

PROJECT SCHEDULE

Copeptin- Relevance as a Perioperative Marker in Pediatric Cardiac Surgery

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1. Summary

Objective:

Vasopressin is a hormone secreted by the posterior pituitary gland. Its main functions are the constriction of blood vessels and reabsorption of water and thereby regulating water balance and hemodynamics of the body. Copeptin is a cleavage product in the synthesis of vasopressin and is being released in equimolar amounts. Its stability makes it easier to determine than vasopressin.

The use of a heart-lung machine in cardiac procedures has important effects on the water balance of the body and affects vasopressin secretion.

This prospective clinical trial aims to figure out if copeptin would be suitable for perioperative risk evaluation in neonates and children with congenital heart disease after surgery with the use of a heart-lung machine.

Hypotheses:

The primary outcome endpoint of this study is the change in copeptin concentration perioperatively in patients with congenital heart disease, with the use of heart-lung machine.

Secondary outcome endpoints are correlations between copeptin concentration and complexity of the procedure (based on the aristotle basic score), clinical status and the perfusion times of the heart-lung machine.

Material and Methods:

The study population consists of 81 subjects, newborns, infants and children up to six years of age with congenital heart disease, undergoing surgery with the use of a heart lung machine.

To determine the main outcome variable, three blood samples will be taken from each patient perioperatively in order to evaluate changes in copeptin concentration.

Relevance and Implications:

This will be the first study focusing on correlations of copeptin concentrations with different variables in pediatric cardiac surgery. Findings may allow to use copeptin perioperatively to evaluate the clinical and hemodynamic situation of patients after congenital heart surgery.

2. Background

Vasopressin - Synthesis and Secretion:

Vasopressin is a polypeptid built of 9 amino acids. It is synthesized in the magnocellular neurons, the paraventricular nucleus and supraoptic nucleus of the anterior hypothalamus. These neurons have axonal projections which connect to the posterior pituitary gland where the hormone is carried through.

After posttranslational processing the product, prepro-AVP-NP11, is being cleaved by endopeptidases into AVP, neurophysin II (95 amino acids) and copeptin (39 amino acids) in equal amounts. By opening voltage-gated calcium channels, neurosecretory granules start fusing with the nerve terminal membrane and release AVP, NP11 and copeptin in equimolar amounts into the circulation.

Neurophysin and copeptin have no known role in peripheral tissues. Since both polypeptides are being released in equal amounts, copeptin can be used as a reliable surrogate marker for vasopressin secretion.

Studies also showed that copeptin is elevated in critically ill patients, after myocardial infarction, septic shock and chronic heart failure.

Cellular Action

Vasopressin binds to G-protein-coupled receptors called vasopressin receptors that are classified into V1, V2 and V3 subtypes. The V1 receptor is found in vascular smooth muscle, liver, platelets and central nervous system and is Gq-mediated and therefore results in an increase of intracellular calcium which leads to vasoconstriction. The V2 receptor is expressed on the basolateral membrane of the collecting ducts of the kidney and stimulates movement of aquaporin-2 to the apical membrane, which allow reabsorption of water. The V3

receptor is found in the anterior pituitary gland and acts synergistically with CRF causing secretion of ACTH.

Copeptin

The measurement of AVP is technically challenging because of its instability and the large amount attached to platelets. Therefore copeptin is used as a surrogate marker. It can be measured in serum and plasma samples by sandwich immunoassay. Its ex vivo stability for at least 7 days in serum and citrate-, EDTA-, and heparin plasma at 4°C was demonstrated. Studies also showed that there is no association in copeptin levels with age.

Heart-Lung Machine

The heart-lung machine takes up the perfusion work of the heart and additionally oxygenates the carried blood. It is a vital tool in cardiac surgery and enables complex procedures to be performed.

It is important to pay close attention to the effects of the heart-lung machine in the postoperative period including: volume shift from intravascular to interstitial, peripheral vasoconstriction, myocardial depression and altered hemodynamics without adaption to recent flow conditions. Therefore it also affects synthesis and secretion of vasopressin.

3. Aims of this study

This prospective clinical trial aims to assess changes in copeptin levels perioperatively in patients with congenital heart disease undergoing corrective or palliative surgery with the use of a heart-lung machine by analysis of three blood samples of each patient.

4. Study population

The study population consists of 81 patients (neonates, infants, children) with congenital heart disease and the need of surgery with a heart-lung machine.

Parental consent after pre-operation discussion and informing of the study is required.

Exclusion criteria are following: need of preoperative mechanical circulatory support, preoperative mechanical ventilation, Diabetes insipidus, preoperative cardio pulmonary resuscitation, no consent of the parents and preoperative shock or sepsis.

5. Methods

After induction of anesthesia every patient undergoing cardiac surgery will get monitored by vascular access. The anesthesiologist will place an arterial catheter for invasive blood pressure and a central venous catheter for applying medication and measurement of central venous pressure. We are planning to use these catheters for taking the blood samples which means that we will not place an additional syringe for the study.

For measurement of copeptin 900µl of blood is taken at three different time points. The first sample will be taken before the surgery in the operation room. The second sample will be taken after the surgery in the operation room and the third sample, one day after the surgery in the intensive care unit. The exact times will be recorded in report sheets.

After the surgery the cardiopulmonary bypass times and the exact procedures will be recorded. The performed procedures are needed in regard to calculating the Aristotle score which determines the complexity of the procedure.

The extubation time and the length of stay in the intensive care unit as well as any important events (any complications) in the postoperative period are going to be recorded.

The samples will be stored at the Meduni Wien Biobank until all samples are collected. After collection of these data for 81 patients the samples will be analyzed by the laboratory and the collected data will be prepared for statistical analysis.

Sample size

Sample size calculation was performed based on a paired t-Test. Due to a prior study we knew that the major change of copeptin concentration would be between the first and second measurement. Assumptions of the needed variables were estimated based on this study.

As a result a sample size of 73 patients will have 80% power to detect a difference in means of -0,25 (e.g. a first condition mean, m_1 , of 0,086 and a second condition mean, m_2 , of 0,336), assuming a standard deviation of differences of 0,75, using a paired t-test with a 0,05 two-sided significance level. Based on clinical experience the dropout rate will be determined at 10%. That implies a total sample size of 81 patients.

6. Statistical analysis

Descriptive Statistics

Statistical analysis will be performed using SPSS. Variables for the description of the population are the distribution of age, gender and weight. For describing numerical data of age and weight will be used, dependent on the distribution, means and standard deviations or median and minimum-maximum values.

Surgery related variables for further descriptive statistics are cardiopulmonary bypass time, aortic cross clamp time and complexity level of aristotle score. Cardiopulmonary bypass and aortic cross clamp time will be described by either mean or median depending on the distribution.

Postoperative data variables for descriptive analysis are length of ICU stay (in days), intubation time (in hours). Length of ICU stay and intubation time will be shown by either mean or median depending on the distribution.

Descriptive statistics of copeptin values consist of mean values and standard deviations of the logarithmized collected data. Three Scattergrams of the logarithmized data will be displayed of the first and second measurement, first and third measurement and second and third measurement.

Relevant descriptive data will be graphically shown in an appropriate manner.

Table of descriptive variables

Variables	Units	Variable type
Age	in years	metric
Gender	girl/boy	nominal
Weight	in kg	metric
CPB Time	in minutes	metric
AoXC Time	in minutes	metric
Complexity level	1-4	ordinal
LOS- ICU	in days	metric
Intubation Time	in hours	metric
Copeptin	in ng/ml	metric

Analytical statistics of copeptin values

Since three samples at different times will be taken of one patient, we are going to compare paired means. To compare the copeptin levels of the first and the second measurements, will be used paired t-test with a 0,05 two-sided significance level. Analyses will be performed using SPSS.

The null hypothesis implies that the means of the first and the second measurements are not significantly different. The alternative hypothesis implies that the difference of both means is significant. As copeptin is not expected to be normally distributed, logarithmic e-transformations will be performed.

This test will be repeated for the second and the third measurements and for the first and the third measurements.

Correlations

Secondary outcome endpoints are correlations between copeptin concentration and complexity of the procedure (based on the aristotle basic score), clinical status (based on length of ICU stay and intubation time) and the perfusion times of the heart-lung machine.

Pearson's correlations coefficient will be used for calculating the correlation of copeptin concentrations with intubation time (in hours), length of ICU stay (in days), the aristotle basic score of each procedure and the times of the heart-lung machine. Data of each correlation will be shown in a scattergram.

7. Ethical aspects

We tried scheduling this study without any disadvantages or discomfort for the subjects. Vascular access for the blood samples will be placed by the anesthesiologists for every patient undergoing cardiac surgery.

Also the amount of blood needed for copeptin measurement will be calculated as low as possible.

Prior to inclusion in the study parents will be asked for written informed consent. Parents or participants can decide to withdraw from the study at any time and can also be removed from the trial by the investigator if exclusion criteria are met. No side effects are expected from taking this amount of blood samples.

The study will be performed in accordance with the Declaration of Helsinki (1964), including all current revisions, and the guidelines for Good Scientific Practice required by the Medical University of Vienna.

First application for approval of this study and all other procedures involved by the Ethics Committee of the Medical University of Vienna will be made in January 2017.

8. Data handling and record keeping

All patients will be coded with an ongoing number (pseudonymized). The data will be collected with protocol sheets and then computerized coded in an SPSS file for analysis. The access for the computer will be restricted to members of the study. Only authorized individuals will have access to the original data.

9. Time schedule

	Sept. 2016	Nov. 2016	Dec. 2016	Feb.201 7	May- Nov. 2017	Dec. 2017	Jan.- March 2018
Literature research							
Sample size calculation							
Project schedule							
Ethics application							
Data collection							
Data analysis							
Writing thesis							

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