Clinical Study Protocol

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

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The following Amendment(s) and Administrative Changes have been made to this protocol since the date of preparation:

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PROTOCOL SYNOPSIS

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

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Study period

Estimated date of first subject enrolled: June 2014

Estimated date of last subject completed: June 2016

Background

Acute kidney injury (AKI) is a major cause of morbidity and mortality in critically ill patients including critically ill children. The etiology of AKI is multifaceted which may partly explain the lack of a causative therapy. The treatment of AKI remains supportive and consists of, in milder cases urine production enhancing medications, and in more severe cases renal replacement therapy. There is evidence that Levosimendan may protect the kidney from injury in conjunction with cardiac surgery in adults [1]. In this study we will use Levosimendan to intervene with renal hypoperfusion and assess its efficacy in reducing creatinine, which is used most frequently as a clinical marker of kidney injury. The study is prospective, randomized, and double-blinded. The trial will contain two study groups, 35 patients in each, where the one group will receive Levosimendan and the second group will receive Milrinone as an inotrope agent perioperatively. Milrinone is currently used as the drug of choice for inotropic therapy in our department like many other pediatric cardiac surgery centers. It remains to see if Levosimendan can exert a kidney protecting function in addition to its inotropic one.

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).

Primary objective

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 levels postoperatively will be the primary endpoint.

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 male and female pediatric patients will be included in the study and undergo heart surgery according to routine procedures at the hospital. Patients will be randomly assigned to receive either Levosimendan (test group) or Milrinone (control group). Creatinine, the common marker of kidney injury, will be measured daily according to the clinical routines. The length of treatment (Levosimendam or Milrinone) is 24 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 4 days after termination of treatment.

Target subject population

Young children, between the age of 1 to 12 months, with congenital heart disease in need of elective heart surgery

Inclusion criteria

The inclusion criteria will be any of the diagnoses:

1. Non-restrictive VSD (corrective surgery)
2. Complete AVSD (biventricular repair)
3. Tetralogy of Fallot

Exclusion criteria

The exclusion criteria will be

1. Unbalanced AVSD

2. Age less than one month and more than one year

3. Acute operation that is unscheduled operation during the first 24 hours after presentation to the department for thoracic surgery

4. Mild, moderate, or severe kidney dysfunction

5. Liver impairment or disease

6. Ongoing infection

7. Use of nephrotoxic drugs (like ibuprofen, angiotensin-converting-enzyme inhibitors, gentamicin, vancomycin) preoperative or postoperative until first post operative day

8. Use of inhibitors of membrane transport proteins (Cimetedin, cetirizine, trimethoprim, probenecid, rifampin and gemfibrosil

9. Allergy to Levosimendan or substance included in the preparation or previous use of Levosimendan

10. Severe arrhythmias needing pace-maker treatment prior to the operation

11. Severe cardiac dysfunction needing for treatment with extracorporeal membrane oxygenation (ECMO) prior to the operation

12. Severe uncorrected hypotension where the initiation of anesthesia is postponed for its correction

13. Re-operation

Investigational product, dosage and mode of administration

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

Control: Milrinone i.v. standard of care

Duration of treatment

24 hours

Duration of study

30 days (24 hours treatment + 4 days follow up + 30-days-mortality registration)

Outcome variable(s):

Creatinine is primary outcome in this study. Inflammatory biomarkers, NGAL, and other relevant biomarkers belong to secondary outcome variables.

Safety

Adverse Events will be recorded. Serious Adverse Events will be reported.

Statistical methods

The number of patients needed for the study will be 70 and is based on a 20% reduction in creatinine in the Levosimendan group compared with the Milrinone group postoperatively. The power of study is set on 80 %.

Patients will be randomly assigned to one of two treatment groups: Levosimendan (n=35) and Milrinone group (n=35).

Analysis of data will be performed by a professional statistician. Univariate analyses will be used where needed. Multivariable regression analysis will be used to identify association between the occurrences of AKI (based on creatinine results) and the measured clinical variables. Descriptive statistics will be applied on the secondary outcomes including clinical data (fluid balance, length of surgery, length of CPB, length of mechanical ventilation postoperatively, length of vasoactive therapy, number of vasoactive medications, total amount of infused vasoactive drugs, hemodynamic parameters).

Safety and tolerability data will be summarized descriptively by treatment and presented in tabular and/or graphical form. All adverse event data will be listed individually and summarized using MedDRA terminology.

STUDY STRUCTURE

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

The following abbreviations and special terms are used in this study protocol.

Abbreviation or special term	Explanation
AE	Adverse event
AKI	Acute Kidney Injury
ARF	Acute Renal Failure
AVSD	Atrioventricular septum defect
CHDs	Congenital heart diseases
CPB	Cardiopulmonary bypass
CRF	Case report form
DAE	Discontinuation due to Adverse Event
EC	Ethics Committee, synonymous to Institutional Review Board (IRB) and Independent Ethics Committee (IEC)
ECG	Electrocardiogram
ECMO	Extracorporeal membrane oxygenation
GCP	Good Clinical Practice
ICU	Intensive care unit
ICH	International Conference on Harmonisation
KATP	Potassium-sensitive adenosine triphosphate channels
MMP-9	Matrix metalloproteinase-9
OAE	Other Significant Adverse Event (ie, adverse events of particular clinical importance, other than SAEs and those AEs leading to discontinuation of the subject from study treatment
NGAL	Neutrophil gelatinase-associated lipocalin
PICU	Pediatric intensive care unit
SAE	Serious adverse event
SIRS	Systemic inflammatory response syndrome
SOPs	Standard Operating Procedures
SUSARs	Suspected unexpected severe adverse reactions
TOF	Tetralogy of Fallot
VSD	Ventricle septum defect

1. INTRODUCTION

1.1 The importance of acute kidney injury

Acute renal failure (ARF), which recently has been refined and defined as acute kidney injury (AKI), is a common and serious condition in critically ill pediatric and adult patients. Due to lack of standard definition of ARF the reported incidence of AKI has varied considerably, namely between 5 and 50% of critically ill patients, both children and adults. The morbidity and mortality of AKI is unacceptably high. The reported mortality rates of about 40-50% in dialysis-dependent AKI, demonstrates the seriousness of the condition. AKI is an independent factor for mortality in critically ill patients, meaning that the patients do not simply die with AKI but also from AKI. The treatment is supportive in addition to reversing the underlying causes. The supportive measures include optimizing fluid and cardiorespiratory status, avoiding nephrotoxic medications and renal replacement therapy [2].

1.2 Etiology of AKI

It is well known that cardiopulmonary bypass (CPB) is associated with AKI [3-6]. Pediatric patients with cyanotic congenital heart disease undergoing CPB are at increased risk for developing AKI [7-9]. The pathophysiology of CPB-related AKI include renal hypoperfusion, ischemia-reperfusion injury and CPB-induced systemic inflammatory response with activation of the blood cascade systems [2]. The complement system is one of the initiators of the systemic inflammation. The cause of systemic inflammatory response syndrome (SIRS) is the contact between blood components and CPB artificial surfaces. This contact is perceived as a danger signal by the body [10]. Other danger signals, and hence SIRS contributors, are ischemia-reperfusion, surgical trauma, and probably endotoxemia. The danger signals activate leukocytes and endothelial cells as well as the plasma cascade systems [11]. The blood cascade systems are comprised by the coagulation, fibrinolytic, kallikrein-kinin, and complement systems. In SIRS there is an unproportional activation of these systems, which is harmful to patients especially in those who have other risk factors. These cascade systems are mutually correlated and "cross talk" to each other. This means that activation of one system may lead to the activation of the other systems [12, 13].

1.3 Diagnosis of AKI

The diagnosis of AKI is based on clinically and laboratory variables like decreased urine output and increased serum creatinine (the cornerstone of the AKI) [14]. However, the fact that creatinine is a late marker of AKI, has led to a considerable search for other

biomarkers enable to early predict the proceeding AKI. Early prediction of AKI is important for timely initiation of prophylactic treatment options. One promising biomarker is NGAL [15].

1.4 Prevention of AKI

There is no causal treatment for AKI, highlighting the importance of the preventive measures. As mentioned previously, renal hypoperfusion is one of the etiologies to AKI. In this study we aim to intervene renal hypoperfusion (by Levosimendan).

1.5 Levosimendan

1.5.1 Mechanism of action

Levosimendan belongs to a relatively new group of drugs which is called calcium-sensitizing. Levosimendan enhances the contraction of heart muscle fibers at the same level of cytosolic calcium concentration. It performs this by holding the calcium-binding site of troponin C in its active conformation which leads to more binding at lower intracardiac calcium concentrations. The advantage of this approach, compared with other inotropes, is the needlessness of increased cytosolic calcium. Hence, the negative effects of increased cytosolic calcium on oxygen consumption and mitochondrial function can be avoided in addition to the avoidance of the activation of various calcium-dependent proteases and phospholipases which mediate systemic inflammation during ischemia-reperfusion. Levosimendan also stimulates membrane potassium-sensitive adenosine triphosphate (KATP) channels. This leads to dilation of peripheral and coronary vessels. Levosimendan also stimulates the same channels in mitochondria and opens them, which may be an important mechanism of pharmacologic preconditioning and cytoprotection. Levosimendan differs from other inotropic agents like dopamine, amrinone, and Milrinone, with regard to maintaining the positive inotropic properties even in the abnormal myocardium. Levosimendan mechanism of action indicates limited, if any, potential to initiation of arrhythmias, which is confirmed by clinical experience. At much greater concentrations, it does inhibit phosphodiesterase III (like Milrinone), but this effect does not appear to be relevant at clinical concentrations [16].

1.5.2 Effects in adults

Clinical effects include improved cardiac output, reduced ventricular filling pressures, and decreased pulmonary vascular resistance during the acute treatment of adult patients with either stable or decompensated heart failure. In addition there are evidence for improved cardiac performance in the post cardiotomy and post bypass

setting. There have also demonstrated beneficial responses in adult patients who appeared to be poorly responsive to other inotropes [17].

1.5.3 Effects and pharmacokinetics in Children

Levosimendan is commonly used in the pediatric intensive care units (PICUs), despite the fact that the drug, like many other drugs in PICUs, is not registered for use in children. In two recent reports [18, 19] the authors assessed the current practices at European hospitals related to the prevention and treatment of low cardiac output syndrome in children undergoing open heart surgery. Results demonstrated that Levosimendan belongs to the most common drug used for this indication in European countries. The most common drugs were Milrinone, dopamine, epinephrine, dobutamine and Levosimendan, which together comprised up to 85.9% of the total drug usage for this indication. The dosage of the off-label usage of Levosimendan in PICUs was usually similar to that of adult dosage regimen, i.e. 12 μg/kg loading dose followed by an infusion of 0.1-0.2 μg/kg/min. This dosage is consistent with current recommendation from the pharmacy at the Queen Silvia Children's Hospital for the use of levosimendan in the PICU. However, in some centers the initial bolus dose was excluded to avoid an eventual iatrogenic hypotension. In some other centers norepinephrine infusion was administrated simultaneous with the start of Levosimendan infusion. The exclusion of a bolus dose in some hospitals occurred when Levosimendan was first introduced and clinicians were more cautious, both in adults and children. Below follows a brief literature review on the use of levosimendan in pediatric cardiac surgery.

The first two case reports on the use of Levosimendan in infants with CHDs were published in 2004 [20, 21]. In the first report Levosimendan was used after cardiac surgery in a two months old infant with complicated postoperative course; acute left ventricle failure and pulmonary vascular hypertension [20]. The infant received 24 μg /kg Levosimendan as a bolus dose followed by an infusion of 0.2 μg /kg/minute for the next 48 hours. Immediately after administration of bolus dose a significant increase in left ventricle function could be demonstrated by echocardiography. In this case Levosimendan caused a breakthrough in the treatment and increasing the function of left ventricle decreased also the vascular resistance. The other case report [21] (in German) was a report on an infant with myocardial infarction due to congenital stenosis of the left coronary artery, left ventricular dysfunction, mitral regurgitation and refractory pulmonary hypertension The authors proposed the use of Levosimendan as a long-term inotropic agent and pulmonary vasodilator in children with depressed cardiac function.

In the same year, the first report on the pharmacokinetics of Levosimendan in children was published [22]. The children were between three months and seven years old and were being evaluated for cardiac surgery in a cardiac catheter laboratory. All children received 12 μg/kg Levosimendan as an intravenous infusion over 10 minutes during the catheterization. The authors of this small study concluded that the pharmacokinetic profile of Levosimendan in children with congenital heart disease was similar to that in adult patients with congenital heart diseases. In 2006, in a small retrospective observational safety study, it was demonstrated that Levosimendan did not worsen the clinical conditions, and the patients did not suffer from adverse events attributable to Levosimendan [23]. The number of patients in this study was nineteen; they were between seven days and ten years old, suffered from established low cardiac output syndrome after congenital heart surgery. The dose of Levosimendan was 12 μg/kg over 60 minutes followed by 0.1 μg/kg/minute for 24 hour. In another retrospective cohort study from the same year, fifteen pediatric patients with ventricular dysfunction were included and the hemodynamics after administration of Levosimendan was analyzed [24]. The patients (aged seven days to eighteen years) had different diagnosis and underwent different procedures. All patients received a bolus dose of Levosimendan (6-12 μg/kg over 10 minutes) followed by either a continuous infusion of 0.1 μg/kg/minute for 24 hours (in thirteen children) or 0.05 μg/kg/minute for 48 hours (in two children). The authors concluded that Levosimendan could safely be administered to infants and children with severe heart failure. In 2007 the first report on the successful use of Levosimendan in a premature infant with congestive heart failure following cardiac surgery was published [25]. The patient was a 32 weeks gestational old premature neonate, weighted 1525 grams and suffered from transposition of great arteries. The postoperative course became complex immediately after surgery. Levosimendan infusion was initiated by a dose of 0.05 μg/kg/minute which was increased to 0.1 μg/kg/minute. The infusion lasted for 24 hours. Within 6 hours after start of Levosimendan infusion a marked clinical improvement could be demonstrated. The authors concluded that Levosimendan was a potent inotropic agent in this patient. In 2009 Osthaus and his colleagues published a report on the intraoperative Levosimendan use in seven infants with body weight between 2.6 and 6.3 kg [26]. In four children Levosimendan infusion was started during weaning from CPB, while in the other three patients it was administered after the termination of CPB but before the chest closure. All patients received Levosimendan as a loading dose of 12 μg/kg over 10 minutes, followed by an infusion of 0.2 μg/kg/minute for 24 hours. The authors demonstrated that the intraoperatively administered Levosimendan was well tolerated in these infants with severe myocardial dysfunction after complex congenital heart surgery. In 2011, Momeni and her colleagues published a paper on comparing the hemodynamical and the

biochemical effects of Levosimendan and Milrinone when used as inotropic support after congenital heart surgery [27]. The study was prospective, randomized and double blinded. Forty-one patients between 0 and 5 years old were enrolled; thirty-six patients completed the study. The patients were randomized to a continuous infusion of either Levosimendan (0.05 μg/kg/minute) or Milrinone (0.4 μg/kg/minute). Epinephrine was started after aortic cross-clamp release in both groups (0.02 μg/kg/minute). The authors concluded that Levosimendan is at least as efficacious as Milrinone after corrective congenital heart surgery. In 2012, Lechner and her colleagues reported a study on the prophylactic use of Levosimendan versus Milrinone in neonates and infants after corrective heart surgery [28]. The study was prospective, randomized and double blind and included 40 infants younger than one year of age. At weaning from CPB, either a 24-hours infusion of 0.1 μg/kg/minute Levosimendan or 0.5 μg/kg/minute Milrinone were administered. The authors observed an increase in cardiac output and cardiac index over time in the Levosimendan group, whereas cardiac output and cardiac index remained stable in the Milrinone group.

The elimination half-life of Levosimendan is about 1 hour, but there is at least one metabolite that has prolonged effects of at least 80 hours. This may account for the observations of sustained benefit after discontinuation of the drug. Levosimendan can be infused simultaneously with any other medications. However, cautious should be taken in case of infusion of arrythmogenic drugs. It should be added that pharmacokinetic data on Levosimendan in children, infants, and neonates is scarce. It is preferable to gain a better understanding of Levosimendan pharmacokinetics in these groups of patients. In the current study we intend to analyze the blood samples in respect to the Levosimendan concentrations in addition to other analyses such as neurobiomarkers, complement system and NGAL.

1.5.4 Dosage and interactions with other medications

As earlier mentioned, Levosimendan is not registered for use in a paediatric population. In the lack of pediatric and neonatal data, the drug has been used with an adult dosage regimen with successful results, which has been presented above. The adult dosage of Levosimendan is 12 μg/kg loading dose followed by an infusion of 0.1-0.2 μg/kg/min. This is the also the recommendation dose of the pharmacy at Queen Silvias Children's Hospital for use of Levosimendan in PICU. The elimination half-life of Levosimendan is about 1 hour, but there is at least one metabolite that has prolonged effects of about 80 hours. This may account for the observations of sustained benefit after discontinuation of the drug. Levosimendan can be infused simultaneously with any other medications. However, cautious should be taken in case of infusion of arrythmogenic drugs.

1.6 Milrinone

Milrinone is a inotrope drug used in infants and children undergoing surgery for congenital heart disease and is indicated for the short-term treatment of postoperative severe heart failure who do not respond to conventional maintenance therapy. It s also indicated for short-term treatment (up to 35 hours) of pediatric patients with acute heart failure, including conditions of low cardiac output after cardiac surgery.

1.7 Research hypothesis

Can Levosimendan reduce the incidence of acute renal dysfunction after cardiac surgery in paediatric children with congenital heart disease?

1.8 Rationale for conducting this study

In this study we will compare the kidney protective ability of Levosimendan compared with Milrinone [29-31]. Levosimendan is an inotrope drug not registered for use in pediatric populations for any indication, however it is currently used as an inotrope in PICUs. Milrinone is another inotrope drug, which is registered for short-term use in pediatric populations . Milrinone is normally used in PICUs in conjunction with pediatric cardiac surgery. There is evidence that Levosimendan, in addition to its inotropic effect, preserves adequate renal perfusion and protects kidneys from injury. This should lead to a less increased creatinine postoperatively. Our aim is to assess the efficacy of Levosimendan in reducing creatinine postoperatively compared to Milrinone.

1.9 Benefit/risk assessment

Levosimendan is primarily indicated in adults for short term treatment of acutely de-compensated severe chronic heart failure in situations where conventional therapy is inadequate or insufficient. However, Levosimendan has also been used also in children and neonates in both Sweden and Europe in connection with complex congenital heart surgery.

Levosimendan, Milrinone, dopamine, epinephrine, dobutamine are all common drugs used in the prevention and treatment of low cardiac output syndrome in European children undergoing open heart surgery. Milrinone is used routinely in our clinic. It is a registered inotropic drug in the paediatric population.

Levosimendan and Milrinone has been considered well tolerated and has a potential benefit on postoperative hemodynamic and metabolic parameters. An initial hemodynamic adverse effect may be a reduction in systolic and diastolic blood pressure.

We estimate that we do not expose patients to any additional significant risk. Infusion of Levosimendan and Milrinone is indicated for use only in hospitals where adequate supervision and experience with inotropic agents are available. Solid routines are followed during surgery, the operation team are well trained in case of emergency and the patients are thoroughly monitored during the stay in the intensive care unit. Prophylactic measures are always taken, e.g. infusion of vasoactive medication for preventing hypotension and insertion of pace-maker leads for treatment of therapy resistant arrhythmias.

1.10 Ethical considerations

This study will be conducted in compliance with ICH Good Clinical Practice and applicable regulatory requirements and in accordance with the ethical principles in the Declaration of Helsinki. For detailed information regarding ethics and regulatory review, informed consent, data protection and audits and inspections see Appendix D (Ethics).

Written approval must be obtained from the regional ethics committees and regulatory authorities prior to start of the trial.

The investigator will ensure that both parents/guardians (legal representative (s) of the child) are given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the study and are notified that they/their child are free to discontinue from the study at any time. The parent/guardian will be given the opportunity to ask questions and allowed time to consider the information provided. Signed and dated informed consent should be obtained before conducting any study specific procedures and stored in the Study Master File. A copy of the signed Informed Consent Form is kept by the parents/guardians (legal representative (s) of the child).

2. STUDY OBJECTIVES AND ENDPOINT(S)

2.1 Primary objective

The aim of this study is to assess the efficacy of Levosimendan to reduce the postoperative acute kidney injury in pediatric patients undergoing surgery for congenital heart disease (CHDs) having creatinine as an endpoint marker.

2.2 Primary endpoint

AKI is usually defined as 50% increase in creatinine within 48 hours. Accordingly, the primary outcome in this study is the postoperative creatinine levels which will be measured according to table 1.

2.3 Secondary endpoints

The secondary endpoints are the hemodynamic parameters, fluid balance, the level of inflammation and other plasma kidney biomarkers (like cystatin C and NGAL), and markers of neural injury. Other parameters will also be assessed such as length of surgery, length of CPB, length of mechanical ventilation postoperatively, length of vasoactive therapy, number of vasoactive medications and total amount of infused vasoactive drugs.

3. STUDY DESIGN

This is a prospective, randomized, parallel-group, and double-blinded study in pediatric patients in need for surgery due to congenital heart disease. The study will be performed at the Department of anesthesia and intensive care unit at The Queen Silvia Children's Hospital, Göteborg, Sweden.

A total of 70 male and female pediatric patients will be included in the study and undergo heart surgery according to routine procedures at the hospital. Patients will be randomly assigned to receive either Levosimendan (test group) or Milrinone (control group). Creatinine will be measured daily according to the clinical routines. The length of treatment (Levosimendam or Milrinone) will be 24 hours. The treatment will be started during the operation at the same time-point at which Milrinone infusion routinely starts. Study-specific blood samples will be obtained at five occasions, during 24 hours perioperatively. Patients will be followed up as by routine as long as they are in PICU. Patients will be followed for an additional 4 days after treatment termination regardless if they are in ICU or they are being cared at in the ward. Final follow-up will occur 30 days after surgery. Overall study design and study activities are shown in Figures 1 and 2 and Table 1.

Figure 1 ***Study design***

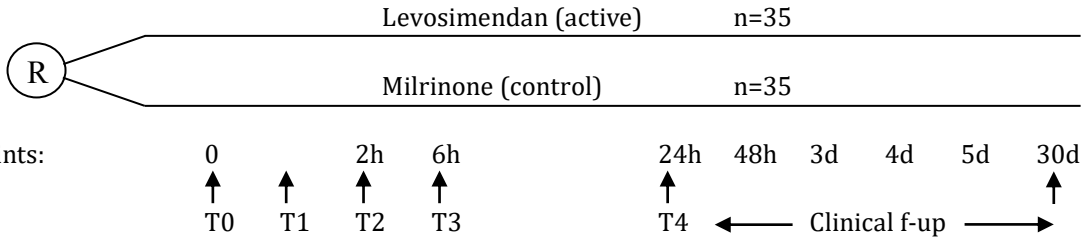
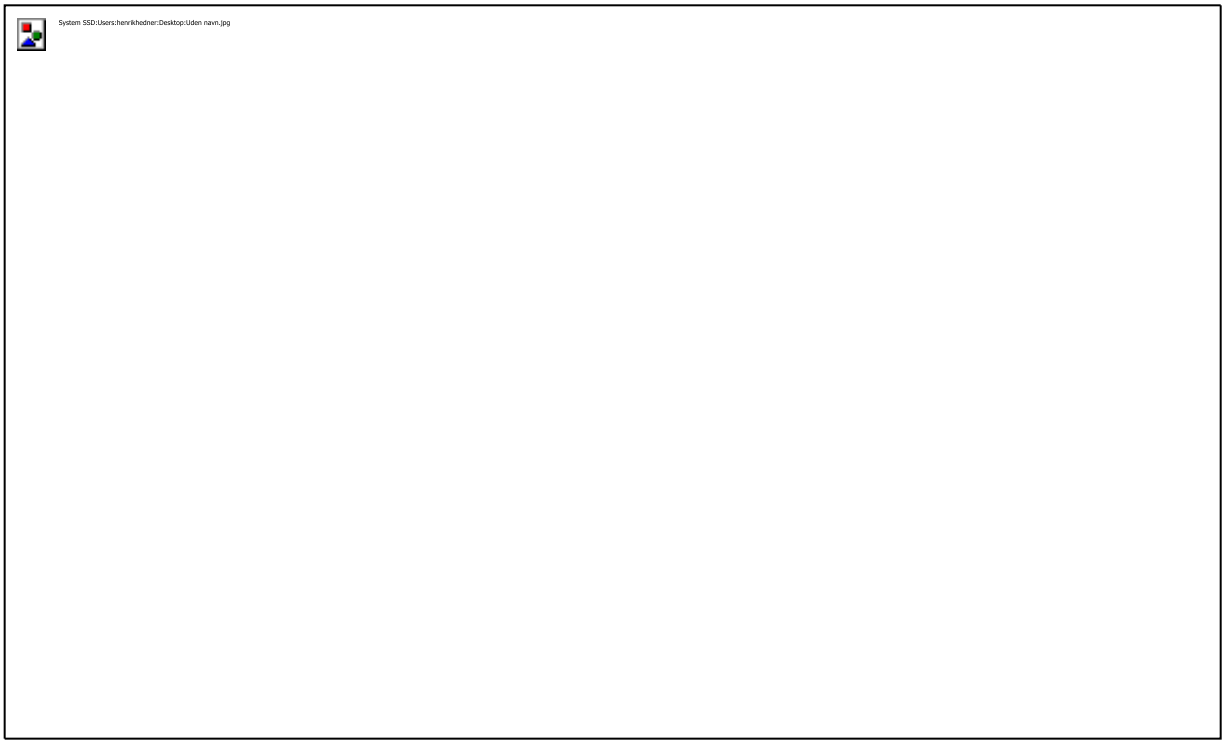


Table 1 Study Activities

	Pre-study	Surgery	Post-surgery				Follow-up		
		T0	T1	T2	T3	T4			
At:		Baseline		2 hours	6 hours	24 hours	48 hours	4, 5 days	30 days
Informed consent	X								
Demography	X								
Weight, height	X								
Medical history	X								
Physical examination	X								
Inclusion/exclusion criteria	X	X							
Randomization		X							
Surgery		X							
Levosimendan/Milrinone		X	X	X	X	X			
Pulse, Blood pressure		X	X	X	X	X	X	X	
Routine laboratory screening (minimum Creatinine, S-K)		X	X	X	X	X	X	X	
Pharmacokinetics					X	X	X	X	
Study specific blood		X	X	X	X	X			
Continuous ECG monitoring		X	X	X	X	X	X		
Continuous hemodynamic and respiratory monitoring		X	X	X	X	X			
Serious Adverse event recording		X	X	X	X	X			
Urine production		X	X	X	X	X		X	
Adverse Events registr*		X	X	X	X	X	X	X	X
Registration of mortality									X
Registration of length of stay (bed-days)									X
Routine echocardiography (preoperative, after CPB termination, first postoperative day, and at discharge)									

* Registration of adverse events will also be performed at day 30 (e.g. telephone contact with the parents if the patient is at home)

Figure 2 Study flow chart



The infusion of Levosimendan/Milrinone will start perioperatively when the patient is on cardiopulmonary bypass. The infusion will thereafter continue in the intensive care unit (ICU) and will be terminated after 24 hours. Patients in both groups will be followed up according the normal clinical routines. Routine echocardiography will be performed at pre-defined time-points as a clinical routine (pre-operatively, after termination of CPB intra-operatively, at first postoperative day, and at discharge). Extra study specific blood samples will be taken from the included patients. Patients who develop AKI will be identified by increase in s-Creatinine >50% from baseline. The usual clinical and laboratory data will be routinely obtained and recorded in the hospital electronic records. Study-specific parameters will be registered in a CRF.

Follow-up of the patients will continue 4 days after termination of Levosimendan /Milrinone infusion. The follow-up parameters, included in case report form (CRF),

include among others treatment-needing hypotension, treatment-needing arrhythmias, s-Creatinine, s-Potassium and albumin.

3.1.1 Stopping criteria for dose change

General stopping criteria are hypersensitivity reactions to the active substance or any recipients.

The children are thoroughly monitored during surgery and postoperatively as per routine at the clinic including continuous blood pressure, pulse, and ECG monitoring. It should be noted that the initiation of Levosimendan/Milrinone occurs during the cardiopulmonary bypass time where cardiopulmonary function parameters (like blood pressure) are optimized before the termination of cardiopulmonary bypass.

3.2 Rationale for study design, doses and control groups

Our department in Sahlgrenska University Hospital, the Department of anesthesiology and intensive care, includes both the adults and children sections. The adult section in our department has been proactive in producing evidence that Levosimendan may protect the kidney from injury in conjunction with cardiac surgery in adults [1]. This study was published in 2013. The overall rationale for the planned children study is the same as we reported in the adult study from 2013. The aim is to investigate whether the findings in adults are applicable in children, given the facts that the impact of cardiac surgery is the same in adults and children and the pharmacokinetics of the Levosimendan, despite scarce of data, are principally the same. In practice, the adult doses of Levosimendan are used in infants and children. Levosimendan in children is used as an inotropic drug, and to our knowledge it has not been used as a kidney protective agent in children yet.

Control group: Milrinone is the inotrope standard of care in this setting. According to Good Clinical Practice if there is a standard of care, it should be used instead for placebo. To our knowledge the kidney-related effects of Milrinone per se has not been investigated yet.

4. SUBJECT POPULATION

A total of 70 children between 1 and 12 months of age will be selected from the pediatric patients who undergo corrective cardiac surgery at the Queen Silvia´s children hospital in Göteborg, Sweden.

Subject population should be selected without bias. Investigator(s) must keep a record of all subjects even those who entered pre-trial screening but were never enrolled. Use a subject screening log. Each subject must meet all of the inclusion criteria and none of the exclusion criteria for this study.

CHDs include some twenty major diagnoses, with many anatomical combinations and physiologic variations as well as prognoses. The birth prevalence of CHD is about 9 per 1000 live births. With a couple of exceptions, the prevalence of any single diagnosis is also very low which influence the research in this group of patients due to limited number of patients treated in each center. In this study we have chosen a study population made of three diagnoses.

4.1 Inclusion criteria

For inclusion in the study subjects must fulfill the following criteria.

1. Provision of informed consent prior to any study specific procedures
2. Female and male children between 1 and 12 months of age
3. Non-restrictive VSD (corrective surgery)
4. Complete AVSD (biventricular repair)
5. Tetralogy of Fallot

4.2 Exclusion criteria

Subjects must not enter the study if any of the following exclusion criteria are fulfilled

1. Unbalanced AVSD
2. Age less than one month and more than one year
3. Acute operation that is unscheduled operation during the first 24 hours after presentation to the department for thoracic surgery
4. Mild, moderate, or severe kidney dysfunction
5. Liver impairment or disease
6. Ongoing infection

7. Use of nephrotoxic drugs (like ibuprofen, angiotensin-converting-enzyme inhibitors, gentamicin, vancomycin) preoperative or postoperative until first post operative day

8. Use of inhibitors of membrane transport proteins (Cimetedin, cetirizine, trimethoprim, probenecid, rifampin and gemfibrosil

9. Allergy to Levosimendan or substance included in the preparation or previous use of Levosimendan

10. Severe arrhythmias needing pace-maker treatment prior to the operation

11. Severe cardiac dysfunction needing for treatment with extracorporeal membrane oxygenation (ECMO) prior to the operation

12. Severe uncorrected hypotension where the initiation of anesthesia is postponed for its correction

13. Re-operation

4.3 Subject enrolment and randomization

The principal investigator will obtain signed informed consent from the potential subject i.e. parent/guardian (legal representative (s) of the child) before any study specific procedures are performed and determine subject eligibility.

Randomization codes will be assigned strictly sequentially as subjects are eligible for randomization according to a pre-defined treatment schedule. If a subject discontinues participation in the study, his/her enrolment/subject number cannot be reused. Discontinued subjects are not allowed to re-enter the study.

4.3.1 Safety and risk minimization in the scope of randomization

A nurse who is not directly involved in the patient care will perform the randomization (by a net-based randomization program) prior to the operation ("randomizer"). Two or three nurses will be trained in working with the randomization soft ware to cover the risk of the absence of randomizer. The randomizers will follow the rule of confidentiality regarding the result of randomization. The randomization result will be kept in a predefined locked safe at our PICU.

Children undergoing surgery for congenital heart diseases belong to different prognostic groups, and their outcomes vary depending on the specific prognostic group they belong

to [32]. The statistical methods of either stratified randomization or minimization should be used in these cases to achieve approximate balance of important characteristics in each group.

In clinical trials with larger sample size and need for several stratifying variables, stratified randomization would be appropriate while in the smaller studies needing several stratifying variables the statistical method called minimization is more appropriate [33, 34]. However, in smaller studies with only one or two stratification variables stratified randomization may be used. In the current study, the diagnosis and the age of the patients constitute known prognostic factors, which should be used as stratifying variables. Hence, we will use stratified randomization with the forehead mentioned stratifying factors for randomization of the patients.

4.4 Discontinuation and Withdrawal of subjects

The parent(s) are at any time free to discontinue his/her child's participation in the study, without prejudice to further treatment. The subjects may be withdrawn from the study at the discretion of the investigator due to safety concerns, adverse events or if judged non-compliant with study procedures. Other reasons for discontinuing a subject are incorrect enrolment and subjects lost to follow-up. Other reasons for discontinuing a subject are incorrect enrolment subjects lost to follow-up, new diagnosis, significant complications. In either case serious adverse events will be followed up.

4.5 Temporary or Premature termination of the study

The Investigator may decide to temporary or permanently stop the trial or part of the trial at any time in case of a substantial amendment or as a part of an urgent safety measure. Urgent safety measures may be taken without prior notification. If a trial is prematurely terminated or suspended, the Investigator should promptly inform the parent/guardian and ensure appropriate therapy and follow-up. Furthermore, the Investigator should promptly inform the Ethics committee and provide a detailed written explanation. The regulatory authority should be informed according to national regulations.

5. TREATMENTS

5.1 Test and control drugs

Levosimendan (test) and Milrinone (control) will be used in the present study.

Table 2 Identity of investigational products

Investigational product	Dosage form and strength	Manufacturer
Levosimendan (Simdax®)	2.5 mg/mL solution. Diluted to 0.05 mg/mL	Orion Corporation, Orionintie 1, FI-02200 Espoo, Finland
Milrinone Abcur	Solution for Injection 1 mg / ml	Abcur AB. Box 1452, 251 14 Helsingborg

5.1.1 Levosimendan

Levosimendan, a calcium-sensitizer (ATC-kod: C01CX08), has been developed for short-time treatment of de-compensated heart failure and is used intravenously when patients with heart failure require immediate initiation of drug therapy and when conventional therapy is insufficient. It increases cardiac contractility and induces vasodilatation.

The pharmacokinetics of Levosimendan is linear at the therapeutic dose range of 0.05-0.2 µg /kg/minute. The short half-life (about 1 hour) of the parent drug, Levosimendan, enables fast onset of drug action, although the effects are long-lasting. The maximum concentrations of metabolites (OR-1855 and OR-1896) are seen on average 2 days after stopping a 24-hour infusion. It is completely metabolised and negligible amounts of unchanged parent compound are excreted in urine and faeces.

Limited data suggest that the pharmacokinetics of levosimendan in children (3 months - 6 years of age) after a single dose is similar to that in adults.

There are no particular risks with short-term use regarding general toxicity and gene toxicity.

Limited data suggest that the pharmacokinetics of Levosimendan in children (3 months-6 years of age) after a single dose is similar to that in adults. The pharmacokinetics of the active metabolite has not been studied in children.

No pharmacokinetic interactions have been observed. However, cautious should be taken in case of all arrythmogenic drugs or those which decrease blood pressure like beta blockades.

5.1.2 Milrinone

Milrinone is a phosphodiesterase inhibitor (ATC-kod: C01CE02). It is a positive inotropic and vasodilating substance. Milrinone is indicated for short term treatment (up to 35 hours) of pediatric patients with acute heart failure, including permits low output after cardiac surgery.

In published studies, selected doses for infants and children were i.v. loading dose of 50-75 g/kg during 30 to 60 minutes or continuous i.v. infusion initiated on the basis of the hemodynamic response and the potential occurrence of adverse events between 0.25-0.75 micrograms/kg/min for a period of up to 35 hours.

Milrinone eliminats faster in children than in adults and neonates have significantly lower clearance than children. It has a mean terminal half-life of 2 to 4 hours in infants and children

Milrinone is slowly injected intravenously into a large vein to avoid local irritation.

5.2 Doses, preparations and treatment regimens

For further details see Appendix B.

5.2.1 Organization for preparation of the study drugs

An experienced specialized nurse in anesthesia or PICU will have the responsibility for preparation of the medications for the day. The candidate nurses will receive extra training regarding the course of the trial. The randomizer delivers the result of the randomization confidentially to the nurse responsible for the drug preparation. The drug preparation responsible nurse will not be involved in the care of that particular patient and will follow the rule of confidentiality regarding the result of randomization.

In our department, the department of anesthesiology and intensive care, there are “medication cards” for drugs used regularly. Each drug has its own card which is approved by the hospital pharmacy. These cards include an exact presentation of the drug as well as the exact process of preparing them. These cards are available in drug preparing rooms and used every day by nurses preparing the drugs. The cards are prepared for pediatric populations where the final concentration of them usually differs from that for adults. The potent drugs, like Milrinon and Levosimendan are prepared only in one standard concentration described in the medication card.

The study drugs will be prepared according to the existing medication cards and in accordance with usual clinical work. There will be neither a change in the final concentration neither of the drugs nor in the process of preparing them.

5.2.2 Preparing final concentrations

Both Levosimendan and Milrinone are diluted in glucose 50 mg/mL and prepared in 50 mL infusion syringes according to the respective medication card. The final concentration of Levosimendan will be 0.05 mg/mL and final concentration of Milrinone will be 0.2 mg/mL.

5.2.3 Infusion rates

With the final concentrations described above and for delivering a normal dose, Levosimendan is infused in a rate of 0.12 mL/kg/h (which delivers 0.1 µg/kg/min) and Milrinone is infused in a rate of 0.15 mL/kg/h (which delivers 0.5 µg/kg/min).

The recommended dose of Levosimendan is 0.1-0.2 µg/kg/min and the recommended dose of Milrinone is 0.3 – 1.0 µg/kg/min.

5.2.4 Dosage scheme in present study

In a double blind study like the present study, it is obvious that the drugs must be infused in one single infusion rate to protect the blindness of the study. In the present study, the infusion rate will be 0.12 mL/kg/h. With this infusion rate the dose delivered will thus be 0.1 µg/kg/min for Levosimendan and 0.4 µg/kg/min for Milrinone.

For delivering a bolus dose we will infuse the study drug at a rate of 1.44 mL/kg/h in 10 minutes (the pump will also be programmed for a maximal infusion volume of 0.24 mL/kg). This will deliver a bolus dose 12 µg/kg of Levosimendan, which is the recommended bolus dose for Levosimendan and 48 µg/kg of Milrinon (recomended bolus dose for Milrinone is 50 µg/kg).

5.2.5 Quality control of the medication preparation

According to the routines of our department drug preparations should always be performed according to the medication cards. The cards should be put on the preparation table and the described steps should be followed step by step. In addition there should be one additional nurse who checks the correctness of the drug preparation for each drug. In this study we will follow the routines of our department in all aspects. The second nurse, who quality controls the preparation of the medication, will not be

involved in the care of the patient and will follow the rule of confidentiality regarding the result of randomization.

5.2.6 Storage conditions

The product should be used immediate after solving but can be stored at 2-8°C for a maximum time of 24 hours.

All study drugs should be kept in a secure place under appropriate storage conditions as specified on the pack label and handled according to the manufacturer's instructions. Handling of Levosimendan/Mirinone will follow recommendations in FASS and the medication cards at the department.

After preparation of the study medication for 24 hours (the length of infusion), the syringes will be coupled to the infusion sets. The infusion sets will be filled with the content of the syringes so that they are ready to be coupled to the patient. A label with the word "Study" and "the inclusion number of the patient" will be paced on each syringe. The syringes and the sets are placed in a drug paper tray and stored in the drug refrigerator in PICU. The first syringe will be used for start of infusion during operation and cardiopulmonary bypass.

5.2.7 Blinding and unblinding procedures

Levosimendan and Milrinone will be prepared in brown syringes originally made for medications needing to be protected from daylight. The reason for this is that the color of prepared Levosimendan is yellow. From the scope of blindness, it is important that Levosimendan and Milrinone have the same appearance. The brown syringes and infusion sets will act well in this regard. For the same reason of blindness, that lumen of the central venous catheter, which will be used for infusion of the study medication, will be covered by surgical soft tape.

The treatment code must not be broken except in medical emergencies when the appropriate management of the subject necessitates knowledge of the treatment randomization.

5.2.8 Labelling

The syringes and study medication will be kept separately from other medications in the surgery ward. The study medication, syringes and related materials will be stored in a box labelled with: study code, i.v. Levosimendan/Milrinone, dosage form, strength, quantity, dosage instructions, medical cards with dosage instructions, name of

investigator (to be filled in at study site), expiry date, storage instruction, “for clinical study use only”, address and telephone number of sponsor/investigator.

5.3 Concomitant and post-study treatment(s)

Medication, which is used as standard therapy in cardiac surgery as well as other medications considered necessary for the subject’s safety and well-being, may be given at the discretion of the investigator.

5.4 Treatment compliance

As treatment will be administered in the hospital, at the intensive care unit, under the supervision of medical staff, compliance is expected to be 100%.

5.5 Accountability

The study drug provided for this study will be used only as directed in the study protocol.

An experienced nurse at the anesthesia and intensive care unit will be appointed as "study medication preparer” for the day and will be responsible for preparing the study medication according to the routines for medicine preparation at the department.

6. STUDY CONDUCT AND METHODS

6.1 Start and termination of study infusion and follow-up

The infusion of Levosimendan/Milrinone starts during CPB and will be continued in our PICU for 24 hours. After 24 hours the infusion rate will be decreased and set to the half infusion rate for 2 hours. If the patient does not tolerate this dose reduction, we will shift from test medication to Milrinone and start a Milrinone infusion. If the patient tolerates the dose reduction for 2 hours, then we will stop completely the infusion of the test medication. Two 2 hours after reducing the infusion rate by 50%, we will stop the infusion completely.

Both groups are followed up according to the normal clinical routines as long as they stay at PICU. Extra blood samples, in addition to those needed for usual clinical care, will be taken from the patients according the specified scheme presented in this document**.**

The trial follow-up of the patients will continue 4 days after termination of Levosimendan/Milirone infusion. CRF includes the clinical and laboratory follow-up

parameters as well as adverse events (like treatment needing hypotension and arrhythmias), adverse reactions and SUSARS (se section 7).

6.2 Efficacy variables

The usual clinical and laboratory data connected to this kind of heart surgery will be routinely obtained prior to surgery, during surgery, and during the care in PICU (see table 4).

6.2.1 Blood for study-specific analysis

Blood samples for the analyses of pharmacokintetics, inflammatory biomarkers like complement components, neurodegenerative biomarkers, NGAL, and other biological markers relevant for AKI will be obtained. Samples will be analyzed after termination of the study.

7. SAFETY

Detailed information regarding safety is seen in Appendix C.

7.1.1 Safety procedures

7.1.1.1 During surgery

Solid routines will be followed during surgery including ECG-monitoring, invasive monitoring of hemodynamics (blood pressure and pulse), respiratory monitoring, and monitoring of cardiopulmonary bypass parameters including blood gases. Hemostasis is monitored with advanced in-site monitoring devices, and blood and blood products as well as coagulation factors are administered if needed. The pediatric cardiac surgery operation theatre is a place for most advanced physiologic monitoring procedures. The cardiac surgery staff is among the most trained health care personnel in dealing with acute cardiac complications.

7.1.1.2 Post-surgery follow-up

The doctors, nurses, and other caring staff are well trained in emergencies related to post cardiac pediatric surgery. The patients are thoroughly monitored routinely during the stay in the PICU. Prophylactic measures are always taken, e.g. infusion of vasoactive medication for preventing hypotension and insertion of pace-maker leads for treatment of therapy resistant arrhythmias. Children will be carefully monitored during the treatment with Levosimendan/Milrinone (24 hours). They will be followed up by an

additional 48 hours after the termination of the treatment. Hypotension, arrhythmias, s-creatinine and albumin will be checked and corrected if needed.

7.1.2 Medical emergencies

The principal investigator(s) is responsible for ensuring that procedures and expertise are available to handle medical emergencies during the study. **A medical emergency usually constitutes an SAE and is to be reported as such.**

It is of the utmost importance that all staff involved in the study is familiar with the content of this section. The principal investigator is responsible for ensuring this.

7.1.3 Common AEs after CHD surgery

Cardiac adverse effects occur in a usual basis in intensive care units after cardiac surgery. Some prophylactic measures are always taken, i.e. infusion of vasoactive medication for preventing hypotension and insertion of pace-maker leads for treatment of therapy resistant arrhythmias (Table 3).

Table 3 Adverse events dependent on the disease or surgical treatment postoperatively

	Occurrence	Treatable	Usual Length of episodes	Prophylactic Measures	Watchfulness and Preparedness for initiation of treatment	Careful monitoring
Hypotension episodes	Usual	Yes	Minutes	Taken routinely	Present	Present
Arrhythmias episodes	Usual	Yes	Minutes to hours	Taken routinely	Present	Present

7.1.4 Adverse reactions of study medication

Data regarding adverse effects of Levosimendanmendan and Milrinone in a pediatric population are scare. The reported adverse reactions of Levosimendanmendan in adults are hypotension and arrhythmias (both ventricular and supra ventricular). Reported adverse reactons of Milrinone are headache, ventricular tachycardia, supraventricular arrhythmias and hypotension. The reactions are probable to occur in the present study and are treatable. Careful and adequate monitoring will be present

7.1.5 Laboratory safety assessment

Routine blood sample for laboratory safety screen will be taken at baseline and every morning in PICU as long as the patient is in PICU according to our clinical guidelines.

Table 4 Laboratory screen

Clinical Chemistry	**Haematology**
S/P-Creatinine	B-Haemoglobin (Hb)
S/P-Cystatin C	B-Platelet count (PPC)
S/P -Bilirubin, total, conjugated/unconjugated	B-Leukocytes, total count (LPC)
S/P -Glucose	B-Leukocytes diff.absolute count:
S/P -Potassium (K)	B-Basofila
S/P -Albumin	B-Eosinof
S/P -Sodium (Na)	B-Lymfocyter
S/P Reactive protein (CRP)	B-Monocyter
	B Neutrofila
	B-Hematocrit (Hct)

The blood samples will be analysed according to routine standards at the Department of Clinical Chemistry at the hospital. Valid reference values of all routine analyses will be obtained. Laboratory values outside the reference limit suspected to be of any clinical significance will be repeated.

7.1.6 Definition of adverse events (AE)

An adverse event is the development of an undesirable medical condition or the deterioration of a pre-existing medical condition following or during exposure to a pharmaceutical product, whether or not considered causally related to the product. An undesirable medical condition can be symptoms (eg, nausea, chest pain), signs (eg, tachycardia, enlarged liver) or the abnormal results of an investigation (eg, laboratory findings, electrocardiogram). In clinical studies, an AE can include an undesirable medical condition occurring at any time, including run-in or washout periods, even if no study treatment has been administered.

The term AE is used to include both serious and non-serious AEs

7.1.7 Definitions of serious adverse event (SAE)

A serious adverse event is an AE occurring during any study phase (i.e, run-in, treatment, wash-out, follow-up), that fulfils one or more of the following criteria:

- Results in death
- Is immediately life-threatening
- Requires in-patient hospitalisation or prolongation of existing hospitalisation
- Results in persistent or significant disability or incapacity
- Is a congenital abnormality or birth defect
- Is an important medical event that may jeopardise the subject or may require medical intervention to prevent one of the outcomes listed above.

For reporting purposes, any suspected transmission via a medicinal product of an infectious agent is also considered an SAE and is reported in an expedited manner. Any organism, virus or infectious particle (for example prion protein transmitting Transmissible Spongiform Encephalopathy), pathogenic or non-pathogenic, is considered an infectious agent.

For further guidance on the definition of a SAE, see Appendix B to the Clinical Study Protocol.

7.1.8 Recording of adverse events

Adverse Events will be collected from, randomization throughout the treatment period including the follow-up period that is five days after start of infusion of Milrinone/Levosimendan. In addition the adverse events will also be registered at day 30 (by telephone conversation with the parents if the patient is at home).

7.1.8.1 Variables

The following variables will be recorded in the CRF for each AE; description of the AE, the date and time when the AE started and stopped, maximum intensity, whether the AE is serious or not, causality rating (yes or no), action taken with regard to investigational product, AE caused subject to discontinue study and outcome.

7.1.8.2 Definitions for intensity rating

- mild (awareness of sign or symptom, but easily tolerated)
- moderate (discomfort sufficient to cause interference with normal activities)
- severe (incapacitating, with inability to perform normal activities)

It is important to distinguish between serious and severe AEs. Severity is a measure of intensity whereas seriousness is defined by the criteria in Section 7.1.7. An AE of severe intensity need not necessarily be considered serious. For example, nausea that persists for several hours may be considered severe nausea, but not a SAE. On the other hand, a stroke that results in only a limited degree of disability may be considered a mild stroke but would be a SAE.

The Investigator will assess causal relationship between Study medication and Adverse Events, and answer "yes" or "no" to the question "Do you consider that there is a reasonable possibility that the event may have been caused by the investigational product.

Causal relationship in cases where the disease under study has deteriorated due to lack of effect will be classified as no, reasonable or possibility.

For SAEs causal relationship will also be assessed for other medication and/or study procedure. Note that for SAEs that could be associated with any study procedure the causal relationship is implied as "yes".

7.1.9 Adverse Events based on examinations and tests

Deterioration as compared to baseline in protocol-mandated laboratory values, vital signs and other safety variables will only be reported as AEs if they fulfil any of the SAE criteria or are the reason for discontinuation of treatment with the investigational product. If deterioration in a laboratory value/vital sign is associated with clinical signs and symptoms, the sign/symptom will be reported as an AE and the associated laboratory result/vital sign will be considered as additional information. Any new or aggravated clinically relevant abnormal medical finding at a physical examination as compared with the baseline assessment will be reported as an AE.

Clinically relevant deterioration in non-protocol-mandated measurements will be reported as AE(s).

Wherever possible the reporting investigator uses the clinical, rather than the laboratory term (eg, anaemia versus low haemoglobin value).

7.1.10 Follow-up of unresolved adverse events

Any AEs that are unresolved at the patient's last AE assessment in the study are followed up by the investigator for as long as medically indicated, but without further recording in the CRF.

7.1.11 Reporting of Suspected unexpected serious adverse reaction (SUSAR)

Any serious adverse reaction that is suspected and unexpected will immediately be reported by sponsor to the EudraVigilance database and to the EC.

Fatal and life-threatening events must be reported within 7 calendar days after they have come to the investigator's knowledge. A detailed written report must be submitted after another 8 days. For other SUSARs 15 days apply.

For the current study, the responsible investigator will delegate registration of SUSARs into the EudraVigilance database to the MPA. The CIOMS form will be used and send electronically to registrator@mpa.se.

All SAEs have to be reported, whether or not considered causally related to the investigational product, or to the study procedure(s). All SAEs will be recorded in the CRF.

The investigators are responsible for informing the Ethics Committee and the Regulatory Authority of the SAE as per local requirements.

An annual safety statement including a summary of all SAEs and SUSARs must be submitted to the MPA and the EPN as long as the study is ongoing. The current reference safety information contained in the SPC in FASS applies.

7.1.12 Registering safety data in CRF

Adverse events will be registered from the time of patient randomization until the end of the follow up period. Registrations will be made in CRFs and in hospital medical journals according to information in this section regarding duration, severity and relationship.

7.1.13 Randomization code breaking

If an AE is assessed to be a SAE or SUSAR, the study code will be broken, the infusion of the study medicine will be terminated and prompt measures for reversing the course of the events will be taken until stability is reached. The patient will be followed up as long as medically indicated. The patient will be included in the intention-to-treat-analysis and described in the Clinical Study Report

7.1.14 Postoperative course after pediatric cardiac surgery is quite eventful

In the 1970s only a small fraction of children with congenital heart diseases could survive. The mortality rates have decreased markedly during the last two three decades, thanks to the continuous development of operation techniques and perioperative care measures. However, the nature of caring for these patients remains quite challenging. A quite many of these children experience an eventful and unstable postoperative course. This partly depends on the patient's diagnoses and partly on the undertaken corrective surgery. The diagnosis alone quite often affects the function of the heart, lungs, and other remote organs profoundly. The corrective surgery in many cases is extensive and demanding. The result is that the postoperative period in these patients is adherent to quite many adverse events. This demanding period craves a highly skilled staff to promptly reverse the AEs, to correctly recognize ARs , and to mange adequately SAEs, and SUSARs.

7.1.15 Evaluation and reversing of AEs

The evaluation of the AEs will be made on the clinical and scientific basis. All caring physicians and nurses are familiar with the measures usually taken in case of adverse events. The adverse events of Levosimendan do not differ from those usually encountered in our PICU. In case of hypotension fluid therapy will be instituted initially. If the patient does not respond to the fluid therapy and hypotension is evaluated as fluid-resistant then therapy with vasoactive medications (noradrenalin, adrenalin) will be instituted. A quite common scenario is that the patient is already receiving vasoactive medication or medications as a continuous infusion or infusions, in this case a dose adjustment of the vasoactive medication or medications will be undertaken. In case of arrhythmia fluid therapy and/or anti-arrhythmic therapy, depending on the clinical situation, will be started. There are established routines in our department for dealing with post cardiac surgery arrhythmia (e.g. fluid therapy, cooling down the patient, and treatment with Amiodarone or Metoprolol). If the arrhythmia is therapy resistant pacing of the heart with routinely pre-inserted pacemaker leads will be instituted. It is noteworthy that the heart is paced routinely postoperatively in many pediatric cardiac surgery patients. In summary, the occurrence of AEs is quite usual in the setting of a post thoracic surgery intensive care unit. In these units, like our PICU, there are clinical routines for prompt recognition, evaluation, and treatment of these AEs. The probable adverse effects of Levosimendan to be encountered in this trial will not differ from the other adverse effects usually encountered in our department in connection with the use of other potent cardiovascular medications and in conjunction with cardiac surgery. A list of AEs is included in the CRF with existing appropriate fields (not related, not likely,

possible, probable, and certain) for evaluating of each AE in respect to a probable causal relationship with Levosimendan.

8. BIOLOGICAL SAMPLING PROCEDURES

8.1 Volume of blood

The patients usually stay in the intensive care unit for 2-4 days depending on the postoperative course. Each day during the stay at the ICU, 10-15 mL blood for routine laboratory screen will be drawn. The amount of blood may increase to 25-30 mL in case of complicated postoperative conditions (disease/operation/post operative course).

In the present study extra blood will be drawn for the analysis of study-specific biomarkers e.g. inflammatory markers and the complement system, NGAL and markers of neuronal injuries.

The total volume of extra blood that will be drawn from each subject in this study is shown in Table 5.

Table 5 Volume of blood to be drawn from each subject

	Time point	**mL blood**
T0	Prior to CPB	2 mL
T1	At the end of CPB	2 mL
T2	2 hours after termination of CPB	2 mL
T3	6 hours after termination of CPB	2 mL
T4	24 hours after termination of CBP	2 mL
At day 5 or at discharge from hospital		*2 mL*
Total		12 mL

The total amount of blood taken extra from the included patients will be 10 mL in the operation day and the first postoperative day. The following table shows the proportion of the extra blood taken from an included patient compared with that amount of the blood needed for normal routine clinical use. The patient in this example has a weight of 3.5 kg and a blood volume of 90 mL/kg (total blood volume 3.5 x 90 = 315 mL). It should be noted that the majority of pediatric cardiac surgery patients receive either blood products or blood (or both), and the length of stay may be considerably longer. In addition to blood drawn according to table 1, at day 5 or at the discharge from hospital 2 mL blood will be drawn for among others the analysis of Levodsimendan metabolites (pharmacokinetics).

Table 6 Blood taken for lab screen and for study-specific analyses

Days in PICU	Routine blood samples *in* ***uncomplicated cases*** mL (% of total blood vol.)	Routine blood samples *in* ***complicated cases*** mL (% of total blood vol.)	Blood taken for **this study** mL (% of total blood vol.)
Day 1 (operation day)	15 (4.8 %)	30 (9.5%)	10 (3.2%)
Day 2	15 (4.8 %)	30 (9.5%)	-
Day 3	15 (4.8 %)	30 (9.5%)	-
Total after 3 PICU days	45 (14.4 %)	90 (28.5 %)	10 (3.2%)

8.2 Handling, storage and destruction of biological samples

The samples for routine analysis will be used up or disposed after analyses and are not applicable for Biobank.

The blood samples will be analysed after finalized study. A minor amount of the biological samples from each patient will be stored in Biobank at the hospital for a maximum of 15 years for future research. The results from future analysis will not be reported in the Clinical Study Report but separately in a Clinical Study Report Amendment and/or Scientific Report or Scientific Publication.

A full chain of custody is maintained for all samples throughout their lifecycle.

The principal investigator keeps full tractability of collected biological samples from the subjects while in storage at the centre.

The sample receiver keeps full tractability of the samples while in storage and during use until used or disposed.

9. STUDY AND DATA MANAGEMENT

9.1 Audits and inspections

A regulatory authority or the Ethics Committee may perform audits or inspections at the centre, including source data verification. The purpose of an audit or inspection is to systematically and independently examine all study-related activities and documents, to determine whether these activities were conducted, and data were recorded, analysed, and accurately reported according to the protocol, Good Clinical Practice (GCP), guidelines of the International Conference on Harmonisation (ICH), and any applicable regulatory requirements.

9.2 Recording of data

Paper case record forms (CRF), will be used to record all data. Data entry, editing and analyses will be done by the responsible investigators and study nurse(s). The investigators will ensure that all data collected in the study will be provided. He/she ensures the accuracy, completeness, legibility and timeliness of the data recorded in the appropriate sections of the CRF and according to any instructions provided.

Clean File/database lock must be documented. The reason for any excluded data or protocol deviations will be described in the study report. Any changes in the database after Clean File/database lock must be documented.

Data will thereafter be entered into an Excel datasheet and analysed with S.P.S.S.19 statistical software.

CRF documentation and other source data will be retained for at least 10 years after finalization of the clinical study.

9.2.1 Source data

All patient source data such as analysis results from the hospital laboratories and other measurements made e.g. ECG readings will be stored at the hospital according to routines.

The CRFs serves as the source for demographic data, medical history and physical examination and measurements as well as blood analysis.

9.3 Training of study site personnel

The principal investigator will ensure that appropriate training relevant to the study is given to all of these staff, and that any new information relevant to the performance of this study is forwarded to the staff involved.

The principal investigator will maintain a record of all individuals involved in the study (medical, nursing and other staff).

9.4 Monitoring of the study

An independent study nurse not directly involved in the study will be appointed for monitoring the study. The monitor will be appropriately trained and informed about the

nature of the study, patient written information, GCP and applicable regulatory requirements. Monitor's qualifications will be documented.

The monitor will verify informed consents of participating subjects, confirm that facilities remain acceptable, that the investigational team is adhering to the protocol that data are being accurately recorded in the Case Report Forms (CRF) and that therapy accountability is being carried out. The monitor will also ensure source data verification (comparison of the data in the CRF with the hospital/practice and other records at the investigational site).

10. STATISTICAL METHODS AND SAMPLE SIZE

10.1 Sample size calculation

The number of patients needed for the study is based on a 20% reduction in 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) will be blinded for the study. The same infusion rate and total volume of Levosimendan and Milrinone will be applied to both groups.

10.2 Statistical analysis

Analysis of data will be performed by a professional statistician. T-test for independent groups will be used for primary outcome. Descriptive statistics will be applied on the secondary outcomes including 30-days mortality, length of hospital stay and kidney function 5 days after start of infusion of Milrinone/Levosimendan. Additionally, clinical data (fluid balance, length of surgery, length of CPB, length of mechanical ventilation postoperatively, length of vasoactive therapy, number of vasoactive medications, total amount of infused vasoactive drugs, hemodynamic parameters) will be registered and analyzed. Univariate analyses will be used where needed. Multivariable regression analysis will be used to identify association between the occurrences of AKI (based on creatinine results) and the measured clinical variables.

Other statistical methods might be used in an exploratory fashion but no formal inference will be made.

Safety and tolerability data will be summarized descriptively by treatment and presented in tabular and/or graphical form. All adverse event data will be listed individually and summarized using MedDRA terminology.

11. STUDY TIMETABLE AND END OF STUDY

The study will be terminated when the follow-up of the last patient included is completed. The follow-up period for each patient included is 4 days after termination of Levosimendan/Milrinone infusion.

First Subject in: June 2014. Last Subject Last Visit: June 2016

Final Study Report: within 12 months from end of study.

12. LIST OF REFERENCES

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Appendix B - The preparation of the study drugs and the doses

13. PREPARATION OF THE STUDY DRUGS

13.1 Logistics

Logistically, it is not possible to use the hospital pharmacy for preparation dilution of the study medications. The hospital pharmacy does not offer such a services. Hence, we should do this by ourselves, using "Drug Cards" currently used in our department; Department of Pediatric Anesthesia and Intensive Care. The Drug Cards has been produced as a result of cooperation between the hospital pharmacy and the clinicians. In our department, the nurses prepare all the drugs we currently use. The nurses, who are either specialist in anesthesia or specialist in intensive care do this according to our strictly regulated routines.

13.2 Levosimendan and Milrinone preparations

Levosimendan and Milrinone, prepared according to the Drug Cards, have different final Concentrations; Levosimendan 0.05 mg/mL and Milrinone 0.2 mg/mL. Despite this, Levosimendan and Milrinone have fairly similar infusion rates to deliver a normal dose of the drugs to the patients; Levosimendan 0.12 mL / kg / hr and Milrinone 0,15 mL / kg / hr. These infusion rates supply the patient with 0.1 µg / kg / min of Levosimendan and 0.5 µg / kg / min of Milrinone

13.3 Infusion rate for a normal dose

Because the study is blinded, we must have the same infusion rate in each included patient. If we have an infusion rate of 0.12 mL / kg / hr, then we deliver 0.1 µg / kg / min of Levosimendan (normal dose) and 0.4 µg / kg / min of Milrinone. The normal dose of Milrinone is 0.3 - 1.0 µg / kg / min. In the clinic, we begin normally infuse Milrinone at a rate of 0.15 mL / kg / hr which corresponds a dose 0.5 µg / kg / min. **Hence, the infusion rate of the drugs in this study is 0.12 mL / kg / hr.** If the patient needs more inotropic support there are two options; delivering a bolus doses (or half-bolus dose) or doubling the dose continuously.

13.4 Bolus dose / half-bolus dose

For administration of bolus doses, we should use the same principle that is the volume of the bolus dose should be constant and this volume should deliver the actual bolus dose for both drugs. The bolus dose for Levosimendan is 12 µg/kg given in ten minutes. An infusion rate of 1.44 ml / kg / hr in ten minutes (a maximum volume 0.24 ml / kg) delivers this volume dose. In case of Milrinone, 1.44 ml / kg / hr in ten minutes (a maximum volume 0.24 ml / kg) delivers 48 µg / kg of the drug. The standard loading dose of Milrinone is 50 µg / kg. **Hence, the bolus dose in this study is administered as 1.44 ml / kg / hr in ten minutes (a maximum volume 0.24 ml / kg).** To deliver a half-bolus dose we infuse 0. 72 mL / kg / hr for 10 minutes (a maximum volume 0.12 ml / kg).

13.5 Doubling the dose continuously

If we double the infusion rate to 0.24 ml / kg / hr, this delivers 0.2 µg / kg / min of Levosimendan (maximum dose of Levosimendan) and 0.8 µg / kg / min of Milrinone. Maximum dose of Milrinone is 1.0 µg / kg / min.

13.6 The calculations

13.6.1 Infusion rate and the dose delivered

What dose do we deliver to the patient with a infusion rate of 0.12 mL/kg/hr?

Infusion rate	***Delivers***
0.15 mL/kg/hr	*0.5 µg/kg/min*
0.12 mL/kg/hr	*X = 0.12 x 0.5 / 0.15 = 0.4 µg/kg/min*

13.6.2 Bolus dose

13.6.2.1 Levosimendan

Is it correct that bolus infusion rate of 1.44 mL/kg/hr in ten minutes delivers 12 µg/kg of Levosimendan? The concentration of Levosimendan is 0.05 mg/mL.

mL	***minutes***	

1.44	60	
x	10	*X = 1.44 x 10 / 60 = 0.24 mL*

This means that in 10 minutes we deliver 0.24 mL of Levosimendan. How much active drug is in this volume?

mL	***mg***	
1	*0.05*	
0.24	*X*	*X = 0.05 x 0.24 / 1 = 0.012 mg = 12 µg*

Hence, it is correct, 1.44 mL/kg/hr in ten minutes delivers 12 µg/kg of Levosimendan.

13.6.2.2 Milrinone

How much active drug is delivered with the bolus infusion rate of 1.44 mL/kg/hr in ten minutes? The concentration of Milrinone is 0.2 mg/mL.

mL	***min***	
1.44	*60*	
X	*10*	*X = 1.44 x 10 / 60 = 0.24 mL*

mL	***mg***	
1	*0.2*	
0.24	*X*	*X = 0.2 x 0.24 / 1 = 0.048 mg = 48 µg*

Appendix C - Definitions of Adverse Events

Definitions:

Adverse Events

Serious Adverse Events

A medical emergency usually constitutes an SAE and is to be reported as such

Intensity rating

Causal relationship

Action taken

Reporting in CRF

Adverse Events based on signs and symptoms

Final outcome assessment

Reporting of serious adverse events

Further guidance on Serious Adverse Events:

Life threatening

Hospitalisation

Important medical event or medical intervention

A guide to interpreting the causality QUESTION

Other significant Adverse Events

Overdose

DEFINITIONS OF ADVERSE EVENTS AND PROCEDURES IN CASE OF PREGNANCY

It is of the utmost importance that all staff involved in the study is familiar with the content of this section. The Principal Investigator is responsible for ensuring this.

Adverse Events

An adverse event is the development of an undesirable medical condition or the deterioration of a pre-existing medical condition following or during exposure to a pharmaceutical product, whether or not considered causally related to the product. An undesirable medical condition can be symptoms (eg, nausea, chest pain), signs (eg, tachycardia, enlarged liver) or the abnormal results of an investigation (eg, laboratory findings, electrocardiogram). In clinical studies, an AE can include an undesirable medical condition occurring at any time, including run-in or washout periods, even if no study treatment has been administered.

The term AE is used to include both serious and non-serious AEs

Serious Adverse Events and suspected unexpected serious adverse reactions (SUSAR)

It is important to distinguish between Serious Adverse Events (SAEs) and severe adverse events (AEs). Severity is a measure of intensity whereas seriousness is defined by the criteria listed below. An AE of severe intensity need not necessarily be considered serious. For example, nausea which persists for several hours may be considered severe nausea, but not an SAE. On the other hand, a stroke which results in only a limited degree of disability may be considered a mild stroke but would be a SAE.

A medical emergency usually constitutes an SAE and is to be reported as such

A serious adverse event is an AE occurring during any study phase (i.e. run-in, pre-entry, screening, treatment, wash-out, follow-up), and at any dose of the investigational product, comparator or placebo, that fulfils one or more of the following criteria:

- results in death
- is immediately life-threatening
- requires in-patient hospitalisation or prolongation of existing hospitalisation
- results in persistent or significant disability or incapacity
- is a congenital abnormality or birth defect

- is an important medical event that may jeopardise the subject or may require medical intervention to prevent one of the outcomes listed above.

For reporting purposes, any suspected transmission via a medicinal product of an infectious agent is also considered an SAE and is reported in an expedited manner. Any organism, virus or infectious particle (for example prion protein transmitting Transmissible Spongiform Encephalopathy), pathogenic or non-pathogenic, is considered an infectious agent.

Definitions for severity rating

0. None – No symptoms

1. Mild - Transient symptoms; awareness of sign or symptom, but easily tolerated. No interference with the subject's daily activities

2. Moderate - Marked symptoms; discomfort sufficient to cause interference with normal activities. Moderate interference with the subject's daily activities

3. Severe - Considerable interference with the subject's daily activities; unacceptable, incapacitating. Inability to perform normal activities

N/A Not Applicable

Causal relationship

The causality of (S)AEs (ie, their relationship to study treatment and/or the investigational procedure) will be assessed by the investigator(s), who in completing the relevant case report form must answer "yes" or "no" to the question "Do you consider that there is a reasonable possibility that the event may have been caused by the drug/the investigational procedure?"

The following terms and definitions are used when assessing the causal relationship between each AE and the relevant trial product(s):

1. Definite - There is no doubt that the incident is related
2. Probable - Good reason and sufficient documentation to assume a causal relationship
3. Possible- A causal relationship is conceivable and cannot be dismissed
4. Unlikely - The event is most likely related to aetiology other than the trial product
5. Not related – The event is not related to the trail product
6. Unknown/Unclassifiable: a report suggesting an adverse event reaction, which cannot be judged because information is insufficient or contradictory and which cannot be supplemented or verified.

N/A Not Applicable

Causal relationship in cases where the disease under study has deteriorated due to lack of effect will be classified as no reasonable possibility.
For SAEs causal relationship will also be assessed for additional study drug and/or other medication and/or study procedure. Note that for SAEs that could be associated with any study procedure the causal relationship is implied as "yes".

Action taken:

0 None
1 Dose of study drug changed
2 Study drug temporarily stopped
3 Study drug stopped
N/A Not Applicable

Reporting in the Case report Form

The following variables will be recorded in the CRF for each AE; description of the AE, the date and time when the AE started and stopped, maximum intensity, whether the AE is serious or not, causality rating (yes or no; if yes specify), action taken with regard to investigational product, AE caused subject to discontinue study and outcome.

Adverse Events based on signs and symptoms

All AEs spontaneously reported by the subject or reported in response to the open question from the study personnel: *"Have you had any health problems since the previous visit?"*, or revealed by observation will be collected and recorded in the CRF. When collecting AEs the recording of diagnoses is preferred (when possible) to recording a list of signs and symptoms. However, if a diagnosis is known and there are other signs or symptoms that are not generally part of the diagnosis, the diagnosis and each sign or symptom will be recorded separately.

Follow-up – Outcome assessment

Any AEs that are unresolved at the patient's last AE assessment in the study are followed up by the investigator for as long as medically indicated, but without further recording in the CRF.

The following terms and definitions are used in assessing the final outcome of an AE:

- Recovered - The subject has fully recovered, or by medical or surgical treatment the condition has returned to the level observed at the first trial-related activity after the subject signed the informed consent.

- Recovering - This term is only applicable if the subject has completed the trial or has died from another AE. The condition is improving and the subject is expected to recover from the event.

- Recovered with sequelae - The subject has recovered from the condition, but with lasting effect due to a disease, injury, treatment or procedure. If a sequela meets an SAE criterion, the AE must be reported as an SAE.

- Not recovered - The condition of the subject has not improved and the symptoms are unchanged, or the outcome is not known at the time of reporting.

- Fatal - This term is only applicable if the subject died from a condition related to the reported AE. Outcomes of other reported AEs in a subject before he/she died should be assessed as “recovered”, “recovering”, “recovered with sequelae” or “not recovered”. An AE with fatal outcome must be reported as an SAE.

- Unknown - This term is only applicable if the subject is lost to follow-up

Reporting of serious adverse events

For studies in countries implementing the EU Clinical Trials Directive, informing Ethics Committees and Regulatory Authorities will be performed by the sponsor.

All Suspected Unexpected Serious Adverse Reactions (SUSARs) have to be reported, whether or not considered causally related to the investigational product, or to the study procedure(s). The Clinical Study Serious Adverse Event Report Form will be used together with other relevant supporting documentation (e.g. ECG, laboratory results, autopsy report) and relevant CRF modules. All SUSARs have to be electronically registered in the EMEAs database.

FURTHER GUIDELINES ON THE DEFINITION OF A SERIOUS ADVERSE EVENT

Life threatening

‘Life-threatening’ means that the subject was at immediate risk of death from the AE as it occurred or it is suspected that use or continued use of the product would result in the subject’s death. ‘Life-threatening’ does not mean that had an AE occurred in a more severe form it might have caused death (eg, hepatitis that resolved without hepatic failure).

Hospitalisation

Out-patient treatment in an emergency room is not in itself a serious AE, although the reasons for it may be (eg, bronchospasm, laryngeal oedema). Hospital admissions and/or surgical operations planned before or during a study are not considered AEs if the illness or disease existed before the subject was enrolled in the study, provided that it did not deteriorate in an unexpected way during the study.

Important medical event or medical intervention

Medical and scientific judgement should be exercised in deciding whether a case is serious in situations where important medical events may not be immediately life-threatening or result in death, hospitalisation, disability or incapacity but may jeopardize the subject or may require medical intervention to prevent one or more outcomes listed in the definition of serious. These should usually be considered as serious.

Examples of such events are:

- Angioedema not severe enough to require intubation but requiring iv hydrocortisone treatment

- Hepatotoxicity caused by paracetamol (acetaminophen) overdose requiring treatment with N-acetylcysteine

- Intensive treatment in an emergency room or at home for allergic bronchospasm

- Blood dyscrasias (eg, neutropenia or anaemia requiring blood transfusion, etc) or convulsions that do not result in hospitalisation

- Development of drug dependency or drug abuse

A guide to interpreting the causality QUESTION
The following factors should be considered when deciding if there is a "reasonable possibility" that an AE may have been caused by the drug.

- Time Course. Exposure to suspect drug. Has the subject actually received the suspect drug? Did the AE occur in a reasonable temporal relationship to the administration of the suspect drug?

- Consistency with known drug profile. Was the AE consistent with the previous knowledge of the suspect drug (pharmacology and toxicology) or drugs of the same pharmacological class? OR could the AE be anticipated from its pharmacological properties?

- Dechallenge experience. Did the AE resolve or improve on stopping or reducing the dose of the suspect drug?

- No alternative cause. The AE cannot be reasonably explained by aetiology such as the underlying disease, other drugs, other host or environmental factors.

- Rechallenge experience. Did the AE reoccur if the suspected drug was reintroduced after having been stopped? A re-challenge would not normally be recommended or supported.

- Laboratory tests. A specific laboratory investigation (if performed) has confirmed the relationship?

A "reasonable possibility" could be considered to exist for an AE where one or more of these factors exist.

In contrast, there would not be a "reasonable possibility" of causality if none of the above criteria apply or where there is evidence of exposure and a reasonable time course but any dechallenge (if performed) is negative or ambiguous or there is another more likely cause of the AE.

In difficult cases, other factors could be considered such as:

- Is this a recognised feature of overdose of the drug?
- Is there a known mechanism?

Ambiguous cases should be considered as being a "reasonable possibility" of a causal relationship unless further evidence becomes available to refute this. Causal relationship in cases where the disease under study has deteriorated due to lack of effect should be classified as no reasonable possibility.

Other significant Adverse Events

An expert will identify other significant Adverse Events (OAEs) during the evaluation of safety data for the Clinical Study Report. Significant adverse events of particular clinical importance, other than SAEs and those AEs leading to discontinuation of the subject from study treatment, will be classified as OAEs. Examples of these are marked haematological and other laboratory abnormalities, and certain events that lead to intervention (other than those already classified as serious), dose reduction or significant additional treatment. For each OAE, a narrative will be written and included in the Clinical Study Report.

Overdose

- An overdose with associated AEs is recorded as the AE diagnosis/symptoms on the relevant AE modules in the CRF.

Appendix D- Ethics and regulatory review

The final study protocol, including the final version of the Written Informed Consent Form and other information given to subjects eg, advertisements must be approved or given a favourable opinion by an Ethics Committee before enrolment of any subject into the study.

Before enrolment of any subject into the study, the final study protocol, including the final version of the Informed Consent Form, is approved by the national regulatory authority or a notification to the national regulatory authority is done, according to local regulations.

It is the responsibility of the Principal Investigator to apply to the Ethics Committee in writing. The application document should:

- contain the name and address of the Ethics Committee
- clearly identify, by title and date, the protocol and other documents submitted for review
- be dated.

In addition, the Principal Investigator should request the Ethics Committee to provide:

- their approval/opinion in a dated document identifying the Principal Investigator's application
- Ethics Committee composition for the meeting when the approval was given
- a statement confirming that the Ethics Committee is organised and operates according to GCP and applicable laws and regulations.

The Principal Investigator is responsible for informing the Ethics Committee of any modifications and amendments to the protocol as per local requirements.

Progress reports and notifications of serious and unexpected adverse drug reactions will be provided to the Ethics Committee according to local regulations and guidelines

All correspondence with the Ethics Committee should be filed by the Principal Investigator in the ISF.

The Principle Investigator is also responsible for obtaining approvals from scientific bodies (eg, to use radiolabelled substances) if necessary for the study.

SUBJECT INFORMATION AND WRITTEN INFORMED CONSENT FORM

The Principal Investigator(s) at each centre will ensure that the subject is given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the study. Subjects must also be notified that they are free to discontinue from the study at any time. The subject should be given the opportunity to ask questions and allowed time to consider the information provided.

The subject's signed and dated informed consent must be obtained before conducting any procedure specifically for the study.

The Principal Investigator(s) must store the original, signed Informed Consent Form in the Investigator's Study File. A copy of the signed Informed Consent Form must be given to the subject.

If a protocol amendment requires a change to the Informed Consent Form, the Ethics Committee must approve modifications that lead to a revised Informed Consent Form before the revised form is used.

SUBJECT DATA PROTECTION

The Informed Consent Form will incorporate (or, in some cases, be accompanied by a separate document incorporating) wording that complies with relevant data protection and privacy legislation.

Extra precautions are taken to preserve confidentiality and prevent genetic or other study data being linked to the identity of the subject. In exceptional circumstances, however, certain individuals might see both the genetic, other study data and the personal identifiers of a subject. For example, in the case of a medical emergency or an investigator might know a subject's identity and also have access to his or her data. Also Regulatory authorities may require access to the relevant files, though the subject's medical information and the genetic files would remain physically separate

AUDITS AND INSPECTIONS

Authorized representatives of Sponsor, a regulatory authority, or an Ethics Committee may perform audits or inspections at the centre, including source data verification. The purpose of an audit or inspection is to systematically and independently examine all study-related activities and documents, to determine whether these activities were conducted, and data were recorded, analysed, and accurately reported according to the protocol, Good Clinical Practice

(GCP), guidelines of the International Conference on Harmonisation (ICH), and any applicable regulatory requirements.

Study Protocol Amendment No 1

EudraCT No. 2013-003105-25

Study Code: MiLe-1

Date: 2014-07-15

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

Investigator:

Doc. Albert Castellheim
Sahlgrenska University Hospital
The Queen Silvia Children's Hospital AnOpIVA
416 85 Gothenburg

Study Site: The Queen Silvia Children's Hospital AnOpIVA

Section of protocol to be amended:

Table 1 in Section 3 of the Study Protocol

Previous text	Revised text
No previous text	New activities in Table 1: Pharmacokinetics 6 hours, 24 hours, 48 hours and 4,5 days Routine echocardiography (pre-op, after CPB termination, first post-op day and at discharge) and 30 days post-op

Reason for Amendment:

Pharmacokinetics at 4/5 days: For a more complete analyse of the time course of drug absorption, distribution, metabolism and excretion.

Echocardiography at day 30: For extended safety surveillance

There will be no negative consequences for the patients rather opposite, it will increase patients' safety and give a better interpretation of the PK

Actions to be taken:

Table 1 in Study Protocol will be revised

Signed agreement to the Amendment:

I agree to the terms of this Protocol Amendment.

Study Code: MiLe-1

2014-07-15	Albert Castellheim
Date (day month year)	Principal investigator

Study Protocol Amendment No 2

EudraCT No. 2013-003105-25

Study Code: MiLe-1

Date: 2014-09-05

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

Investigator:

Doc. Albert Castellheim
Sahlgrenska University Hospital
The Queen Silvia Children's Hospital AnOpIVA
416 85 Gothenburg

Study Site: The Queen Silvia Children's Hospital AnOpIVA

Section of protocol to be amended:

Exclusion criteria in Protocol Synopsis and in Section 4-2 of the Study Protocol

Previous text	Revised text
Use of nephrotoxic drugs (like ibuprofen, angiotensin-converting-enzyme inhibitors, gentamicin, vancomycin) preoperative or postoperative until first post operative day	7. Use of nephrotoxic drugs (like ibuprofen, gentamicin, vancomycin) preoperative or postoperative until first post operative day

Reason for Amendment:

We have included the Ross score in to the protocol after an agreement with our pediatric cardiologist. Ross score grades the patients according to the severity of their heart failure.

According the same agreement with pediatric cardiologist peers there will be no negative consequences for the patients rather the opposite; the study will include those patients who receive angiotensin-converting-enzyme inhibitors for their heart failure.

Actions to be taken:

Change of the exclusion criteria text

Signed agreement to the Amendment:

I agree to the terms of this Protocol Amendment.

Study Code: MiLe-1

2014.09.05	Albert Castellheim
Date (day month year)	Principal investigator

Study Protocol Amendment No 3

EudraCT No. 2013-003105-25

Study Code: MiLe-1

Date: 2014-09-09

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

Investigator:

Doc. Albert Castellheim
Sahlgrenska University Hospital
The Queen Silvia Children's Hospital AnOpIVA
416 85 Gothenburg

Study Site: The Queen Silvia Children's Hospital AnOpIVA

Section of protocol to be amended:

At the end of the Study Protocol

Previous text	Revised text
No previous text	Research assistant: Maria Thorson

Reason for Amendment:

The study logistics necessitates involving of a research assistant.

Actions to be taken:

Adding the revised text to the section Collaborators in the Study Protocol.

Signed agreement to the Amendment:

I agree to the terms of this Protocol Amendment.

Study Code: MiLe-1

Study Protocol Amendment No 3

EudraCT No. 2013-003105-25

Study Code: MiLe-1

Date: 2014-09-01

2014.09.09	Albert Castellheim
Date (day month year)	Principal investigator

Study Protocol Amendment No 4

EudraCT No. 2013-003105-25

Study Code: MiLe-1

Date: 2017.02.22

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

Investigator:

Albert Castellheim; associate professor
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The Queen Silvia Children's Hospital AnOpIVA
416 85 Gothenburg

Study Site: The Queen Silvia Children's Hospital AnOpIVA

Amendment: Definition of adverse events (AE) in MiLe-1

Reason for Amendment:

Lack of definition of AEs, in contrast to SAEs which were well-defined.

Actions to be taken:

AEs, according to following criteria, for all included patients will separately be registered.

AEs Criteria:

1. Heart rate > 200/min at these time points: 2, 6, 10-12 hours after CPB weaning, first postop morning at 6 o'clock and 24 hours after CPB weaning [1]

2. Arrythmias (rhythm other than sinusrythm). Same time points as in no 1 [2,3]

3. Inotropic score > 20 at the same time points as in no 1 [4]

4. Thrombocytes < 50.000 on the first postoperative morning [3]

5. Hypokalemia < 3.5 at the same time points as in no 1 [2]

References:

1) Advanced Paediatric Life Support (APLS)

2) Pharmaceutical Specialities in Sweden (Farmaceutiska Specialiteter i Sverige: Fass)

3) Hoffman TM. Cirkulation 2003;107:996-1002

4) Gaies MG. Pediatr Crit Care Med 2010; 234-238

Signed agreement to the Amendment:

Study Code: MiLe-1

Principal investigator: Albert Castellheim

Date: 2017.02.22