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Therapeutic Atrial Natriuretic Peptide infusion in Acute Kidney Injury after surgery for Pediatric Congenital Heart Disease

Master Thesis in Medicine

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ABSTRACT

Background

Acute kidney injury (AKI) is a common complication after Cardio Pulmonary Bypass (CPB) Surgery in the pediatric population. Diuretics are used worldwide to treat this condition. Human Atrial Natriuretic Peptide (hANP) is a diuretic that has been used to treat acute kidney injury at Queen Silvia Children's Hospital (DSBUS) for a decade. Despite this, no previous studies have been done on the effects of hANP among the pediatric AKI patients.

Aim

The aim is to evaluate the effects of hANP in the pediatric AKI population, with ambition to identify whether hANP treatment is associated with improved outcomes or not.

Methods

This is a retrospective cohort study on pediatric patients undergoing CPB surgery at DSBUS from January 1st 2010 through December 31st 2013. Two study groups (hANP and no-hANP) were used. The data was extracted from the patients' journals. Odds Ratio (OR) to assess the risk for dialysis was calculated using binary logistic regression. Non-parametric tests were used to calculate differences between median values regarding Length of Stay in the Pediatric Intensive Care Unit (PICU LOS), CPB duration and time to dialysis initiation.

Results

A total of 75 patients were included (hANP, n=45, no-hANP, n=30). No significant differences could be seen between the groups regarding CPB duration, incidence of dialysis or time to dialysis. However, the median PICU LOS were 3 days longer in the hANP group (7 days vs. 4

days, p=0.043) and for every ten minutes on the CPB machine, a 13 % increased risk for dialysis-dependent AKI (p=0.017) was seen, regardless of hANP administration.

Conclusions

Longer time in CPB surgery is associated with an increased risk for dialysis-dependent AKI. Because of the limited options for selection of population, the risk of selection bias is high. Hence, any conclusions based on this study should be resulting in an understanding that further studies are needed on this topic.

Key Words

Acute kidney injury, Cardio Pulmonary Bypass, human Atrial Natriuretic Peptide, Dialysis, Pediatric Congenital Heart Disease

ABBREVIATIONS

ACE	Angiotensin Converting Enzyme
ADH	Anti Diuretic Hormone
AKI	Acute Kidney Injury
AKIN	Acute Kidney Injury Network
ANP	Atrial Natriuretic Peptide
ARDS	Acute Respiratory Distress Syndrome
ATN	Acute Tubular Necrosis
BUN	Blood Urea Nitrogen
CKD	Chronic Kidney Disease
COX	Cyclooxygenase
CPB	Cardio Pulmonary Bypass
DSBUS	Queen Silvia Children's Hospital
EPO	Erythropoietin
FiO_2	Flow Index of Oxygen
FO	Fluid Overload
GFR	Glomerular Filtration Rate
hANP	human Atrial Natriuretic Peptide
KDIGO	Kidney Disease: Improving Global Outcomes
LOS	Length of Stay
MAP	Mean Arterial Pressure
MOF	Multi Organ Failure
NO	Nitrogen Oxide
NSAID	Non-Steroid Anti Inflammatory Drugs

PASK	Pre-ANP Creatinine
PD	Peritoneal Dialysis
PICU	pediatric Intensive Care Unit
PIP	Positive Inspiratory Pressure
POD	Post-Operative Day
RAAS	Renin-Angiotensin-Aldosterone System
RIFLE	Risk, Injury, Failure, Loss and End-Stage Renal Disease
sCr	Serum Creatinine
TICU	Thoracic Intensive Care Unit

BACKGROUND

ANATOMY OF THE KIDNEY

Location

The kidneys are located retroperitoneal in the abdomen, ranging from the 12th thoracic vertebrae to the 3rd lumbar vertebrae, and are surrounded by protecting layers. These layers are the pararenal fat, the renal fascia (Gerota's fascia) and closest to the kidney, the perirenal fat. [1].

Blood Supply

The blood supply of the kidney arises from the paired renal arteries, which originates from the abdominal aorta, and drain into the renal veins[1]. After entering the kidney, each renal artery branches into the afferent arteriole, which thereafter divides into the glomerular capillaries. The glomerular capillaries run inside of the Bowman's capsule, where most of the filtration takes place to form the primary urine. The glomerular capillaries exit Bowman's capsule to turn into the efferent arteriole (figure 1). The efferent arteriole thereafter becomes the peritubular capillaries, which surrounds the renal tubules. This is where the renal arterial system and the renal venous system are connected[2].

The renal veins and arteries as well as the lymphatic vessels, nervous supply and the ureter runs through the renal hilum.

Parenchyma and Calyxes

The parenchyma of the kidney is divided into the outer, renal cortex and the inner, renal medulla. Based on the appearance of the renal medulla, the parenchyma is divided into 8-10 pyramids. Roughly the tubular system can be divided into three parts; the proximal tubule,

the loop of Henle and the distal tubule. Ultimately all distal tubules from one pyramid merge together becoming the collecting duct. These pyramids drain into the minor calyxes via the renal papilla. The minor calyxes merges to create the major calyxes, which are connected to the renal pelvis and thereby the ureter [1].

The Functional Unit – The Nephron

Each kidney has approximately 1 million nephrons [3]. Nephrons are the urine-producing structure of the kidney. There are both cortical and juxtamedullary nephrons, which are named after the location of the renal corpuscle and the length of the loop of Henle.

The cortical nephrons have their glomeruli in the outer cortex and a short Loop of Henle, which only touches the renal medulla, whereas the juxtamedullary nephron's glomeruli are located in the cortex as well, but closer to the renal medulla[3]. The juxtamedullary nephrons have longer Loop of Henle, which penetrates the deeper parts of the renal medulla. In the juxtamedullary nephrons, the previously described arterial system of the nephrons does not entirely apply. In this case, the efferent arteriole extends along the loop of Henle. When reaching the outer medulla, it divides into specialized peritubular capillaries, the so-called Vasa Recta, which then empties into the renal vein.

Basically, the nephron can be subdivided into the renal corpuscle and the renal tubule. The corpuscle consists of the afferent arteriole from the renal artery, becoming a capillary network inside of Bowman's capsule, exiting as the efferent arteriole. This complex structure is called a glomerulus or the renal corpuscle. The tubular system is originating from the Bowman's capsule[1]. As mentioned, there are three parts to the tubular system. The proximal and the distal tubules are located in the renal cortex. Meanwhile the loop of Henle, divided into a descending and an ascending part, runs through the renal medulla [2].

In the distal tubule, there is an area of specialized cells, Macula Densa. Macula Densa is a part of the juxtaglomerular complex, which control renal blood flow and glomerular filtration rate (GFR)[3].

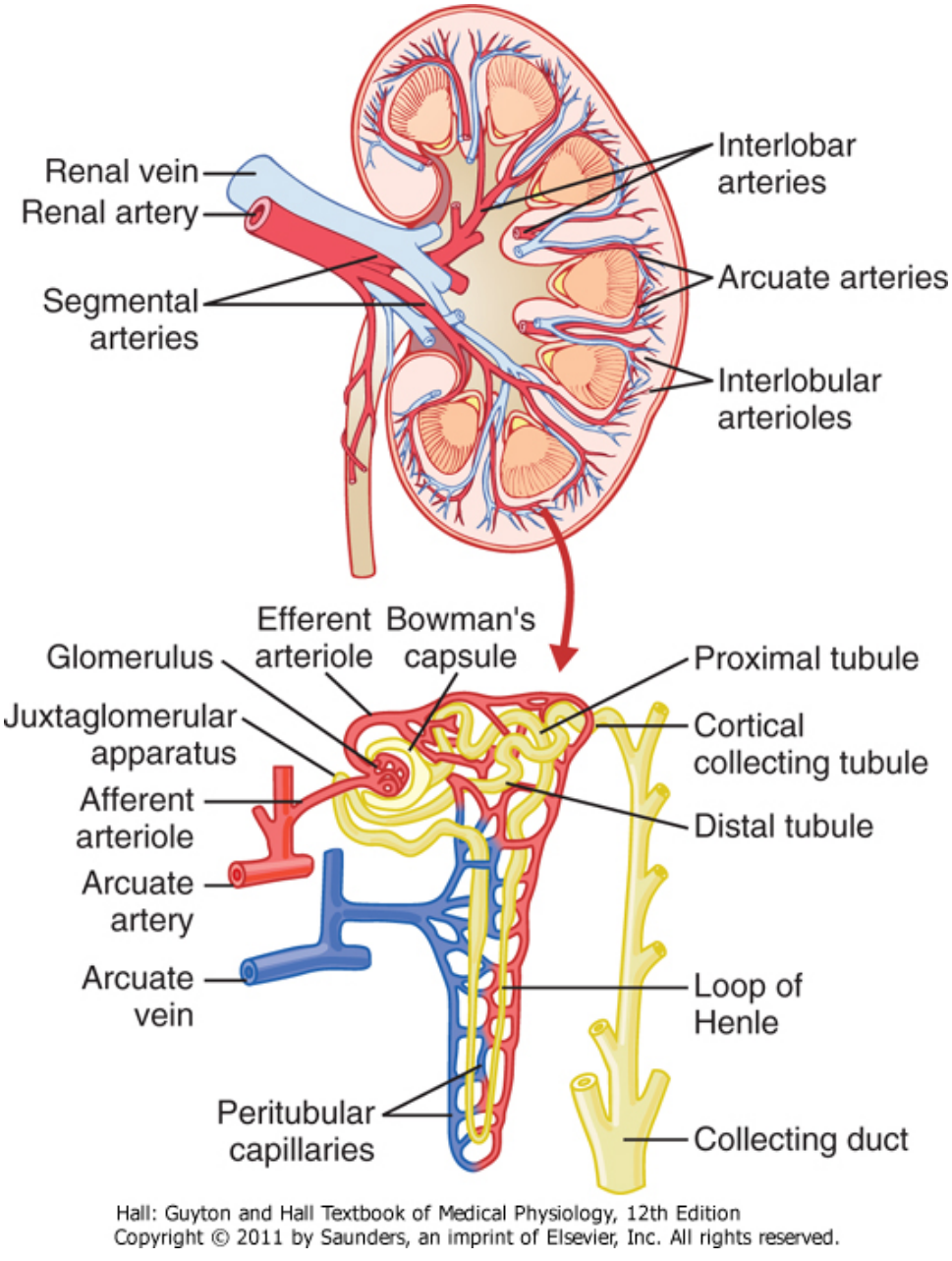

Figure 1. The anatomy of the kidney.

PHYSIOLOGY

Functions

First and foremost, the kidney participates in homeostasis, which means regulation of body fluids and the oncotic pressure. In other words, keeping the balance of electrolytes, nutrients and fluid constant.

Blood pressure is partly regulated via the renin-angiotensin-aldosterone system (RAAS), which is activated by the juxtaglomerular cells in the kidney.

The kidney controls the acid-base homeostasis by reabsorption and production of bicarbonate when acidotic, and reabsorption of hydrogen ions when basic.

By eliminating waste products, such as creatinine and nitrogenous waste products, the kidneys function as the body's waste excreting station.

The endocrine actions are other crucial factors when speaking of the kidney's role in the human body.

Urine Formation

In order to maintain homeostasis, the kidneys produce urine. The urinary excretion is a result of glomerular filtration, reabsorption and secretion. The glomerular filtration takes place in the glomerulus. Fluids and most substances (except from proteins) are filtered due to high hydrostatic pressure in the capillaries and high oncotic pressure in Bowman's capsule. Most substances pass, since the endothelium, the basal membrane and the podocytes together create a highly permeable membrane for selected substances.

The urine is modified in the tubules, by reabsorbing water and solutes from the filtered fluid into the blood stream and secreting substances from the blood into the tubular lumen[3].

Blood Pressure

Macula Densa is an important structure in regulating the blood pressure. By sensing the concentration of sodium chloride in the distal tubule, the Macula Densa reacts to low plasma sodium concentration. Also, a decrease in blood pressure leads to an increase in sodium reabsorption in the proximal tubule and thereby a lower level of sodium in the distal tubule[4].

When reduced blood pressure or low concentration of plasma sodium, the Macula Densa dilate the afferent arteriole to increase GFR. Macula Densa also signals to the juxtaglomerular cells, which react by converting prorenin to renin and thereafter secrete renin straight into the systemic circulation. The reduced perfusion pressure in the juxtaglomerular cells has a direct effect of triggering the cells to release renin as well[3, 5]. Renin functions through enzymatic actions to hydrolase angiotensinogen (from the liver) to angiotensin I. Through angiotensin converting enzyme (ACE) on the endothelial cells, mostly found in the pulmonary and renal circulation, angiotensin I is converted to angiotensin II[4]. Angiotensin II is a sodium-retaining hormone, which stimulates sodium reabsorption in the loop of Henle, the distal tubules and the collecting ducts and is a vasoactive peptide, causing constriction of the arterioles. Also, angiotensin II stimulates the adrenal cortex to secrete aldosterone, which increases sodium reabsorption and thereby results in an increase in blood pressure[3, 4].

Other effects of angiotensin II are activation of the sympathetic nervous system and stimulation of the pituitary gland to secrete Anti Diuretic Hormone (ADH).

Circulation

The kidneys are predisposed to ischemic events. The circulation of the kidneys is vulnerable. Approximately 25% of the Cardiac Output is directed to the kidneys, which is why changes in

hemodynamics affects the kidneys. The kidneys have high demands of oxygen supply, and when decreased blood flow this oxygen supply is not enough. The vulnerability can be demonstrated partly as the blood flow and the tissue oxygenation, having a gradient from cortex to the inner medulla (table 1). Therefore, the inner medulla is more sensitive for hemodynamic changes. Also, renal vascular resistance is substantial, where a systemic arterial pressure of 100mmHg decreases to 4 mmHg in the renal vein[3].

Table 1. Blood flow and oxygenation of the kidney

Parameter	Cortex	Medulla	Ischemia
Blood flow (mL/g/min)	5	2.5 (outer medulla) 0.6 (inner medulla)	?
Tissue oxygenation (PO_2) mmHg	50 mmHg (6.67 kPa)	15 mmHg (2 kPa)	?

Endocrine Actions

The kidney also function as an endocrine organ, which produces Erythropoietin (EPO). EPO stimulates the bone marrow to produce red blood cells [6]. The kidney plays a central role in the calcium homeostasis, not only because of the electrolyte transports in the tubules, but also due to the fact that the kidneys activate vitamin D3. Activated vitamin D affects the gastrointestinal tract to increase calcium reabsorption. Also Parathyroid hormone increases the tubular reabsorption of calcium[3]. P304 and 965

The RAAS system, which regulates blood pressure, is a hormonal system as well.

ACUTE KIDNEY INJURY IN CHILDREN

Acute kidney injury

Acute kidney injury (AKI) is a common post-operative complication among children undergoing cardiac surgery, and is associated with adverse outcomes.

AKI is defined as an abrupt change in renal function, affecting fluid and electrolyte status, acid-base and hormonal regulation [7]. The Kidney Disease | Improving Global Outcome (KDIGO) organization defines AKI as either serum-creatinine (sCr) increase by more than 26.5 µmol/l (0,3mg/dl) within 48 hours, an 1.5 fold increase in sCr compared to baseline or urine output less than 0.5 ml/kg/h for at least 6 hours. Note that creatinine and urine output are only surrogates for a decrease in GFR [7]. Thereafter, the severity is graded relatively to creatinine and urine output (Table 2). There are other ways to define AKI as well, for instance by using the RIFLE and AKIN criteria, both regarding the sCr levels and urine output [7].

Studies show that the incidence of AKI after pediatric cardiac surgery with cardio pulmonary bypass (CPB) ranges from 10-64%[8-12].

Table 2. KDIGO AKI definition and staging

AKI Stage	Serum creatinine	Urine output
1	1.5-1.9 times baseline OR ≥ 0.3 mg/dl (≥26.5μmol/l) increase	<0.5 ml/kg/h for 6-12 hours
2	2.0-2.9 times baseline	<0.5 ml/kg/h for ≥12 hours
3	3.0 times baseline OR ≥4.0 mg/dl (≥353.6μmol/l) OR Initiation of renal replacement therapy OR, In patients <18 years, decrase in eGFR to <35ml/min per 1.73	<0.3 ml/kg/h for ≥24 hours OR Anuria for ≥12 hours

KDIGO = Kidney Disease: Improving Global Outcome. AKI = Acute kidney injury

There are difficulties when measuring sCr in neonates, as a result of present maternal creatinine, creatinine reabsorption in the proximal tubules, lower GFR and due to individual differences in maturation [13]. The importance of considering changes in fluid status when measuring sCr is substantial. Reckoning the fluid status change enables finding changes in sCr due to a true decrease in renal function, as opposed to changes in sCr as a consequence of abrupt changes in weight[14].

An alternative way of measuring creatinine is by correcting for fluid balance, using the following formula:

Corrected creatinine = Measured creatinine x [1+(accumulated fluid balance/total body water)] [15]. Using corrected creatinine for AKI assessment gives reliable results regarding incidence[16].

Studies have shown that assessing AKI by using both serum creatinine and urine output optimize the AKI diagnosis [17, 18].

The nitrogenous waste product blood urea nitrogen (BUN) is another commonly used biomarker, which can be helpful in evaluating the kidneys' condition[7]. However, there are other ways to detect AKI as well, for instance by measuring cystatin C[19, 20]. Although, cystatin C itself is not a strong enough independent factor for the purpose of detecting AKI[20].

Defining AKI collectively is a necessity to be able to use it practically, but also for research purposes.

It is known that AKI increases mortality and length of stay (LOS) amongst patients in the pediatric Intensive Care Units (PICU)[18, 21, 22]. Chertow et al. established an independent association between an increase in creatinine (>26.5μmol) and mortality[22].

Fluid overload (FO), an imbalance in fluid input and fluid output, often occurs along with significant AKI. %FO also considers body weight when admitted to the ICU. Calculating %FO the following formula is used:

%FO=((fluid intake-fluid output)/PICU admission weight) x100 [23] Studies have shown that FO >10-20% increases the risk of mortality, independent of illness severity and multi organ failure (MOF) status, when compared with FO <10% [23, 24]. A risk factor for FO is Cardio Pulmonary Bypass surgery[25].

Mechanisms Behind AKI in Children Undergoing CPB Surgery

Acute kidney injury can be subdivided into three groups – prerenal, intrarenal and postrenal. Basically, prerenal AKI is most commonly a systemic circulatory issue, where the renal blood flow and blood pressure is reduced. It is called intrarenal AKI when the kidney itself is affected. Postrenal AKI is a consequence of obstruction in the urinary collecting system [3].

In neonates and children undergoing CPB surgery a few mechanisms behind AKI are described. Mostly, in these children the pre-renal factors are of importance. At first, there is an inflammatory response, with elevated cytokine levels, during cardiopulmonary bypass surgery [26]. The inflammatory response is mainly due to the blood's exposure to foreign material in the cardio pulmonary bypass machine, resulting in an increase in capillary permeability. This increase in permeability leads to redistribution of intravascular fluid and a true decrease in blood volume, culminating in hypotension and reduced renal blood flow [27, 28]. The renal ischemia following CBP is also a risk factor for developing AKI[8].

Children with congestive heart failure are predisposed to AKI events due to reduced renal perfusion. This reduction in renal perfusion is due to the decreased effective blood volume, and not a true decrease in blood volume, as a consequence of the underlying congestive heart disorder [28].

When treating children, medications that lack trials regarding dosing and efficacy as well as safety for this population, are often required. Thereby, using these untried drugs, children are at larger risk of side effects[29]. This includes a few nephrotoxic substances. Nephrotoxic induced AKI is the most avoidable cause in the neonate AKI population, due to the possibility to monitor exposure and evaluating kidney status [29]. Moffet et al. showed that 52% of the nephrotoxic exposure were antimicrobial agents[30]. For instance, aminoglycoside exposure increases risk for Acute Tubular Necrosis (ATN) and might lead to complete renal failure[29, 31]. One third of children treated with aminoglycosides develop AKI[32]. Other common nephrotoxic substances are ACE inhibitors, which dilates the efferent arteriole and thereby decrease hydraulic pressure and GFR. Cyclooxygenase (COX) inhibitors such as Non Steroid Anti Inflammatory Drugs (NSAID) decrease the prostaglandin production. Prostaglandins are

important in dilating the afferent arteriole, and when inhibited the afferent arteriole constricts which lead to a decrease in glomerular perfusion and glomerular filtration[29].
It is important to monitor the amount and intensity of exposure, as well as other risk factors, to be able to prevent drug induced AKI [29, 30].

AKI Treatment in Children

Diuretics are commonly used in managing fluid overload and AKI in children, despite the fact that results of previous studies are discrepant regarding renal recovery and mortality when using diuretics[33-35].
Usually, in critically ill children, the oncotic pressure is reduced, which leads to fluid redistribution to the interstitial fluid. This activates counter-regulatory hormones such as angiotensin II and the sympathetic nervous system, in order to increase sodium retention [23]. Therefore it is of importance to normalize oncotic pressure to get a satisfactory effect of the diuretics treatment[23]. Since albumin is an important factor in maintaining the oncotic pressure, hypoalbuminaemia needs correction in order to maximize the effect of diuretics treatment[36].
Kwiatkowski et al. [37] showed a decrease in morbidity (shorter mechanical ventilation, less fluid overload and fewer happenings with disturbed electrolytes) when using peritoneal dialysis (PD) to treat AKI compared with furosemide treatment. Although the study did not establish any differences in mortality or LOS[37].

Chronic Kidney Disease after Acute Kidney Injury

A 5-year follow-up in children after pediatric cardiac surgery, regarding kidney outcome, showed that the kidney associated complications hypertension and chronic kidney disease

(CKD) were common (17% and 18%). No correlation between AKI and the incidence of hypertension or CKD among these patients could be seen [38]. Although a 6-months follow up in children with drug induced AKI 70% had residual kidney damage [31]. Whereas, Coca et al. implemented a Meta-analysis of adults, showing a significant increased risk for developing CKD for patients surviving an AKI event[39].

ATRIAL NATRIURETIC PEPTIDE

Physiology

Atrial Natriuretic Peptide (ANP) is a 28-amino acid peptide, which increases natriuresis and diuresis [40]. ANP is secreted by the atrial myocytes, in response to atrial wall distention[3]. Among other things, ANP's direct effects are vaso- and venodilation. Also, ANP has an inhibitory effect on the sympathetic nervous system and the renin-angiotensin-aldosteron system. The natriuresis is due to a decrease in sodium reabsorption in the kidney, which forces sodium to exit the body, as a consequence water follows[41]. As mentioned, ANP inhibits RAAS by direct effects on the renin secretion. When renin is inhibited it leads to a reduction in Angiotensin II formation. Considering the angiotensin-II-induced anti-natriuresis, when angiotensin II is constrained, the action will lead to an even larger natriuresis[3, 41].

Most studies on healthy subjects and patients with normal renal function have shown that ANP causes an increase in GFR [42]. When ANP is present, the pre-glomerular vascular resistance decreases and the post-glomerular vascular resistance increases, causing higher hydraulic pressure within glomerular capillaries. This increase in hydraulic pressure has also been shown in animal studies, which coincide with the results in humans[43].

As mentioned earlier, ANP functions as a natriuretic peptide, due to the collaboration between the ANP induced increase in GFR and the tubular effects of ANP, resulting in an increased sodium excretion [44-46].

Renal oxygen consumption (VO_2) is strictly correlated to the tubular sodium reabsorption. This means, when GFR increases, more water and sodium will enter the tubular system, demanding the renal tubules to reabsorb more sodium. Due to the correlation between VO_2 and tubular sodium reabsorption, an increase in GFR leads to greater renal VO_2 [47, 48]. As ANP, through pre-glomerular vasodilation and post-glomerular vasoconstriction, increases GFR, it also inhibits the tubular sodium reabsorption [3]. Despite ANP's inhibitory effects on tubular reabsorption, Swärd et al. showed a higher renal VO_2 in patients receiving ANP vs. the ones receiving furosemide [49].

ANP Treatment – Previous Studies

A randomized, double-blinded, placebo-controlled trial with adults undergoing CPB surgery, where the intervention group received continuous hANP-infusion post-operatively, affirmed a lower incidence of dialysis-dependent AKI than the placebo group [50]. Other studies, where an ANP- analog (anaritide) has been used, have not been able to prove a significant improvement neither in renal outcomes regarding need for dialysis nor dialysis free survival [51, 52]. However Allgren et al. found that anaritide improved dialysis-free survival in patients suffering from oliguria [51].

Although, a review article, including adult patients, showed that low dose ANP improved outcomes when preventing and managing postsurgical AKI, as well as shortening LOS among these patients [53].

AIM

The purpose of this study was to evaluate the effects of hANP treatment in the pediatric AKI population after corrective cardiac surgery.

The aim was to improve AKI treatment among infants and neonates in the PICU after corrective cardiac surgery, in order to prevent dialysis-dependent AKI, by comparing hANP and furosemide treatment with the commonly used furosemide treatment.

MATERIALS AND METHODS

Ethics

When conducting studies on pediatric populations, it is of importance to put the benefits in relation to the possible harm. Since hANP has been used for a decade in the PICU at Queen Silvia Children's Hospital (DSBUS) it is crucial to determine the effects of hANP treatment, in order to give these children best possible treatment.. This ethical dilemma is the motive force for this retrospective study.

Study population

In this study, 2 study groups are used, one intervention group (hANP group) and one control group (no-hANP). Patients in the hANP group received hANP and furosemide treatment, while the no-hANP group only received furosemide treatment.

We chose to study pediatric cardiac surgery patients who received hANP treatment January 1st 2010 through December 31st 2013 in the PICU at DSBUS in Gothenburg.

Only patients undergoing their first major surgery with Cardio Pulmonary Bypass during this period were included. The children, who had already been through a major surgery, might have had a previous AKI event. If that is the case, there is an immediate risk that the kidneys are already damaged, which could delude the results.

PICU LOS longer than 30 days were excluded due to the fact that other complicating factors might be present, affecting the results.

The no-hANP group was collected from DSBUS as well. We received a list of all patients undergoing corrective cardiac surgery from January 1st 2010 through December 31st 2013. Patients were organized according to date of surgery. Data from the first 6 patients who had their surgery during each year: 2010, 2011, 2012 and 2013, which passed the eligibility criteria were extracted. Thereafter an additional 6 patients were extracted to collect a group of 30 patients. The 6 patients were randomized regarding year and month of surgery.

Full inclusion and exclusion criteria are summarized in table 3. The same inclusion and exclusion criteria apply for the no-hANP group as the hANP group. The original study population (hANP group) contained 89 patients. After applying eligibility criteria, 45 patients remained. Patient flow is displayed in figure 2.

Table 3. Inclusion and Exclusion Criteria

Inclusion Criteria
Corrective cardiac surgery with CPB between Jan 1st 2010 and Dec 31st 2013
Exclusion Criteria
Cardio Pulmonary Bypass time <90 minutes
PICU LOS >30 days
Previous major surgery
Previous cardiac surgery
Extra Corporeal Membrane Oxygenation

CPB = Cardio Pulmonary Bypass. PICU LOS = Pediatric Intensive Care Unit Length of Stay

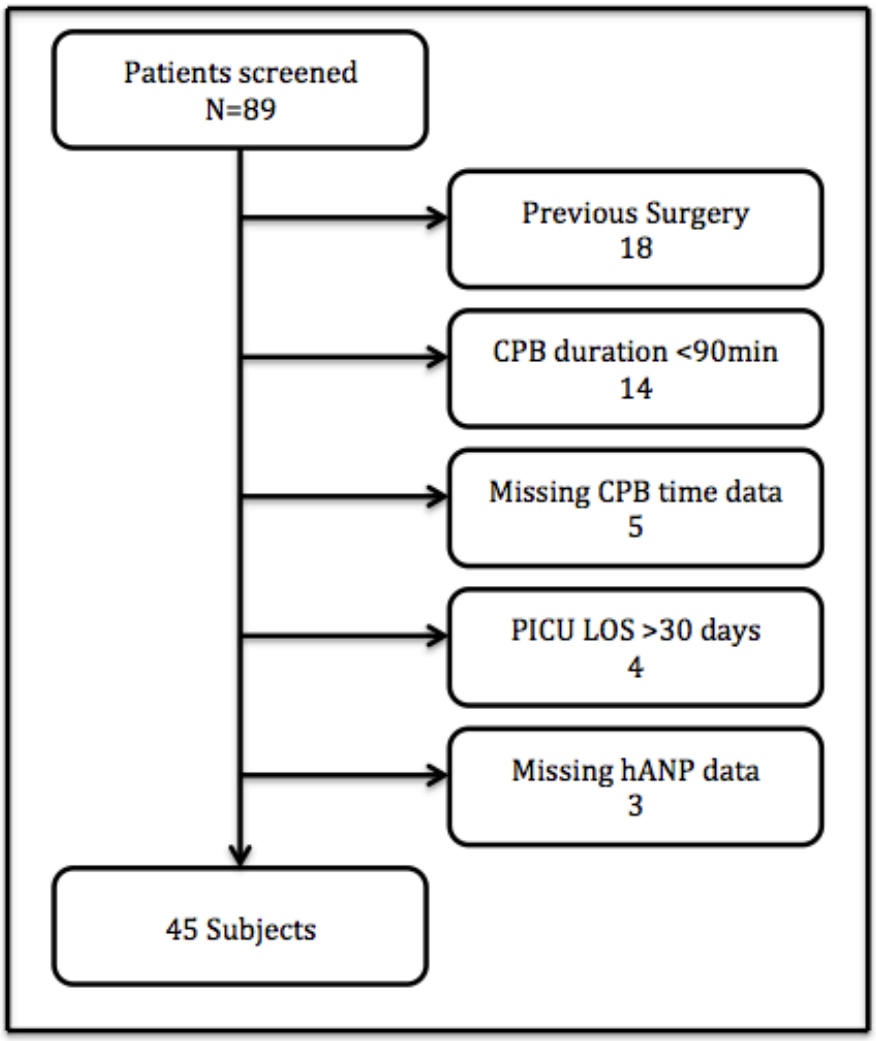

Figure 2. Patient Flow, hANP group. PICU = Pediatric Intensive Care Unit. LOS = Length of Stay. hANP = human Atrial Natriuretic Peptide. CPB = Cardio Pulmonary Bypass.

Data Collection

By retrieving data from the patients' journals we received information regarding distribution of age at surgery, CPB duration and the length of stay in the PICU.

The length of hANP treatment as well as start date for furosemide treatment was collected. Also, data on which patients that required dialysis was retrieved. Among the patients receiving dialysis, data on which postoperative day dialysis was initiated was verified.

We decided on several other factors, important in diagnosing and following the pattern of development regarding AKI in our collection of data to be able to better assess the effects of hANP treatment. Those factors are listed below.

Creatinine

The primary biomarker is creatinine. We chose creatinine to be able to follow the development of AKI, but also to evaluate treatment efficacy. Creatinine will be registered pre-operatively, the first postoperative day (POD), right before initiation of hANP infusion (pre-ANP creatinine, PASK) and at the time when sCr is reduced to 50% of the PASK level. When reduced to 50% of the PASK level, the kidneys are thought to have reversed the AKI and hANP treatment is supposed to have been phased out and terminated.

Corrected Creatinine

Due to difficulties in measuring creatinine in the neonatal and pediatric population we will use a formula to calculate corrected creatinine, to adjust for fluid status. Total body water equals 0.6 of the total body weight in kilograms [16].

Corrected creatinine = Measured creatinine x [1+(accumulated fluid balance/total body water)] [15]

Body Weight and Fluid Overload

To be able to calculate corrected creatinine, we will follow total body weight at the times when creatinine is registered. Weight is also necessary to calculate %FO.

Fluid balance, in other words, the balance between fluid intake (liters) and fluid output (liters), is important when evaluating the kidney function. We will not register the fluid balance during the surgery, due to difficulties in following the fluid and drug input properly. Thereby there will be a repeated systematic error, which we consider not affecting the

outcome. When calculating %FO, fluid balance and PICU admission weight are the basic elements.

%FO=((fluid intake-fluid output)/PICU admission weight) x100 [23]. That is why fluid input and fluid output will be registered throughout the PICU stay.

Urea

Urea is a biomarker, which usually correlate with kidney function. Since the kidneys function is the body's waste excretion station for nitrogenous products, urea level is registered at the same time as creatinine.

Other factors

Creatinine is the central biomarker, but these following factors will also be evaluated right before initiation of ANP infusion and when PASK is reduced to 50%. These factors will be looked at, in order to evaluate whether hANP treatment improves not only creatinine and kidney function but other factors regarding circulatory, inflammatory and respiratory status as well.

Systemic Vascular Function

In order to evaluate the main systemic vascular function, the need for inotrope and vasopressor support will be looked upon, starting the first postoperative day. Epinephrine (ηg/kg/min), Norepinephrine (ηg/kg/min), Milrinon (μg/kg/min), Nitrogen oxide (NO) (ppm) and other vasoactive substances will be registered. Mean arterial pressure (MAP) will also be monitored for the purpose of main systemic vascular function.

Inflammation

Inflammation status is to be registered through extracting CRP and white blood cells, LPK. Those biomarkers ought to reflect the inflammatory response caused by the surgery, as well as whether infection is present or not. Use of antibiotics, which might have a direct nephrotoxic effect, is registered.

Respiratory factors

Cardiac surgery affects a lot of different vital systems. When evaluating the respiratory variables, a few different methods will be used. Initially, the arterial blood gas will be used to analyze paO_2 and pCO_2, in order to estimate oxygenation and carbon dioxide retention. Also, the respiratory settings, the flow index of oxygen (FiO_2) and the positive inspiratory pressure (PIP) are extracted. PIP and FiO_2 are important for monitoring what is required to maintain an acceptable respiratory status, and if that changes with improvement in creatinine after hANP treatment.

Once FiO_2 and PaO_2 are extracted, those factors are used to calculate a ratio to see if Acute Respiratory Distress Syndrome (ARDS) is present. 1 kPa equals 7.5 mmHg, and by multiply the PaO_2 with 7.5 it will convert into mmHg.

ARDS equals $(PaO_2 x 7.5)/FiO_2 < 200$.

Matching the groups

The length of CPB is the best way of matching the control and intervention group, regarding exposure of inflammation and ischemia during surgery. CPB duration also accounts as a surrogate for the complexity of the underlying cardiac disorder.

Outcomes

The primary outcome in this study is the difference in risk for dialysis-dependent AKI between the hANP group and the no-hANP group. Does the risk for dialysis increase with CPB duration? Patients who ended up on dialysis – are there any difference between the groups regarding when dialysis is initiated? Also, can hANP treatment affect the PICU LOS?

Statistics

The data was analyzed using IBM Statistical Package for Social Science.

Binary logistic regression was used for calculation of risk for dialysis. Descriptive statistics and quantitative methods were used to calculate median values and quartiles. Independent samples median test was used to illustrate differences in median values between the groups.

RESULTS

Patient Characteristics

A total of 75 patients were included in the study (hANP, n=45; no-hANP, n=30). Patient characteristics are illustrated in figure 3 and table 4.

Age at surgery is summarized in table 5.

Results

When analyzing the risk for dialysis, there was no significant difference between the hANP and the no-hANP group. Although, when correcting for CPB duration, we could see a 13% increase in risk for dialysis for every 10 minutes staying on the CPB (p=0,017) (Table 6), regardless of hANP treatment.

Figure 4 shows the CPB duration distribution in relation to whether dialysis was needed or not. Median CPB duration was 183 minutes in the PD group and 135 minutes in the no-PD group (p= 0.018).

The median PICU LOS was 3 days longer in the hANP group (7 days vs. 4 days) (p=0.043). This difference is illustrated in figure 5.

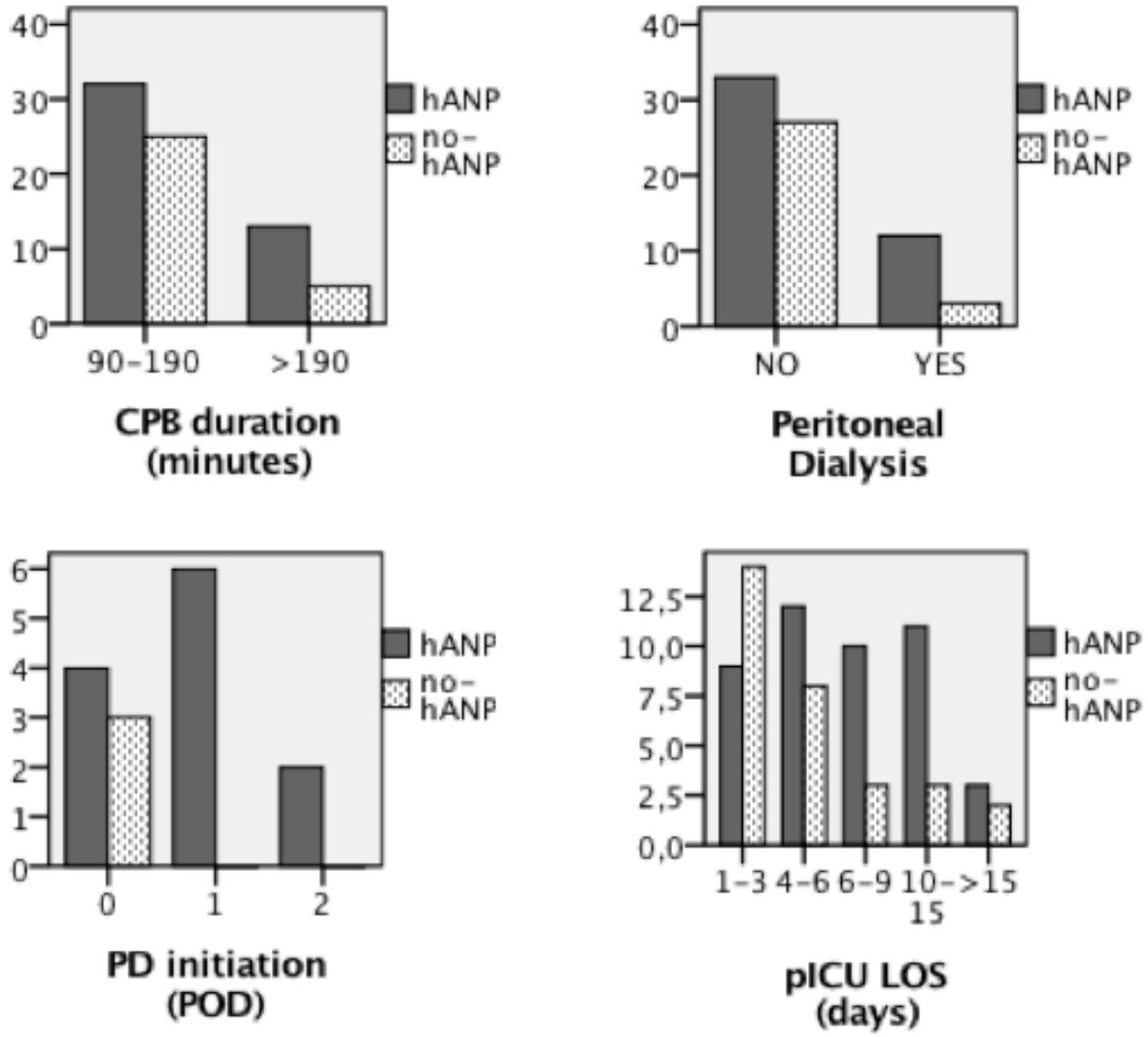

Figure 3. Patient Characteristics. This panel shows the distribution between the hANP and the no-hANP group. The X-axis showing the variable and the Y-axis showing number of subjects. Data is collected from table 4. For additional information, please see table 4. CPB = Cardio Pulmonary Bypass. PD = Peritoneal Dialysis. POD = Post-Operative Day. PICU LOS = pediatric Intensive Care Unit Length of Stay.

Table 4. Patient Characteristics

		hANP (n=45)		No-hANP (n=30)	
		n	%	n	%
CPB time	90-190 minutes	32	71.1	25	83.3
	>190 minutes	13	28.9	5	16.7
hANP treatment	<6 days	31	68.9	-	-
	6-10 days	11	24.4	-	-
	>10 days	3	6.7	-	-
PICU LOS	1-3 days	9	20.0	14	46.7
	4-6 days	12	26.7	8	26.7
	7-9 days	10	22.2	3	10.0
	10-15 days	11	24.4	3	10.0
	>15 days	3	6.7	2	6.7
PD	YES	12	26.7	3	10.0
	NO	33	73.3	27	90.0
PD initiation	POD0	4	33.3	3	100.0
(POD)	POD1	6	50.0	-	-
	POD2	2	16.7	-	-

CPB = Cardio Pulmonary Bypass. hANP = human Atrial Natriuretic Peptide
PICU LOS = Pediatric Intensive Care Unit Length of Stay. PD = Peritoneal Dialysis.
POD = Post-Operative Day.

Table 5. Age at Surgery

		hANP		No-hANP	
		n	%	n	%
Age at surgery	<1 month	23	51.1	16	53.3
	1-6 months	15	33.3	8	26.7
	6-12 months	6	13.3	5	16.7
	>12 months	1	2.2	1	3.3

Table 6. Risk for Peritoneal Dialysis. Variables in the Equation

								95% C.I.for OR	
		B	S.E.	Wald	df	p-value	OR	Lower	Upper
Step 1[a]	hANP	.800	.732	1.196	1	.274	2.227	.530	9.347
	CPB time, 10min	.123	.051	5.734	1	**.017**	1.131	1.023	1.250
	Constant	-4.007	1.016	15.558	1	.000	.018		

a. Variables entered on step 1: hANP treatment and CPB time 10 minutes.

Risk for peritoneal dialysis, no significant difference could be seen between the hANP and the no-hANP group. When correcting for CPB time, Odds Ratio equals 1.13, which means a 13% increase in risk for every 10 minutes spent on the CPB (P=0.017). Risk was calculated using binary logistic regression methods. C.I. = Confidence Interval. OR = Odds Ratio. CPB = Cardio Pulmonary Bypass.

39 out of 45 (86.7%) in the hANP group started furosemide treatment on POD0, 5 (11.1%) on POD1 and 1 (2.2%) on POD2. In the no-hANP group, 27 out of 30 (90.0%) received furosemide treatment POD0, 2 (6.7%) on POD1 and 1 (3.3%) on POD2. hANP treatment was

initiated POD0 (n=17, 37.8%), POD1 (n=25, 55.6%) or POD2 through POD9 (n=3, 6.7%), and the length of hANP treatment varied from 1 to 14 days.

Dialysis was initiated POD0 in all 3 cases in the no-hANP group. In the hANP group, dialysis was initiated at POD0 (n=4, 33.3%), POD1 (n=6, 50.0%) and POD2 (n=2, 16.7%) (Fig 3, Table 4). No significant differences could be seen between the groups (Table 7).

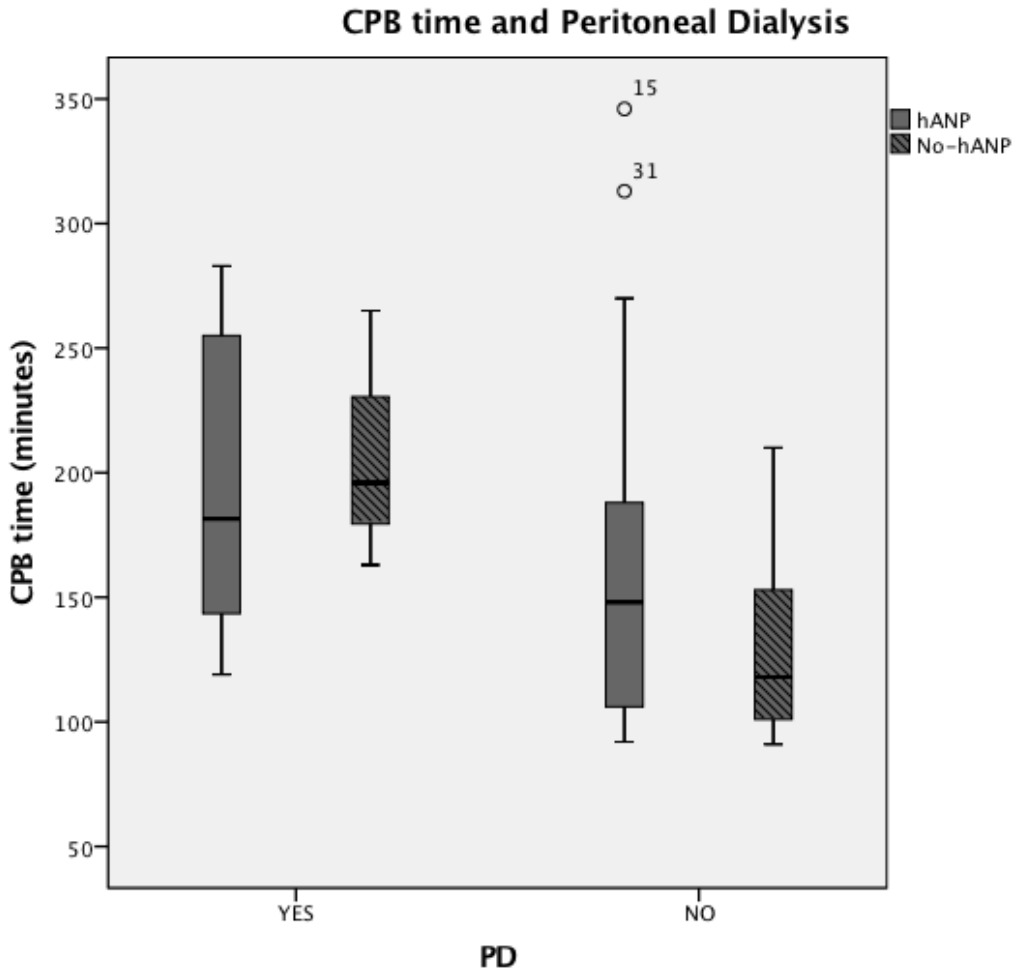

Figure 4. CPB time and Peritoneal Dialysis. The median CPB time was higher in the group receiving Peritoneal dialysis (183 minutes) vs. the ones not receiving Peritoneal Dialysis (135 minutes) (p=0.018). Significance was calculated using independent samples median test (non-parametric test). CPB = Cardio Pulmonary Bypass. PD = Peritoneal Dialysis.

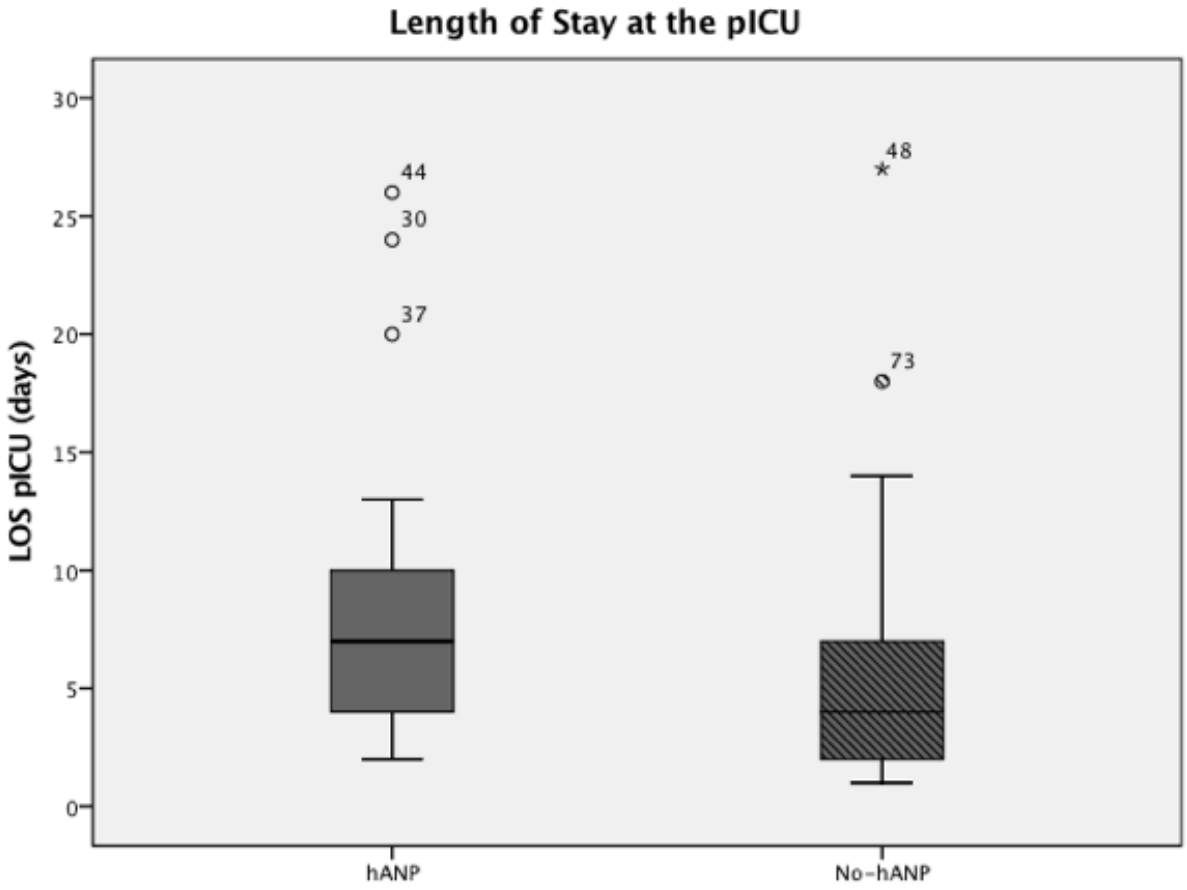

Figure 5. PICU LOS. Patients receiving hANP had a 3 days longer median LOS than the no-hANP patients (p=0.043). Significance was calculated using independent samples median test (non-parametric test). LOS = Length of Stay. PICU = pediatric Intensive Care Unit.

Table 7. Comparison of perioperative parameters in the study groups

	hANP	No-hANP	
	Median (Q1-Q3)	Median (Q1-Q3)	p-value
Age at surgery (months)	1.00 (0.2-6.0)	0.80 (0.3-5.3)	*0.89*
CPB time (minutes)	154 (119-205)	131 (102-154)	*0.12*
hANP treatment (days)	4 (2-7)	-	-
PICU LOS (days)	7 (4-10)	4 (2-7)	**0.043**
PD initiation (POD)	1 (0-1)	0 (0)	*1.00*

This table shows median values and range Q1-Q3, as well as p-values. Using Non-parametric (Independent Samples Median) Tests for analyzing significance. Only the difference of PICU LOS was significant. Q1 = quartile 1 (25^{th} percentile). Q3 = quartile 3 (75^{th} percentile). CPB = Cardiopulmonary Bypass. PICU LOS = pediatric Intensive Care Unit Length of Stay. PD = Peritoneal Dialysis. POD = Post-operative day.

DISCUSSION

Discussion

The most important finding of present study was the significant increased risk for dialysis in correlation with CPB duration. No correlation between type of diuretics treatment and dialysis was found. The correlation between CPB duration and dialysis is in concordance with previous findings by Chan et al. [54]. This is further established by Pedersen et al. [55], where the use as well as the duration of CPB is associated with an increased risk for requiring dialysis after cardiac surgery.

However, it has previously been discussed whether there is an actual correlation between CPB duration and dialysis or not. A large observational study on an adult population, showed

that after adjusting for confounders, no significant association between CPB duration and dialysis-dependent AKI could be found [56].

Previous studies on hANP and hANP-analogues effects on AKI have only been done on adult subjects, showing various results [50-53]. Swärd et al. [50] showed a lower incidence of dialysis amongst the post-cardiac surgery patients receiving continuous hANP infusion in the adult thoracic ICU AKI population in a large randomized controlled trial.

Since there are no previous studies done on the effects of hANP in the pediatric population, it is crucial to determine how this matter stands regarding hANP treatment effects. In the PICU at DSBUS, hANP has been used to treat AKI for a decade. The PICU and the tICU in Gothenburg are the only centers where the hANP treatment algorithm in post-cardiac surgery is consensus.

hANP has become a part of the post-operative care in pediatric patients after cardiac surgery at Queen Silvia Children's Hospital. The natural course is to use furosemide postoperatively. When furosemide treatment does not have desirable effect, hANP is administered. Once furosemide and hANP treatment is failing, dialysis is initiated.

Sometimes dialysis is initiated in conjunction with the surgery. When dialysis is initiated in connection with the surgery, the patients are assessed not to be able to maintain homeostasis by only diuretics administration. Although sometimes, dialysis is initiated on basis of fluid overload due to the surgery or hyperkalemia, but that was not the case in this study.

All patients in the no-hANP group ended up on dialysis in connection with the surgery - or not at all. This might imply that the ones ending up on dialysis in the no-hANP group are the most critically ill children. Whereas children in the hANP group receiving peritoneal dialysis on POD0 were treated with diuretics postoperatively prior to the PD initiation.

As mentioned earlier, the natural course of treatment is to start with furosemide, in order to add hANP and thereafter use dialysis. Therefore, it is righteous to consider the no-hANP patients to be either ill enough to be in need of dialysis right away, or not ill enough to require hANP at all.

One of the limitations in this study is that the no-hANP group and the hANP group both originate from DSBUS. Since hANP has been used at DSBUS for 10 years, the way of using it has become consensus. Thus, only using furosemide reverses the no-hANP patients' AKI, and helps them recover kidney function. The furosemide non-responders receive hANP, which thereby puts them in the hANP group. When acknowledging this fact, it can be understood that the patients receiving hANP treatment possess individual factors that might indicate a more therapy resistant AKI than the no-hANP group. As well as patients receiving dialysis in connection with the surgery avoid the hANP administration entirely and are put in the no-hANP group automatically. This leads to a distorted distribution of patients between groups and a selection bias.

Further, a prolonged PICU LOS could be seen in the hANP group. This prolonged PICU LOS rather corroborates the selection bias than questioning the effects of hANP on the LOS. Patients receiving hANP are in need of a more aggressive approach and supposedly have a more therapy resistant AKI. Therefore it is natural to assume that patients requiring hANP also tend to be in need of longer care than the ones improving on only furosemide treatment. Also, it is important to mention that furosemide is a treatment that can be relocated into other wards than the PICU, meanwhile hANP is an infusion that is only administered at the PICU. Thus, patients improving on furosemide can get transferred earlier than patients requiring hANP.

Therefore, there are difficulties when trying to conclude the effects of hANP, since both groups originate from a population in a ward where hANP is part of the protocol on how to treat AKI. The patients who do not respond to furosemide inevitably end up in the hANP group. This divides the population into two subpopulations – one in which all the furosemide responders end up, and one where all the furosemide non-responders end up. To be able to avoid this selection bias, we would be in need of a no-hANP group, originating from a ward where hANP treatment is not part of standard treatment. On this basis, the no-hANP group we used was not optimal considering the aim of this study.

Methodological difficulties

Initially, a lot of factors were considered important in order to evaluate the effects of hANP. Due to the fact that the patient's PICU journals were all handwritten and scanned in, the possibility to retrieve all the predetermined data was limited. The scanned in journals were often 200-500 pages long and therefore hard to overlook. Some important pages and data were missing. This, in combination with the limited time for this study (20 weeks) resulted in a reevaluation regarding which factors to prioritize. Due to the inconsistency in laboratory analyzes, it would rather lead to inconclusive results than clarity to analyze them all.

Creatinine

Creatinine was the benchmark biomarker. There were difficulties in finding the baseline creatinine in a lot of cases. Without the baseline level, it would not be possible to evaluate the hANP treatment’s effects on creatinine. Also, creatinine was not measured frequently enough to be able to follow its path. Since we would be in need of the creatinine analyze for

specific times, it aggravated the extraction further. Therefore we decided on not to use creatinine as the baseline biomarker of the study. In order to evaluate the hANP-effects on creatinine (and other biomarkers) a protocol on when to measure creatinine is needed.

Body Weight and Corrected Creatinine

Body weight was not updated continuously, and in a lot of cases the PICU admissions weight was the only registered weight throughout the stay. When not being able to extract body weight, in combination with the previously described difficulties with creatinine, corrected creatinine was not possible to calculate. Thereby, creatinine and corrected creatinine could not be used to assess AKI status and evaluate the effects of hANP treatment.

Fluid Balance

Difficulties in finding explicit data, applied for fluid balance as well. It was possible to find proper data in some cases, but due to a lot of missing data and the massive material, we decided on not to extract fluid balance. This lead to not being able to calculate %FO, which would have been an interesting factor to evaluate, considering the previously described findings regarding adverse outcome when %FO >10-20% [23, 24].

Other Variables

Likewise, a lot of other laboratory analyzes were missing, and therefore concluding whether other laboratory factors were improved or not was not possible. For instance, PASK was the set point for when the other biomarkers and factors were to be looked upon. Usually, there were no laboratory analyzes available for that specific moment. Also, at times only one or

two analyzes were found, and to evaluate the effect of hANP on such variables would not be feasible.

Considering these difficulties arterial blood gas analyzes were rarely in accordance with when we wanted to analyze them. That resulted in exclusion of PaO_2 and PCO_2. When excluding PaO_2, it was no longer possible to calculate the ARDS ratio. Thereby the importance of extracting FiO_2 disappeared.

The previously described reasoning applies for the systemic vascular variables and the inflammatory variables, as well as for the respirator variables.

Limitations

The limitations of this study are, as mentioned, the lack of randomization regarding the groups. There is a selection bias when considering the origin of the groups, since hANP treatment was applicable only to the patients suffering from furosemide resistant AKI.

Another limitation is the fact that a lot of factors that were supposed to be evaluated, was not possible to extract, and therefore it is not possible to express a collected opinion on which factors hANP might affect and improve.

Of course, all of the factors ought to be studied to be able to conclude whether hANP treatment is beneficial as additional treatment in the pediatric post-cardiac surgery population. If another retrospective cohort study will be attempted, it is important to find a control group from a ward where hANP is not part of standard treatment, in order to avoid selection bias. Although, a randomized controlled study would be preferable to evaluate the actual effects of hANP in the pediatric population.

CONCLUSION

There are a lot of interesting findings in this study. The major finding was that the risk for dialysis increased along with CPB duration. No association could be seen between incidence of dialysis and hANP treatment.

The significant longer LOS in the hANP group is another finding that ought to be of interest. Due to the selection bias this finding should be considered inconclusive.

Selection bias is a common issue when speaking of retrospective cohort studies. In this case, as discussed, the selection bias leads to impossibility to conclude anything regarding the actual effects of hANP treatment. Therefore further studies are needed to determine whether hANP treatment is beneficial or not.

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POPULÄRVETENSKAPLIG SAMMANFATTNING

Akut njurskada är en vanlig komplikation hos barn efter korrigerande hjärtkirurgi med hjärt-lungmaskin. Akut njurskada innebär att njurfunktionen drastiskt försämras temporärt. Försämringen mäts ofta med hjälp av urinproduktion samt biomarkörer så som kreatinin. För att diagnostisera akut njurskada krävs minst en 50%ig ökning av kreatininvärdet alternativt en urinproduktion under 0,5 ml/kg/h under 6 h.

Akut njurskada är en svårhanterad komplikation som kan kräva aggressiv terapi och få förödande konsekvenser.

Vanligtvis används vätskedrivande läkemedel (furosemid) för att vända förloppet. Då furosemid inte har fullgod effekt kan dialys initieras.

På Drottning Silvias Barnsjukhus i Göteborg har ett annat vätskedrivande preparat, humant ANP (hANP), använts i kombination med furosemid det senaste decenniet.

Behandlingstrappan kan beskrivas som initial behandling med furosemid. Då furosemid inte har fullgod effekt tillsätts hANP. Därefter kan dialys startas vid behov. ANP är en kroppsegen substans som produceras i hjärtats förmak. Därefter påverkar ANP blodflödet i njuren för att på så vis kunna öka filtreringen och blir därmed vätskedrivande. Dessutom har ANP effekter på hormonella system vilket leder till att natriumupptaget från urinen hämmas, och därmed har en additiv effekt vad kommer till ökat urinflöde.

Syftet med den här studien var att utvärdera effekten av kombinationsbehandling med hANP och furosemid med den sedvanliga behandlingen furosemid. Vi skulle observera huruvida någon skillnad i antal dagar på intensivvårdsavdelningen, risk för dialys samt vilken dag efter kirurgin som dialysbehandlingen initierades. Faktorer som skulle användas för detta ändamål inhämtades från patienternas journaler. Alla patienter var barn som mellan 1a januari 2010

och 31a december 2013 hade genomgått hjärtkirurgi med hjärt-lungmaskin på Drottning Silvias Barnsjukhus.

Resultaten visade inte på någon ökad risk för dialys behandlingarna emellan. Inte heller någon skillnad i antalet dagar från operation till start av dialys. Däremot kunde en ökad risk för dialys sättas i relation till antalet minuter spenderade på hjärt-lungmaskinen.

Utöver detta kunde vi se ett större antal dagar på intensivvårdsavdelningen bland de patienter som fick kombinationsbehandlingen.

Med tanke på att hANP numera är en del av behandlingstrappan vid akut njurskada på Drottning Silvias Barnsjukhus, är det dessvärre svårt att dra några slutsatser av denna studie. Då de barn som inte svarar på furosemidbehandling kräver en mer aggressiv behandling är det också rimligt att de behöver kvarvara på intensivvårdsavdelningen under längre tid. Därför är det viktigt att beakta att vidare studier är nödvändiga för att kunna utvärdera hANPs faktiska effekt på utfall efter akut njurskada hos barn som genomgått korrigerande hjärtkirurgi med hjärtlungmaskin.

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