outcome)
8) Materially inadequate record keeping
9) Intentional deviation from protocol or GCP

Minor protocol deviations

1) Study drug protocol not followed appropriately
2) One or more of following blood samples are missing or mishandled: Serum Creatinine postop day 1 or 2. Any of plasma samples T0 – T5
3) 28 days follow-up information (28 days outcome) is missing

5.4 Screening, preoperative data and Preop samples taken in OR from art and cvc line

Demographics and baseline characteristics will be summarized by treatment group for the ITT and PP populations and analyzed according to the methods described in section “General Methodology” above.

5.5 Efficacy Analyses

5.5.1 Primary Efficacy Analysis

Logistic regression of AKI (50% increase in corrected creatinine within 48 hours) with treatment group independent variable will be used for the primary outcome. If differences in corrected creatinine at baseline differs between groups adjustment for baseline corrected creatinine will be made by adding baseline creatinine as an independent variable. The analysis will be two sided and with alpha 0.05.

5.5.2 Secondary Efficacy Analyses

Complementary analyses of the primary analysis will be made up to 72 hours and also one analysis up to 96 hours in the same fashion as the primary analysis.

Secondary variables will be presented at each timepoint and changes from baseline (screening, pre-op) will be calculated where applicable. T-test for independent groups will be used for secondary continuous outcomes if the data not is clearly non-normal distributed. If baseline values differs between groups adjustment for baseline values will be made using a covariance analysis will be made. If the outcome is clearly non normal distributed Fishers non parametric permutation test will be used instead of t-test.

Categorical (no/yes) variables will be analysed between groups by using Fisher exact test.

Univariable and multivariable logistic regression analysis will be used to identify association between the occurrences of AKI (based on creatinine results) and the measured clinical variables.

5.5.3 Exploratory Efficacy Analyses

Selected correlation analyses will be performed between secondary variables/outcomes.

5.6 Safety Analyses

5.6.1 Adverse Events

A summary of subjects reporting at least one of the following AEs will be presented in an overview table:

- Any AE
- Any SAE
- Any treatment-related AE (Definite, Probably and Possible)
- Any treatment-related SAE (Definite, Probably and Possible)
- Any AE leading to discontinuation
- Death

Summaries presenting n (%) of AEs and n (%) of subjects with at least one AE will be provided for:

- All AEs (includes all serious and non-serious AEs)
- All treatment-related AE (Definite, Probably and Possible)
- All SAEs
- All treatment-related SAE (Definite, Probably and Possible)

6 INTERIM ANALYSES

No interim analyses planned.

7 CHANGES OF ANALYSIS FROM PROTOCOL

Specify all major changes from protocol by subdividing into before and after database lock and unblinding.

8 LISTING OF TABLE, FIGURES AND LISTINGS

8.1 Listing of Tables

Table Number	Table Title
14.1.1	Patient Disposition and Data Sets Analyzed
14.1.2	Screening data (ITT Population)
14.1.3	Preoperative data (ITT Population)
14.1.4	Preoperative samples taken in OR from art and cvc line (ITT Population)
14.2	Concomitant treatments and medications by day (ITT Population)
14.3.1	AKI and corrected creatinine (ITT population)

14.3.2.1	Hemodynamic parameters by timepoint (ITT population)
14.3.2.2	Arterial blood gas test by timepoint (ITT population)
14.3.2.3	Rate of vasoactive drugs by timepoint (ITT population)
14.3.2.4	NGAL by timepoint (ITT population)
14.3.3.1	Fluid balance by timepoint (ITT population)
14.3.3.2	Morning lab results by timepoint (ITT population)
14.3.3.3	Morning Hemodynamics by timepoint (ITT population)
14.3.3.4	Blood gas at 6 am by timepoint (ITT population)
14.3.3.5	Rate of vasoactive drugs at 06:00 am by timepoint (ITT population)
14.3.3.6	Cardiopulmonary bypass and use of cortisone in OR by timepoint (ITT population)
14.3.3.7	Other parameters by timepoint (ITT population)
14.4.1	Univariable and multivariable analyses of AKI (ITT population)
14.4.2	Correlation analyses (ITT population)
14.5.1	Summary of Adverse Events (Safety Population)
14.5.2	All AEs (includes all serious and non-serious AEs) (Safety Population)
14.5.3	All treatment-related AE (Definite, Probably and Possible) (Safety Population)
14.5.4	All SAEs (Safety Population)
14.5.5	All treatment-related SAE (Definite, Probably and Possible) (Safety Population)

8.2 Listing of Figures

Figure Number	Table Title
14.1	AKI by 24, 48, 72 and 96 hours
14.2	AKI stage by 24, 48, 72 and 96 hours
14.3	FO % day 1 to 4
14.4	Creatinine and corrected creatinine day 1 to 4
14.5	eGFR creatinine day 1 to 4
14.6	eGFR Cystatin C day 1 to 4
14.7	Combined eGFR day 1 to 4

8.3 Listing of Listings

All analysis data will be exported to Excel and delivered to sponsor. No formal listings will be produced.