COpeptin in critically ill
Paediatric and Neonatal Intensive Care patients
and its association with arterial hypotension
A single-centre prospective observational study
The COPNIC-Study

Type of Research Project:	Research project in which biological material is sampled from humans and health-related personal data is collected
Risk Categorisation:	Risk category A
Project Identifier:	COPNIC-Study
Project Leader:	Vincenzo Cannizzaro, MD, PhD University Children`s Hospital Zurich Steinwiesstrasse 75 CH-8032 Zurich Telefon +41 44 266 7111 Vincenzo.cannizzaro@kispi.uzh.ch www.kispi.uzh.ch
Health condition / problem	Critical illness and hypotension in paediatric and neonatal intensive care patients
Project Duration	36 months
Project Plan Version and Date:	2.2 17/08/2017

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SIGNATURE PAGE(S)

Project number   COPNIC-Study
Project Title   COpeptin in critically ill
                    Paediatric and Neonatal Intensive Care patients
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The project leader and the methodologist *(Verena Gotta)* have approved the research plan 2.2 17/08/2017, and confirm hereby to conduct the project according to the plan, the current version of the World Medical Association Declaration of Helsinki, the Principles of Good Clinical Practice (GCP) and the local legally applicable requirements.

Project Leader:
Vincenzo Cannizzaro, MD, PhD

Zurich, 7.9.17
Place/Date   Signature

Project Methodologist:
Verena Gotta

Basel, 07.09.2017
Place/Date   Signature

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SYNOPSIS (SUMMARY)

Project Leader (or Sponsor)	PD Dr. med. Vincenzo Cannizzaro, PhD Universitäts-Kinderspital Zürich Steinwiesstrasse 75 CH-8032 Zürich Telefon +41 44 266 7111 Vincenzo.cannizzaro@kispi.uzh.ch www.kispi.uzh.ch
Project Title:	Zusammenhang zwischen dem neuen Blutwert Copeptin und niedrigem Blutdruck bei Kindern und Jugendlichen auf der Intensivstation
Short Title / Project ID:	COPNIC-Study KEK 2017-00451
Project Plan Version and Date:	2.2 17/08/2017
Risk categorisation:	Risiko Kategorie A
Type of Research:	Forschungsprojekt, während dessen biologisches Material von Menschen entnommen wird und gesundheitsbezogene persönliche Daten gesammelt werden.
Project design:	Prospektive Beobachtungsstudie an einem Zentrum
Background and Rationale:	Auf einer Intensivstation leiden kritisch kranke pädiatrische Patienten häufig unter niedrigem arteriellem Blutdruck. Dies kann verschiedene Gründe haben und in verschiedenen klinischen Situationen mit Herz-Kreislauf- und Atemversagen auftreten, z. B. im septischen Schock, bei peripartaler Asphyxie, bei Schädel-Hirn-Trauma und nach verschiedenen Operationen. Die intensivmedizinischen Standardbehandlungen für arterielle Hypotension bestehen aus intravenösen Volumengaben und Katecholamintherapie. Beide Behandlungen können sehr effektiv sein, bergen aber auch schwerwiegende Risiken für Komplikationen: Lungenödem, verlängerte Beatmungszeit, verlängerter Aufenthalt auf der Intensivstation, Arrhythmien, erhöhter Sauerstoffbedarf des Herzmuskels, etc. Es ist wünschenswert, die Volumen- und Katecholamingaben sinnvoll einzuschränken, um die Nebenwirkungen zu reduzieren. In den letzten Jahren konnte gezeigt werden, dass ein Teil der intensivmedizinischen Patienten einen zumindest vorrübergehenden Mangel des körpereigenen, stark vasoaktiven Hormons Vasopressin aufweist und dass diese Patienten von einer intravenösen Vasopressingabe profitieren könnten. Kinder und Jugendliche sind bisher nur in wenigen und kleinen Studien untersucht worden und es gibt bisher in der klinischen Routine keine etablierte Möglichkeit, Patienten mit einem Vasopressinmangel zu erkennen. In dieser Studie wird zu diesem Zweck Copeptin im Blut gemessen, da Vasopressin zum einen selbst schwer zu messen ist, und zum anderen, weil Copeptin äquimolar zum Vasopressin in die Blutbahn ausgeschüttet wird. Mittels Copeptin-gesteuerter intravenöser Vasopressintherapie bei Patienten mit einem Mangel an körpereigenem Vasopressin könnte die risikoreiche Volumen- und Katecholamintherapie gezielt unterstützt werden und Flüssigkeitsvolumen und Katecholamine eingespart werden
Objective(s):	In dieser prospektiven Beobachtungstudie wird der Verlauf des Biomarkers Copeptin im Blut kritisch kranker Kinder und Jugendlicher untersucht. Speziell werden die Assoziationen mit arterieller Hypotension und verschiedenen Covariablen/Krankheiten geprüft, um Rückschlüsse auf einen relevanten Vasopressinmangel zu ziehen.

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Endpoint(s):	Primärer Endpunkt

	Mittlere Veränderung von Blut-Copeptinspiegeln bei kritisch kranken Kindern und Jugendlichen mit arterieller Normo- und Hypotension über 48 Stunden nach Aufnahme auf die Intensivstation des Kinderspitals Zürich. In einem zweiten Schritt werden der Verlauf und die Variabilität der Blut-Copeptinspiegel über einen Zeitraum von bis zu 7 Tagen charakterisiert.
	
	Es werden Assoziationen mit folgenden klinischen Co-Variablen geprüft:
	• Arterieller Blutdruck
	• Septischer Schock
	• Herzinsuffizienz
	• Schädelhirntrauma, Hirntumor und/oder intrakranielle Operation
	• Perinatale Asphyxie
	• Krankheitsschwere
	• Alter
	
	Sekundäre Endpunkte
	• Prädiktive Assoziationen von Copeptin mit folgenden klinischen Outcome-Parametern:
	  – Dauer des Aufenthaltes auf der Intensivstation.
	  – Dauer der respiratorischen Unterstützung
	  – Dauer und Dosis der Katecholamintherapie
	  – Todesrate bei 28 Tagen
	• Dynamischer Zusammenhang zwischen Blutdruck, Copeptinspiegeln und Katecholamintherapie.

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Inclusion / Exclusion criteria:	Einschlusskriterien: Alle kritisch kranken pädiatrischen Patienten unabhängig von deren Alter, Erkrankung oder Krankheitsschwere kommen für den Einschluss in diese Studie in Frage. Die Blutcopeptinwerte von Patienten mit arterieller Hypotension werden mit den Werten von Patienten verglichen, die einen normalen Blutdruck aufweisen und keine medikamentöse Blutdruckunterstützung benötigen. Diese letztgenannten Patienten werden aufgrund fehlender international anerkannter Normalwerte zur Berechnung der Copeptin-Referenzbereiche benötigt. Es werden daher alle Patienten im genannten Altersbereich, die auf die Intensivstation des Kinderspitals Zürich eintreten, in diese Studie eingeschlossen, sofern die folgenden Einschlusskriterien und keines der Ausschlusskriterien erfüllt sind: • Alter: Erster Lebenstag bis <18 Geburtstag. • Fähigkeit der für das Kind sorgeberechtigten Person oder der/des Jugendlichen (falls ≥14 Jahre), mündliche und schriftliche Informationen sowie die Einwilligungserklärung in Deutsch zu verstehen. Ausschlusskriterien: • Sorgeberechtigte Person oder Patient(-in) (falls ≥14 Jahre) nicht fähig, mündliche und schriftliche Informationen sowie die Einwilligungserklärung in Deutsch zu verstehen. • Unmöglichkeit der Einwilligung aus anderen Gründen (beispielsweise Abwesenheit von sorgeberechtigten Personen oder tiefe Sedation der/des Jugendlichen (falls ≥14 Jahre) für >24 Stunden bei Abwesenheit eines gesetzlichen Vertreters). • Ablehnen der Studienteilnahme durch die sorgeberechtigte Person oder den/die Jugendliche (falls ≥14 Jahre und sediert für < 24h). In diese Studie werden vulnerable Patientengruppen eingeschlossen: Neugeborene, Kinder und Jugendliche. Zum Teil sind diese Patienten nicht auskunftsfähig und die Eltern oder die gesetzlichen Vertreter müssen an ihrer statt in die Studie einwilligen. Der Einschluss der genannten Patienten ist unumgänglich, da zum einen die Daten zu pädiatrischen Patienten bisher nur in geringem Ausmaß vorhanden sind. Zum anderen leiden die jüngeren Patienten besonders stark unter den Nebenwirkungen der Katecholamin- und Flüssigkeitstherapie. Die Entwicklung eines Biomarkers zur Identifikation von Patienten, die auf eine intravenöse Vasopressingabe ansprechen und somit weniger Katecholamin- und Flüssigkeitstherapie benötigen, ist besonders bei diesen Patientengruppen wünschenswert.
Project assessments, procedures:	Während der Routinebehandlung kritisch kranker Kinder und Jugendlicher auf der Intensivstation des Kinderspitals Zürich wird zu verschiedenen Zeitpunkten (Aufnahme, 12, 24, 48, 96, 168 Stunden) das Blutcopeptin der Patienten gemessen. Der Zeitpunkt der Blutentnahme wird durch anderweitig medizinisch indizierte Blutentnahmen festgelegt, damit keine zusätzlichen Manipulationen an zentralvenösen oder arteriellen Kathetern und keine zusätzlichen schmerzhaften Blutentnahmen erforderlich werden. Es wird die Assoziation des Copeptins mit verschiedenen klinischen Diagnosen, Vital- und Laborparametern untersucht, die im klinischen Alltag alle open-label vorhanden sind. Diese Co-Variablen werden auf den Papier-Case-Report-Forms notiert und sind zusammenfassend im SCHEDULE OF ASSESSMENTS, Seite 11 dieses Protokolls, einzusehen.
Number of Participants:	Diese Studie erreicht eine Power von 0.8 bei einer Effektstärke von 0.36 und einem Signifikanzniveau von 0.05 bei 62 Patienten (siehe unten; Statistical Analysis incl. Power Analysis). Da innert den ersten 48 Stunden eine Drop-Out-Rate von 60% durch geplante Entlassung von der Intensivstation zu erwarten ist und bis zu 10% der Copeptinwerte außerhalb des Messbereichs liegen, muss die Patientenzahl entsprechend höher angesetzt werden. Insgesamt werden daher: 62 x 2.5 x 1.1 = **170 Patienten** in diese Studie eingeschlossen.
Project Duration, schedule:	Gesamtdauer: 36 Monate Patienteneinschlussphase: 18 Monate (01.10.2017 - 31.03.2018). Datenanalyse, Konferenzen und Publikation: 18 Monate (01.04.2019-30.09.2020).

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Project Centre(s):	Universitäts-Kinderspital Zürich Steinwiesstrasse 75 CH-8032 Zürich
Statistical Considerations:	Diese Studie ist eine Beobachtungstudie mit zu erwartenden deskriptiven Ergebnissen. Die Studie wird gepowert für den primären Endpunkt, der mittleren Veränderung des Blut-Copeptins in den ersten 48 Stunden nach Aufnahme auf die Intensivstation bei kritisch kranken Kindern und Jugendlichen. Folgende Faktoren sind dabei zu berücksichtigen: • Basierend auf den verfügbaren publizierten Daten wird bei 48 Stunden eine mittlere Copeptin-Differenz (zum Aufnahmezeitpunkt; d) von 0.3 ng/ml (Inter-Quartilenbereich: 1.1 ng/ml) erwartet. Daraus ergeben sich die Standardabweichung (sd) von 0.82 ng/ml und die Effektstärke (d/sd) von 0.36. • 60% der Patienten werden potentiell innert 48 Stunden verlegt. • 90% der gewonnenen Werte liegen im messbaren Bereich. • Mit den Voraussetzungen 0.8 Power, Signifikanzniveau 0.05, Effektstärke 0.36, 60 % Drop-out, 10% außerhalb des messbaren Bereichs errechnet sich eine Patientenzahl von 170 Patienten.
Other methodological Considerations:	Alle eingeschlossenen Patienten werden in die Analyse für den primären Endpunkt aufgenommen. 60% der Patienten der Intensivstation werden innert 48 Stunden verlegt, daher muss die Patientenzahl entsprechend erhöht werden. Von besonderem Interesse sind darüber hinaus die Patienten, die länger als 48 Stunden hospitalisiert bleiben, dies sind in der Regel schwer kranke Patienten. Daher werden zunächst die Blut- Copeptin-Werte aller eingeschlossenen Patienten innert den ersten 48 Stunden analysiert und in einem zweiten Schritt die Copeptin-Werte der Patienten analysiert, die bis zu 7 Tagen bleiben (13% aller Patienten bleiben länger als 7 Tage auf der Intensivstation des Kinderspital hospitalisiert).
Risk-Benefit statement:	Das Wesen dieser Studie besteht aus einem Vergleich von Blut-Copeptin-Werten mit routinemäßig vorhandenen klinischen Parametern. Die entnommene Blutmenge (600 µl) ist nicht relevant für die Patientenpopulation. Für dieses Projekt werden alle geplanten Blutentnahmen zusammen mit anderweitig medizinisch indizierten Blutentnahmen durchgeführt. Es werden keine zusätzlichen Blutentnahmen durchgeführt, um keine zusätzlichen Manipulationen an arteriellen oder zentralvenösen Kathetern mit erhöhtem Infektionsrisiko zu verursachen. Des Weiteren wird auf zusätzliche invasive Blutentnahmen verzichtet, um keine zusätzlichen Schmerzen zu verursachen. Die Belastung der Patienten durch die Studie wird als gering eingeschätzt. Die eingeschlossenen Patienten profitieren nicht von der Studie, können aber potentiell mithelfen, in Zukunft eine gezielte Unterstützung von schwerkranken, hypotensiven Patienten durch Vasopressin zu fördern und somit den risikoreichen und schädlichen Gebrauch von Katecholaminen und Flüssigkeit zu vermindern.

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ABBREVIATIONS

DoH	Declaration of Helsinki
EC	Ethics Committee
EGEP	Essentials of Good Epidemiological Practice
FOPH	Federal Office for Public Health
HRA	Federal Act on Research involving Human Beings (Human Research Act, HRA)
HRO	Ordinance on Human Research with the Exception of Clinical Trials (Human Research Ordinance, HRO)
ID	Identification
IIT	Investigator-initiated Trial
SE	Serious event
STROBE	Strengthening the reporting of observational studies in epidemiology
PIM	Paediatric Index Of Mortality
SAPS	Simplified Acute Physiology Score
CRIB	Clinical Risk Index for Babies
ICU	Intensive Care Unit
PaO_2	PaO_2 Partial arterial oxygen pressure
$PaCO_2$	$PaCO_2$ Partial arterial carbon dioxide pressure
CT	Computed tomography
ALT	Alanin-Transaminase
MRI	Magnetic Resonance Imaging
AVP	Arginine-Vasopressin

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SCHEDULE OF ASSESSMENTS (FLOW OF RESEARCH PROJECT)

Project Periods					If patients still in ICU	
Time (hours)	Admission ICU	12*	24*	48*	96*	168*
Information, Informed Consent	x					
In- /Exclusion Criteria	x					
Age (years + months); if neonatal (<28 d): postnatal days + postconceptional age	x					
Diagnosis	x					
Disease-Severity-Scores[a]	x					
Operation, perioperative parameters[b]	x	x	x	x	x	x
Weight	x					
Sex	x					
Primary Variables assessment						
Blood sample for copeptin (600 µl)	x	x	x	x	x	x
Arterial blood pressure	x	x	x	x	x	x
Secondary Variables assessment						
Vital parameters[c]	x	x	x	x	x	x
Respiratory support	x	x	x	x	x	x
Vasoactive Drugs	x	x	x	x	x	x
Other Drugs	x	x	x	x	x	x
Fluid administration/24h each day	x	x	x	x	x	x
Laboratory values[d]	x	x	x	x	x	x
Neurological/neonatal findings[e]	x	x	x	x	x	x
Organ dysfunction/mechanical resuscitation	x	x	x	x	x	x
Serious (Adverse) Events/Death	x	x	x	x	x	x
Discharge date from ICU/Hospital	When applicable					

a) Paediatric Index Of Mortality, PIM II; Simplified Acute Physiology Score, SAPS II; Clinical Risk Index for Babies, CRIB II

b) Preoperative medication, anaesthetics, type of surgery. If cardiac surgery in addition: cardiac lesion, echocardiographic and cardiac catheter results, periods of cardiopulmonary bypass, cross clamping, deep hypothermic arrest, modified ultrafiltration, postoperative echocardiographic results.

c) Respiratory rate, peripheral oxygen saturation, arterial oxygen saturation, heart rate, peripheral pulses, capillary refill, urine output.

d) Sodium, potassium, chloride, calcium, troponin, creatine kinase, creatine kinase MB, pH, partial arterial oxygen pressure (PaO2), partial carbon arterial carbon dioxide pressure (PaCO2), base deficit, arterial lactate, glucose, alanine transaminase (ALT), creatinine, C-reactive protein, haemoglobin, white blood cell count, total bilirubin; all if medically indicated.

e) Glasgow Coma Scale, CT-findings (abnormal cisterns, midline shift >5mm, traumatic subarachnoidal hemorrhage), seizures, intracranial and cerebral perfusion pressure (if invasively monitored); Apgar-score, arterial umbilical cord pH, results of neurological imaging (cranial ultrasound and/or MRI), and performance of neonatal cooling as prevention of neurological damage.

* Exact time points will be determined by other medical indications of blood sampling.

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1. ADMINISTRATIVE STRUCTURE

Project Leader

Name: Vincenzo Cannizzaro, MD, PhD
Address: University Children`s Hospital Zurich
Steinwiesstrasse 75
CH-8032 Zurich
Email: Vincenzo.cannizzaro@kispi.uzh.ch
Phone: +41 44 266 7111
Fax: +41 44 266 7168

Project site(s) and responsible researcher:

Institution: University Children`s Hospital Zurich
Name: Philipp Baumann, MD
Address: Steinwiesstrasse 75, CH-8032 Zurich
Email: philipp.baumann@kispi.uzh.ch
Phone: +41 44 266 7111
Fax: +41 44 266 7168

Key Persons involved in research project:

Name: Markus Deisenberg, MD
Address: Steinwiesstrasse 75, CH-8032 Zurich
Email: kevin.schmid@kispi.uzh.ch
Phone: +41 44 266 7111
Fax: +41 44 266 7168

Other personnel

Biostatistician:

Name: Verena Gotta
Address: Pediatric Pharmacology and
Pharmacometrics
University of Basel Children's Hospital
Spitalstrasse 33, CH-4056 Basel
Email: Verena.Gotta@ukbb.ch
Phone: + 41 61 704 1212

Laboratory:

Institution: Institute of Clinical Chemistry
University Children`s Hospital Zurich
Name: Martin Hersberger, PhD
Address: Steinwiesstrasse 75, 8032 Zurich
Email: martin.hersberger@kispi.uzh.ch
Phone: +41 44 266 75 41
Fax: +41 44 266 71 69

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2. ETHICAL AND REGULATORY ASPECTS

2.1 Ethical Conduct of Study

The research project will be carried out in accordance to the research plan and with principles enunciated in the current version of the Declaration of Helsinki (DoH), the Essentials of Good Epidemiological Practice issued by Public Health Schweiz (EGEP), the Swiss Law and Swiss regulatory authority's requirements as applicable, the full responsibility for this lies with the project leader. The EC and regulatory will be informed about project start and termination.

2.2 Risk categorisation

This is an observation study with risk category A involving human beings (critical ill children) without any kind of medical intervention. Blood samples will be taken during regular clinical blood sampling to measure blood levels of copeptin. Blood sampling and data storage are considered to be of minor clinical and personal relevance for the study patients.

2.3 Ethics Committee (EC) and Competent Authorities (CA), FOPH

Kantonale Ethikkommission Zürich
Stampfenbachstrasse 121
8090 Zürich

2.4 Participant Information and Informed Consent

All patient care takers and adolescents (if age≥14 years) have to give written informed consent. Informed consent forms will be only available in German. For enrolment, patients, parents, family members or other care takers must have sufficient knowledge of German to understand the instructions, given by their treating intensive care specialist. Written informed consent for study inclusion should be obtained from patients (if ≥ 14 years of age), patients` parents or care takers before first study relevant blood sample is taken and before first patient data are recorded.

In this study we have to include vulnerable patients, please see chapter 3.3 risk-benefit assessment.

This study may include patients, who themselves or whose caretakers may not be able to give written informed consent in emergency situations. This may be due to acute clinical deterioration, resuscitation and/or sedation of the patients. Further, care takers may not be present or accountable for formal medical explanations and may not be able to give informed consent at the time of intensive care unit admission due to the deterioration of their child. In this case, blood samples for copeptin will be taken, but not analyzed. As soon as possible, but latest 24 hours following intensive care unit admission, written informed consent will be obtained from care takers, if patient < 14 years or if patient ≥ 14 years and sedated for more than 24 hours. If the adolescent (≥14 years of age) stays sedated or unable to give informed consent for more than 24 hours, the presumptive will of the adolescent has to be taken into account by the legal care takers when giving informed consent in her/his place. Written informed consent will be obtained from the patient her-/himself, if ≥ 14 years and sedated for less than 24 hours in agreement with HFG Art. 30, Abs. 2. If a legal care taker gives informed consent in place of an adolescent (≥14 years of age), the informed consent must be obtained from the adolescent her-/himself as soon as the adolescent is able to give consent her-/himself. If the adolescent is not willed to give consent, all data and blood products obtained until then will be destroyed in this case. The volume of blood necessary for the copeptin analysis (600 µl each sample) is not relevant for any age group including neonates. The additional clinical data needed for primary and secondary endpoint assessment is open-label. Study relevant data is available in medical files and electronic patient data management systems of the intensive care ward anyway. In addition, copeptin analyses will be performed only for blood samples from patients with written informed consent using a pooled batch of blood samples several weeks later.

In case written informed consent cannot be obtained within 24 hours after admission, the patient will not be included in the study, all blood samples will be destroyed and no patient data will be analyzed (in agreement with HFG Art. 31 Abs.1+2).

The patients included in this study will not directly benefit from the study. However, the expected added scientific and clinical value has the potential to significantly improve future patient safety and management in emergency and critical care situations.

2.5 Participant privacy and safety

The Project Leader affirms and upholds the principle of the participants' right to dignity, privacy and health and that the project team will comply with applicable privacy laws. Especially, anonymity of the participants will be guaranteed when presenting data at scientific meetings or publishing in scientific journals.

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Individual participant medical information obtained as a result of this research project is considered confidential and disclosure to third parties is prohibited. Participant confidentiality will be further ensured by utilising identification code numbers to correspond to medical information in the computer files.

For data verification purposes, authorised representatives of the Sponsor, a competent authority or an ethics committee may require direct access to parts of the medical records relevant to the project, including participants' medical history.

According to HFG Art. 30 Abs. 1 lit. c it is necessary to obtain written confirmation of a physician not related to the study, that the personal and legal rights of the unconscious patient are granted. The volume of blood necessary for the copeptin analysis (600 µl each sample) is not relevant or harmful for any age group. Therefore, it is deemed, that it is not necessary to obtain the mentioned written confirmation by a physician not related to the study.

2.6 Early termination of project

This study compares blood values with clinical conditions in an observational manner. Given the small blood volume sampled for each copeptin measurement (600 µl) serious adverse events related to the study and leading to early termination of the study are highly unlikely. Therefore, no termination criteria have to be defined.

However, the premature end or interruption of the research project for any reason will be reported to the EC within 90 days by the project leader upon completion of the project (HRO Art. 22).

2.7 Amendments, Changes

Significant changes to the project plan will have to be approved by the EC (amendments, if applicable according HRO Art. 18).

The Project Leader will submit to the EC any application documents specified in Annex 2, which are affected by the change. At the same time, the project leader will provide information on the reasons for the change.

Substantial amendments are only implemented after approval of the EC.

3. INTRODUCTION

3.1 Background

The aim of this prospective observational study is to characterize the course and progression of the biomarker copeptin in critically ill neonates and children. Copeptin values have the potential to be associated with outcome and to serve as a therapeutic guide for exogenous vasopressin administration in severe arterial hypotension and shock.

Paediatric intensive care patients very often suffer severe arterial hypotension in various clinical situations with cardiac and respiratory failure, e.g. septic shock, asphyxia, brain injury, and after several types of operations. Septic shock is associated with a very high mortality up to 50% mainly due to an excessive rate of hypoperfusion-related multiorgan dysfunction (up to 80% in severe sepsis or septic shock)[1]. Treatment for shock in general includes fluid and catecholamine administration to maintain adequate blood flow and sufficient oxygen transport to peripheral tissues[2-6]. Fluid resuscitation may lead to volume overload and pulmonary edema bearing additional risks[7]. In addition, large volumes of fluids administered over a short period of time may lead to heart failure[8]. Though catecholamines deliver strong fluid sparing arterial pressure support, they can place the patient with instable cardiovascular state at risk for severe complications (i.e. arrhythmia, right and left ventricular after load augmentation, reduced tissue blood flow)[9-12].

In recent years, several authors have reported the use of intravenous vasopressin in addition to or instead of standard antihypotensive treatment[6,13-20]. Vasopressin is not a catecholamine but a natural endogenous hormone with less toxic side effects. It may help to reduce fluid and catecholamine use. Its main endogenous function is maintenance of water- and electrolyte balance, as renal V2-Arginine-Vasopressin (AVP) receptors in the collecting duct mediate water reabsorption via induction of intracellular cAMP (Cyclic adenosine monophosphate) production and reversible translocation of the water channel "aquaporin 2" from cytoplasmic vesicles to the apical cell membrane[21]. But in addition, it has strong dose-dependent vasoconstrictive effects on the peripheral vessels mediated via the V1-vascular AVP-receptor[22].

The treatment of arterial hypotension with exogenous vasopressin has been proven to be safe and effective in adults, especially in septic vasodilatory shock as shown in a recent metaanalysis[23]. Following the first successful adult case reports, research activity on vasopressin treatment in children and adolescents augmented, but results so far are not as clear as in adults.

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Studies in children and neonates could demonstrate that exogenous AVP-administration (or administration of its long-lasting analogon terlipressin) can be effective to reduce catecholamine requirements and catecholamine-related side effects[15,24]. In addition, it is safe also in very vulnerable patients as extremely low birth weight infants (i.e. birth weight <1000g)[25]. Therefore, the exogenous AVP-use in neonates suffering arterial hypotension increases Europe-wide[26]. However, not all children treated with vasopressin reacted with an adequate rise of blood pressure[17,18,27]. An explanation may be that most paediatric patients (70%) react to critical cardiovascular situations with a strong elevation of endogenous vasopressin lasting up to 120 hours in vasodilatory shock[28,29]. This may at least partly explain the ability of children to maintain high blood pressure longer than adults in the early phase of beginning shock. Those patients with high endogenous AVP levels do not benefit from additional exogenous vasopressin and intravenous vasopressin treatment would be futile[30-32]. But a fraction of paediatric patients (30%), adolescents and children, show rather low endogenous vasopressin levels on several occasions including septic and hypovolemic shock. Exogenous vasopressin infusion seemed to be especially helpful for supporting blood pressure in those children, whose endogenous vasopressin levels were low (i.e. endogenous vasopressin insufficiency), but numbers of children identified so far differ, as pediatric studies are small[13,27,30].

Clinical identification of endogenous AVP-deficiency is difficult so far, as no clinical parameters help to establish the diagnosis of AVP-deficiency. In this situation, a blood biomarker indicating AVP-deficiency could be a solution to this problem. The measurement of endogenous AVP levels would be ideal for the identification of individuals in need of additional exogenous AVP, but measurement of endogenous blood AVP is not suitable for clinical use for the following reasons. First, laboratory assessment of endogenous vasopressin itself is time consuming and cumbersome due to its instable character *in vivo* (half life 24 minutes) and *ex vivo*, even frozen at -20°C[33,34]. Second, a large fraction of endogenous vasopressin is platelet-bound, potentially causing erroneous measurements[35]. Therefore, actual research activity focus on the simplified approach to measure blood copeptin levels instead of vasopressin. Copeptin is the 39-amino acid long C-terminal portion (C-terminal proAVP, CT-proAVP) of pre-provasopressin. It is cleaved in a 1:1 ratio to vasopressin from the precursor hormone pre-provasopressin in the hypothalamus and then released into the portal circulation of the neurohypophysis. The endogenous function of copeptin is unclear today. It is stable at room air for 7 days and up to 14 days when cooled at 4°C and therefore much more suitable for routine clinical use[36]. It correlates well with endogenous AVP levels in adults, especially in non-septic cases[37-39]. In children, the correlation to AVP was not favourable in septic shock, but excellent in young patients undergoing open heart surgery involving cardiopulmonary bypass[27,40]. But again, numbers in children are small. Furthermore, copeptin has been recognized as an excellent biomarker for non-favourable outcome in a number of pathologies and for stress- and severity of disease-assessment in children[41-44] as well as in adults[45-48]. As data on copeptin in the paediatric population are derived from small and observational studies so far, general further insight into copeptin dynamics in critically ill children and adolescents would be desirable for the evaluation of potential clinical use.

This study is designed to investigate the natural course and variability of copeptin concentrations in critically ill children and neonates. Specifically, this study aims to investigate patient characteristics that are associated with both arterial hypotension and low endogenous copeptin levels, and to explore the association of copeptin levels with clinical outcomes. This may allow identifying patients that could benefit the most from a copeptin-guided exogenous vasopressin administration as treatment of arterial hypotension. The individual reaction to exogenous vasopressin treatment will however not be investigated in this observational study, which would be subject to a follow-up interventional trial.

3.2   Rationale for the research project

Based on the background described above, the rationale for this study can be summarized as followed.
1) Many critically ill children and neonates need intensive care therapy to maintain adequate blood flow and arterial blood pressure. 2) The side effects of abundant fluid administration and catecholamine therapy may be harmful for patients in critical care. An additional fluid and catecholamine sparing therapy is desirable. 3) Some patients with persistent vascular hypotension show low endogenous vasopressin levels. 4) These patients have to be identified to administer exogenous vasopressin. 5) Identification of these patients can only be achieved via determination of the stable vasopressin-by-product copeptin rather than via direct laboratory assessment of the instable endogenous vasopressin.

Accurate identification of patients with persistent low vasopressin/copeptin levels would facilitate future individual vasopressin therapy to support and replace, at least in part, catecholamines and fluids in severe arterial hypotension and shock.
As numbers studied for the correlation of copeptin and arterial hypotension in paediatric and neonatal critical care settings are small, this prospective observational study has to be performed.
The actual effect of exogenous vasopressin therapy in patients with low blood copeptin levels will have to be tested in a follow-up interventional trial.

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3.3 Risk-Benefit Assessment

The study period will cover up to 168 hours in which a maximum of 6 blood samples (minimum 600 µl each) from peripheral venous lines or central venous and arterial catheters will be analysed for baseline copeptin distribution. The required amounts of blood volume for analyses are clinically not relevant for any age group. As critically ill patients frequently require invasive arterial blood pressure monitoring and central venous access for fluid and medicament administration, the required catheters for blood withdrawal are generally in place. Importantly, each study blood withdrawal will be <u>grouped together with otherwise medically indicated blood withdrawals</u>, causing no additional catheter manipulation, pain or harm for the patient. The comparison of blood copeptin and arterial hypotension will define the primary endpoint.

All further laboratory parameters proposed in "Schedule of Assessments (Flow of Research Project)" are considered to be secondary endpoints. Analyses of these laboratory values will only be included in the study, if their sampling is indicated for any other medical reason.

Children and neonates included in this study do not directly benefit from the study. However, the recruited patients help to identify future patient cohorts, which could benefit from individualized vasopressin administration supporting and replacing at least a part of the current standard fluid and catecholamine therapy in hypotension and shock. Moreover, the potential association between copeptin levels and outcome might provide new insights when it comes to counselling parents experiencing an emotionally challenging period of their lives.

Security of the collected data is given through data collection in especially signed folders (including case report forms) in the intensive care ward and through data storage in an especially signed electronic file (University Children`s Hospital Zurich). Data in the folders on the ward contain only data stored in clinical patient files anyway. Data will be managed strictly confidential, after initial data acquisition data will be coded through electronic storage with a study number and age (years + months, if <28 d in days); access to original data will only be given to the study personal and to institutions mentioned above. There will be no transfer of non-coded data to other storage systems outside the University Children`s Hospital Zurich.

4. OBJECTIVES, ENPOINTS/OUTCOMES AND OTHER STUDY VARIABLES

4.1 Objectives

Primary objective

The primary objective is to investigate the average change of blood copeptin levels in critically ill children and neonates with both arterial normo- and hypotension over 48h after intensive care unit (ICU) admission, and in a second step to describe the natural course and variability of copeptin levels over a period of up to 7 days after ICU admission. In this scope we will also investigate the association of patient characteristics (covariates) with blood copeptin baseline levels at ICU admission and the course of blood copeptin levels during ICU stay. In particular the association with the following factors will be investigated:
- Arterial blood pressure (in particular hypotension vs. normotension), to identify patients that may most benefit from exogenous AVP treatment
- Septic shock
- Heart failure
- Traumatic head injury, brain tumor and/or intracranial operation
- Perinatal asphyxia
- Severity of disease (disease score)
- Age

Secondary objective

The secondary objective is to explore the association of individual copeptin levels over up to 7 days with the following clinical outcomes:
a. Length of stay on the intensive care unit
b. Length of respiratory support
c. Length and dose of catecholamine therapy (as a marker of severity of disease and degree of arterial hypotension)
d. Rate of death at day 28,

and to explore the temporal and dynamic relationship between the course of blood pressure, blood copeptin levels and catecholamine therapy over time.

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4.2 Primary and secondary endpoint/outcome(s)

The primary endpoint of this study is descriptive, since the primary objective is simply to better understand which paediatric patients admitted to the ICU are likely to present with arterial hypotension in the presence of low copeptin levels over an observation period of up to 7 days. Results are expected to allow defining a group of patients that may benefit the most from exogenous AVP therapy. The effectiveness of exogenous AVP administration to patients with low AVP would have to be tested in a follow-up interventional trial. The patients participating in this present observational study will not receive any study medicaments.

Definition and measurement of variables relevant to primary endpoint:

Copeptin levels

Blood copeptin values measured by the B·R·A·H·M·S Copeptin KRYPTOR (Thermo Fisher Scientific, Hennigsdorf, Germany) in all critical ill children and neonates admitted to the intensive care unit (ICU) will be analysed. Time points for blood sampling will be at ICU admission and then determined by other medical indications but scheduled to be performed with approximately 12, 24, and 48. Time points for all patients staying longer than 48 hours will be 96 and 168 hours to capture the presumed copeptin pattern in children. For the endogenous active hormone AVP it could be shown by Lee et al. in 2013 that AVP stays elevated for 120 hours/5 days after onset of shock, but actual duration of AVP/copeptin elevation is unknown in children. Therefore, a last measurement with seven days will be included to show a potential decline of copeptin after the resolution of shock[28].

Arterial blood pressure and hypotension

Hypotension is defined as systolic arterial pressure below age specific 5th percentiles according to reference values established in the context of the last International Pediatric Sepsis Consensus Conference (published 2005): newborn 0 days to 1 week 59 mmHg, neonate 1 week to 1 month 79 mmHg, infant 1 month to 1 year 75 mmHg, toddlers and preschool children 1 to 5 years 74 mmHg, school age children 5 to 12 years 83 mmHg, adolescent and young adult 12 to 18 years 90 mmHg[49]. Blood pressure will be measured invasively via indwelling central or peripheral arterial catheters or non-invasively via auscultatory or oscillometric techniques.

Septic shock

For the definition of "septic shock" sepsis, systemic inflammatory reaction (SIRS), and septic shock have to be defined[50].

Sepsis will be defined as systemic inflammatory reaction (SIRS) in the presence of suspected or proven infection. SIRS will be defined as the presence of at least two of the following four criteria, one of which must be abnormal temperature or leukocyte count: 1) Core temperature of >38.5°C or <36°C. 2) Tachycardia, defined as a mean heart rate >2 SD above normal for age in the absence of external stimulus, chronic drugs, or painful stimuli; or otherwise unexplained persistent elevation over a 0.5- to 4-hr time period OR for children <1 year old: bradycardia, defined as a mean heart rate <5th percentile for age in the absence of external vagal stimulus, beta-blocking drugs, or congenital heart disease; or otherwise unexplained persistent depression over a 0.5-hr time period. 3) Mean respiratory rate >2 SD above normal for age or mechanical ventilation for an acute process not related to underlying neuromuscular disease or the receipt of general anaesthesia. 4) Leukocyte count elevated or depressed for age (not secondary to chemotherapy-induced leukopenia) or >10% immature neutrophils. For all reference values see Table 1).

TABLE 1

Age Group	Heart Rate Tachycardia Beats/min	Heart Rate Bradycardia Beats/min	Respiratory Rate Breaths/Min	Leukocyte Count 10³/mm³	Systolic Blood Pressure mmHG
0 days to 1 wk.	>180	<100	<50	>34	<59
1 wk. to 1 mo.	>180	<100	<40	>19.5 or <5	<79
1 mo. to 1 yr.	>180	<90	<34	>17.5 or <5	<75
2-5 yrs.	>140	NA	<22	>15.5 or <6	<74
6-12 yrs.	>130	NA	<18	>13.5 or <4.5	<83
13 to <18 yrs.	>110	NA	<14	>11 or <4.5	<90

Age-specific vital signs and laboratory variables (5th percentile for lower values for heart rate, leukocyte count, and systolic blood pressure and 95th percentile for upper values for heart rate, respiration rate, or leukocyte count)[49,50]

Infection may be suspected or proven. Infection may be suspected by a clinical syndrome associated with a high probability of infection. Evidence of infection includes positive findings on clinical exam, imaging, or laboratory

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tests (e.g. white blood cells in a normally sterile body fluid, perforated viscus, and chest radiograph consistent with pneumonia, petechial or purpuric rash, or purpura fulminans). Infection will be defined as proven by positive culture, tissue stain, or polymerase chain reaction test and may be caused by any pathogen.

Septic shock is defined as sepsis AND cardiovascular organ dysfunction ("Organ dysfunction criteria", section on cardiovascular dysfunction later this chapter).

Heart failure

All patients with structural or functional impairment of heart function, leading to an inadequate amount of blood being pumped through the body, will be analysed as subgroup. Type of cardiopathy, cardiac lesions, preoperative medication, echocardiographic and cardiac catheter results, periods of cardiopulmonary bypass, cross clamping, deep hypothermic arrest, modified ultrafiltration, anaesthetics used, type of surgery, and the postoperative echocardiographic and laboratory results will be recorded for each patient. Patients will be defined as having preoperative congestive heart failure if they are treated with digoxin, diuretics, inhibitors of angiotensin converting enzyme, beta-blockers, or milrinone, or if they have congestive cardiac failure listed as diagnosis.

Traumatic head injury, brain tumour and brain operation

All children and adolescents with traumatic head injury, brain tumour and/or brain operation will be analysed as subgroup. Glasgow coma scale on admission and post resuscitation, presence and history of seizures, CT abnormalities as abnormal cisterns, midline shift >5mm, traumatic subarachnoidal haemorrhage, performance and type of intracranial surgery will be noted for each patient. In addition, intracranial pressure values and cerebral perfusion pressures will be recorded at each visit.

Perinatal asphyxia

Perinatal asphyxia causes neonatal encephalopathy. This is a clinically defined syndrome of disturbed neurologic function in the earliest days of life in an infant born at or beyond 35 weeks of gestation, manifested by a subnormal level of consciousness or seizures, and often accompanied by difficulty with initiating and maintaining respiration and depression of tone and reflexes. To assess children with neonatal asphyxia and consecutive encephalopathy, the following neonatal signs consistent with an acute peripartum or intrapartum hypoxic event must be met for study subgroup inclusion: Gestational age ≥35 weeks AND an Apgar score of <5 at 5 minutes and 10 minutes AND a foetal umbilical artery acidaemia: foetal umbilical artery pH <7.15, and/or base deficit ≥12 mmol/L AND presence of multisystem organ failure consistent with hypoxic-ischemic encephalopathy (two or more dysfunctional organ systems mentioned in section "Organ dysfunction criteria")[51]. One important point defining hypoxic-ischemic encephalopathy is neuroimaging evidence of acute brain injury seen on brain magnet resonance imaging (MRI) consistent with hypoxia-ischemia (i.e. deep nuclear grey matter (basal ganglia or thalamus) and/or watershed cortical injury). As this imaging is usually performed after 96 hours of patient cooling in moderate and severe cases, it cannot be mandatory for study inclusion, but if available will be part of endpoint analysis.

For endpoint assessment, the following will be noted: gestational age, Apgar-score, arterial umbilical cord pH, blood gas analysis, lactate, presence of arterial hypotension as defined as mean arterial pressure below the gestational age limit treated with intravenous corticosteroids, fluids and/or catecholamines, results of neurological imaging (cranial ultrasound and/or MRI), and performance of neonatal cooling as prevention of neurological damage.

Organ dysfunction criteria:
Cardiovascular dysfunction
Despite administration of isotonic intravenous fluid bolus ≥40 mL/kg in 1 hr:
- Decrease in BP (hypotension) <5th percentile for age or systolic BP <2 SD below normal for age

OR
- Need for vasoactive drug to maintain BP in normal range (dopamine, dobutamine, epinephrine, norepinephrine or vasopressin at any dose)

OR
- Two of the following:
   Unexplained metabolic acidosis: base deficit >5.0 mmol/L
   Increased arterial lactate >2 times upper limit of normal (2.0 mmol/L)
   Oliguria: urine output <0.5 mL/kg/hr
   Prolonged capillary refill: >5 s
   Core to peripheral temperature gap >3°C

Respiratory dysfunction
- PaO_2/FIO_2 <40 kPa in absence of cyanotic heart disease or pre-existing lung disease (i.e. acute respiratory distress syndrome (ARDS)) and acute lung injury (ALI). ARDS must include a PaO_2/FIO_2 ratio ≤26.6 kPa,

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bilateral infiltrates, acute onset, and no evidence of left heart failure. ALI is defined identically except the PaO2/FIO2 ratio must be ≤40 kPa.

OR

- PaCO$_2$ >2.6 kPa over baseline PaCO$_2$

OR

- Proven need (assumes oxygen requirement was tested by decreasing flow with subsequent increase in flow if required) or >50% FIO$_2$ to maintain saturation ≥92%

OR

- Need for nonselective invasive or non-invasive mechanical ventilation (postoperatively, this requirement can be met if the patient has developed an acute inflammatory or infectious process in the lungs that prevents him or her from being extubated)

Neurologic dysfunction

- Glasgow Coma Score ≤11

OR

- Acute change in mental status with a decrease in Glasgow Coma Score ≥3 points from abnormal baseline

Hematologic dysfunction

- Platelet count <80,000/mm^3 or a decline of 50% in platelet count from highest value recorded over the past 3 days (for chronic haematology/oncology patients)

OR

- International normalized ratio >2

Renal dysfunction

- Serum creatinine ≥2 times upper limit of normal for age or 2-fold increase in baseline creatinine
- Reference values: newborn 27 to 88 µmol/L; infant 18 to 35 µmol/L; child 27 to 62 µmol/L; adolescent 44 to 88 µmol/L[52]

Hepatic dysfunction

- Total bilirubin ≥4 mg/dL (not applicable for newborn)

OR

- Alanine transaminase (ALT) 2 times upper limit of normal for age
- Reference values (95th percentile): newborn – 18 month male: 60 U/L, female 55 U/L;18 month – 5 years male 40 U/L, female 35 U/L[53]; 5 – 8 years male 35 U/L, female 35; 9 – 13 years 44 U/L, female 35 U/L; 13 - 18 years male 69 U/L; female 35 U/L[54]

Severity of disease:
Only one of the following disease scores will be evaluated per patient as appropriate for the respective age group

- Paediatric Index of Mortality 2[55] (PIM 2): results in "predicted mortality risk" (range: 0-100%), calculated from available patient data at admission
- Simplified Acute Physiology Score[56] (SAPS II): results in an integer point score between 0 and 163 and a predicted mortality between 0% and 100%, calculated from 12 routine physiological measurements collected during first 24h after ICU admission (age, heart rate, systolic blood pressure)
- Clinical Risk Index for Babies[57] (CRIB II) : results in a score ranging from 0 to 27 and mortality probability ranging from 0-100%, calculated at admission from five variables (sex, birth weight, gestational age, temperature, base excess).

Age:
- Patients` age will be noted on study entrance.

Definition and measurement of variables relevant to secondary endpoints:

a) Length of ICU stay will be noted in hours.
b) Length of respiratory support will be noted in hours, stratified by invasive and non-invasive ventilatory support.
c) Length of catecholamine therapy will be noted in hours separately for every drug. In addition, the inotropic score for the estimation of amount of vasopressors used will be calculated with the equation by Wernovsky modified by Choong et al.: 1 point will be given for every µg/kg/min dopamine or dobutamine, 10 points will be given for every µg/kg/min milrinone and 100 points will be given for every µg/kg/min adrenaline or noradrenaline[40,58].
e) Death will be noted, rate of death at 28 days will be correlated with blood copeptin level.

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4.3 Other study variables

The following endpoints will be noted, as copeptin results may differ when stratified according to:

- Weight
- Sex
- Postnatal days and postconceptional age in neonates
- Exogenous vasopressin administration

5. PROJECT DESIGN

5.1 Type of research and general project design

The proposed study is designed as a single-centre prospective observational study.

This design was chosen to gather data on all or at least on a large fraction of critically ill children and neonates admitted to our intensive care unit, assuming patients` or care takers informed consent. Further, as data on copeptin in critical ill children is scarce, no randomization or intervention other than taking blood samples will be necessary. Limitation: The observational study design will not be able to answer any kind of causative questions related to study findings.

5.2 Procedures

As soon as written informed consent is obtained from care takers, the study period will begin for the included children and neonates. The primary study period is defined as 48 hours. For all patients discharged from the ICU within 48 hours after admission, the study period will end with discharge from ICU. 62.3 % of patients stay less than 48h in the ICU of the University Children`s Hospital Zurich (median (10th- 90th percentile): 1.3 (0.4-9.2) days). The sample size was calculated to compensate for these patients discharged early. Patients staying longer than 48 hours (37.7%) are generally those patients with a higher degree of disease severity and are of specific interest. For all patients staying longer than 48 hours, the study period will end with discharge from ICU or with 168 hours, whichever comes first,

On admission demographic baseline data will be recorded on paper bedside case report forms as outlined in "Schedule of assessment (Flow of research project)" (i.e. age (years + months; if <28 days: postnatal days and postconceptional age), Apgar-score, arterial umbilical cord pH; diagnosis; disease-severity-scores; operation, preoperative parameters; weight; sex). Blood samples (600 μl) will be taken from all study patients on specific points of time (on admission, with 12, 24, 48, and, if still in ICU with 96, and 168 hours following admission), processed and stored at -80 °C for batch analyses later. Just before or after blood sampling the following clinical data will be recorded: arterial blood pressure; vital parameters (respiratory rate, peripheral oxygen saturation, arterial oxygen saturation, perfusion index, heart rate, peripheral pulses, capillary refill, urine output); laboratory values (sodium, potassium, calcium, chloride, troponin, creatine kinase, creatine kinase MB, pH, partial arterial oxygen pressure (PaO_2), partial carbon arterial carbon dioxide pressure ($PaCO_2$), base deficit, arterial lactate, glucose, alanine transaminase (ALT), creatinine, C-reactive protein, haemoglobin, white blood cell count, total bilirubin); neurological parameters (Glasgow Coma Scale, CT-findings (abnormal cisterns, midline shift >5mm, traumatic subarachnoidal haemorrhage, seizures, intracranial and cerebral perfusion pressure (if invasively monitored)); neonatal parameters (results of neonatal neurological imaging (cranial ultrasound and/or MRI) and performance of neonatal cooling as prevention of neurological damage). Importantly, laboratory parameters will only be assessed, if their analysis is otherwise medically indicated for limitation of blood loss and phlebotomy. As most critically ill patients have to be equipped with invasive hemodynamic monitoring allowing repetitive blood withdrawal, no additional phlebotomy should be necessary. Copeptin values will only be analysed, when the study blood sample can be taken alongside with other medically indicated blood samples.

If the study patient is discharged from the ICU, the study period ends and no further blood samples or clinical data will be taken or analysed. Length of ICU stay will be noted. Every patient will be followed by study investigators for a total of 28 days to note date of discharge from ICU, Hospital and date of death, if applicable.

As outlined in 2.4 "Participant Information and Informed Consent" this study may include patients, who themselves or whose caretakers may not be able to give written informed consent in emergency situations due to acute clinical deterioration, resuscitation and/or sedation of the patients. Further, care takers may not be present or accountable for formal medical explanations and may not be able to give informed consent at the time of intensive care unit admission due to the deterioration of their child.

In this case, blood samples for copeptin will be taken, but not analysed. As soon as possible, but latest 24 hours following intensive care unit admission, written informed consent will be obtained. If no informed consent can be obtained within 24 h after ICU-admission, all blood samples will be destroyed and the patient will be excluded.

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5.3 Recruitment and Screening

All care takers of eligible critically ill children or neonates admitted to the intensive care unit of the University`s Children Hospital Zurich will be consecutively assessed during daily clinical routine by study investigators. One hundred and seventy study patients are targeted and should be enrolled within 18 months, as a total of 1800 children and adolescents are admitted to the department of Intensive Care Medicine and Neonatology at the University`s Children Hospital Zurich.

5.4 Methods of minimising bias

This is an observational study comparing blood values to clinical conditions. As blood values have to be obtained via one of the procedures most routinely used in the intensive care setting (i.e. venous, arterial or capillary blood sampling), no relevant bias is expected to influence this point. All clinical parameters will be available open-label to treating physicians and study personal in medical charts, no bias is expected here either. Treating physicians will be blinded to copeptin values. Staff analysing copeptin levels at the BRAHMS laboratory will be blinded to clinical course of the patient.

Taken together, no special bias entry points are relevant to this study.

6. PROJECT POPULATION

6.1 Inclusion criteria

This is a study designed to explore Copeptin values in all kinds of critically ill paediatric patients. Patients with normal blood pressures will allow calculating Copeptin reference values for the ones requiring cardiovascular support. Therefore, all patients, irrespective of their medical condition (e.g. medical or surgical, mildly or severely affected) and their age (neonates to adolescents) admitted to the Intensive Care Unit of the University`s Children Hospital Zurich will be eligible, as long as they fulfil the following conditions:

- Age: first day of life until 18th birthday.
- Ability of the care taker or the adolescent (if≥14 years of age) to understand verbal and written instructions and informed consent in German.

6.2 Exclusion criteria

Patients will not be included in the study for the following reasons:

- Care taker or adolescent (if ≥14 years of age) unwilling to give written informed consent.
- Care taker or adolescent (if ≥14 years of age) not understanding German and without a family member able to translate.
- Adolescent (if ≥14 years of age) unwilling to give written informed consent following sedation < 24 hours.
- Care takers of long-term sedated (>24 hours) adolescents (if ≥14 years of age) unwilling to give written informed consent or not present within 24 hours.

6.3 Criteria for withdrawal / discontinuation of participants

For the following reasons the study period will end immediately for the included patients.

- Withdrawal of informed consent
- Discharge from Intensive Care Unit
- Death

7. PROJECT ASSESSMENTS

7.1 Project flow chart(s) / table of procedures and assessments

Please see study flow chart under "SCHEDULE OF ASSESSMENTS (FLOW OF RESEARCH PROJECT)", page 8.

7.2 Assessments of primary endpoint/outcome

The following primary endpoint variable will be obtained in laboratory analysis and stored in an electronic database:

- Blood copeptin values. All study blood samples will be send labelled with patients` names and study numbers to the internal laboratory of the University Children`s Hospital Zurich for centrifugation, aliquot preparation and storage. The stored aliquots assigned to copeptin analysis will be sent in batches to

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BRAHMS (Thermo Fisher Scientific) in Hennigsdorf, Germany. Before the aliquots leave the Children`s Hospital Zurich, all aliquots will be re-labelled manually by study staff to be coded with study number only. All results will be received from BRAHMS in a coded form and stored in a coded database inside the University Children`s Hospital Zurich.

The following primary endpoint variables will be obtained and noted on bedside paper case report forms:

- Arterial blood pressure: obtained via invasive (indwelling peripheral or central arterial catheters) or non-invasive (oscillometric or auscultatory) measurements.
- Diagnosis.
- Vital parameters: respiratory rate, peripheral oxygen saturation, arterial oxygen saturation, perfusion index, heart rate, peripheral pulses, capillary refill, urine output.
- Laboratory values: sodium, potassium, calcium, chloride, troponin, creatine kinase, creatine kinase MB, pH, partial arterial oxygen pressure (PaO_2), partial carbon arterial carbon dioxide pressure ($PaCO_2$), base deficit, arterial lactate, glucose, alanine transaminase (ALT), creatinine, C-reactive protein, haemoglobin, white blood cell count, total bilirubin; all if medically indicated.
- Fluid administration/24h each day.
- Operation and perioperative parameters, i.e. preoperative medication, anaesthetics, type of surgery; if cardiac surgery in addition: cardiac lesion, echocardiographic and cardiac catheter results, periods of cardiopulmonary bypass, cross clamping, deep hypothermic arrest, modified ultrafiltration, postoperative echocardiographic results.
- Neurological findings: Glasgow Coma Scale, CT-results (abnormal cisterns, midline shift >5mm, traumatic subarachnoidal haemorrhage, seizures, intracranial and cerebral perfusion pressure (if invasively monitored)).
- Neonatal findings: Apgar-score, arterial umbilical cord pH, results of neurological imaging (cranial ultrasound and/or MRI), and performance of neonatal cooling as prevention of neurological damage.
- Organ dysfunction/mechanical resuscitation.
- Disease-Severity-Scores (PIM 2, SAPS II, CRIB II).

7.3   Assessment of secondary endpoint/outcome(s)

The following secondary endpoint variables will be obtained and noted on bedside paper case report forms at the scheduled time points of assessment and blood sampling:

- Discharge date from ICU as determined by the moment of decision made by treating physicians for discharge, even though immediate discharge may not be possible due to capacity reasons of the targeted peripheral ward.
- Respiratory support.
- Vasoactive Drugs.
- Serious (Adverse) Events/Death.

7.4   Assessment of other study variables

The following further study variables will be obtained and noted on bedside paper case report forms:
- Weight
- Sex
- Age in years and months. If neonatal (<28 d): postnatal days + postconceptional age
- Other drugs, exogenous vasopressin administration

7.5   Assessment of safety and reporting

7.5.1   Definition of Serious Events (SEs)

A **serious event** is any unfavourable event for which a causal relationship to sampling of biological material or the collection of health related personal data cannot be ruled out, and which:
- requires hospitalisation or prolongation of an inpatients' hospitalisation,
- results in persistent or significant disability or incapacity, or
- is life-threatening or results in death,

If a serious event occurs the research project will be set on hold.

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7.5.2   Assessment and Documentation of SEs

The assessment by the project leader with regard to the project-specific measure relation is done according to the following definitions:

- Unrelated: The occurrence of the event has no temporal relationship to the project-specific measures applied and can be explained by the underlying disease or other factors.
- Related: There is a plausible temporal relationship between the occurrence of the event and the project-specific, applied measures and cannot be explained by the underlying disease or other factors.

All SEs are to be documented in the participants´ file and on the SE report form. A sample form is appended to the Protocol and can be downloaded at www.swissethics.com.

7.5.3   Reporting of SEs, Safety and Protective Measures

The project leader shall report any occurring SE to the responsible EC within 7 days (and to the FOPH in case of involved radioactive sources). He/she shall also submit a report which evaluates the relationship between the event reported and the methods of collecting health related personal data or sampling of biological material within that project, furthermore proposals how to proceed with the project.
The project leader will notify the EC within 7 days of any immediate other safety and protective measures, which have to be taken during the conduct of the research project. In addition, the project leader will explain the circumstances, which necessitated the safety and protective measures.

8.   STATISTICAL METHODOLOGY

8.1   Determination of Sample Size

The sample size calculation is based on the primary objective, namely to investigate the average change of blood copeptin levels (mean difference between time-points, i.e. from admission to 12h, from 12h to 24h, from 24h to 48h) in critically ill children and neonates with both arterial normo- and hypotension over 48h after ICU admission.

*Assumptions***:**
1. We hypothesize that the mean difference in blood copeptin levels between two time-points is *not* 0 (as assessed by a two-sided t-test, assuming equal variance).
2. We base corresponding sample size calculations on the data in the study from Lee et al. (2013)[28] since this paper provides blood copeptin levels at different time points from patients with septic shock admitted to the PICU, i.e. a population similar to a part of the population expected in the present study:

Table 2: Blood copeptin levels measured in the study of Lee et al. (2013).

Time	Healthy n=70 Median (IQR) [ng/mL Copeptin]	Sepsis n=53 Median (IQR) [ng/mL Copeptin]	Septic shock (PICU), n=13 Median* (IQR) [ng/mL Copeptin]	Difference in means between period [ng/mL]	Interquartile range (IQR) [ng/mL]
0-24 h	12 (0.8-1.8)	1.5 (1.0-2.2)	0.9 (0.8-1.2)	-	0.4
48 h			1.2 (0.7-1.8)	0.3	1.1
72 h			1.25 (0.8-1.8)	0.05	1.0
96 h			1.5 (1.3-1.7)	0.25	0.4
120 h			2.0 (1.0-2.2)	0.5	1.2

*we assume normal distribution and use the reported median as mean in the following, as discussed below.

3. For the relevant time points the maximum mean difference between time points is d = 0.3 ng/mL (from 0-24h to 48h, highlighted in blue in the Table 2 above), the interquartile range (IQR) is approximately 1.1 ng/mL.
4. We assume normality, which seems reasonable given the data only seem slightly skewed (median – 0.5, median +0.6). The IQR is then approximately 4/3 of a standard deviation (sd), i.e. sd = 0.75 x 1.1 ng/mL = 0.82 ng/mL.

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5. For the power calculation, we fix the significance level at 5%, and vary the power to determine the effect on possible sample size (Table 3). We use the standard difference in means calculation (t-test).

Table 3: Power calculation without adjustment for drop-out or other reasons of missing data. *effect* = d/sd

Power	Sample size for effect* = 0.36 (0.5)
0.8	62 (26)
0.9	82 (37)
0.95	101 (45)

6. The median PICU length of stay was 6 days, whereas internal data of Zurich's PICU suggests an even shorter median length of stay (1.3 days, see below), with only 40% of patients staying at least 48h. We thus assume a 60% drop out before the end of the targeted observation period of 48 hours (see above), which means that we need to multiply our sample size by 10/4.
7. We assume that at least 90% of the blood copeptin values are within the measurable range of the assay (0.02-4.8 ng/mL), which means that we need to multiply our sample size by 10/9 to account for an additional potential 10% of missing data.

Conclusion: Assuming 80% power, 5% significance level a **sample size of** 62 x 2.5 x 1.1 = **170 patients** with an effect size of 0.36 (**mean difference d=0.3 ng/mL**, sd=0.8 ng/mL, 60% drop out, 10% of data below lower limit of quantification) is needed for this study.

8.2 Planned analysis

8.2.1 Datasets to be analysed
Analysis population
- The primary analysis population for the assessment of the mean difference in blood copeptin levels between time points within the first 48 hours after ICU admission is the full analysis set of all included patients (intention-to-treat analysis). Associations of copeptin levels with the following covariates will be analysed in all included patients: arterial blood pressure, severity of disease and age. Associations with further patient characteristics as septic shock, heart failure, traumatic brain injury, perinatal asphyxia will be analysed in affected patients only.
- The secondary analyses (association of copeptin levels with clinical outcome) will be done in all patients for whom the respective information is available (see 8.2.2 Handling of Missing data).

Analysis tool
All relevant clinical and laboratory parameters obtained during the study period will be analysed using SPSS 22 (IBM, Armonk, NY, USA), R (R Core Team, Vienna, Austria), and NONMEM (Icon Development Solutions, Ellicott City, MD, USA).

Endpoint analysis
Primary endpoint
The mean difference in blood copeptin levels between time points (admission – 12h, 12h-24h, 24h-48h) will be analysed using a two-sided t-test.

The course of individual blood copeptin levels over up to 7 days will be analysed using linear and non-linear mixed-effect regression modelling, which allows to characterize both the individual and overall typical (median) time course simultaneously, and to quantify associated inter-individual variability. The influence of/association with covariates (e.g. age, blood pressure, particular underlying diseases such as septic shock or heart failure) on both the baseline and the course of copeptin levels will be investigated in this scope ("copeptin kinetic model").

The final "copeptin kinetic" regression model will be used for stochastic (Monte-Carlo) simulations to determine the proportions of patients below a specific blood copeptin level (in particular >1.12 ng/mL, which has been suggested to indicate vasopressin insufficiency[27]) at any time point and to evaluate its discriminatory potential to identify patients with hypotension that may most benefit from exogenous AVP treatment. The model-predicted proportions will be evaluated by comparing them to the empirically calculated proportions of patients with blood copeptin levels < 1.12 ng/mL at each specifically measured time point with 95% confidence intervals.

Secondary endpoints

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Finally, measured blood copeptin levels and/or model-derived individual parameters characterizing the course of individual blood copeptin levels will be investigated as predictor variables for length of ICU stay, length of respiratory support and survival in a time-to-event analysis, and for severity of disease (including dose of catecholamine therapy) in a regression analysis.

In a more advanced analysis, also the time-course of blood pressure measurements will be analysed and characterized in a mixed-effect regression analysis. The temporal and dynamic relationship between blood copeptin and blood pressure will be furthermore assessed.

8.2.2   Handling of missing data
Primary outcome:
Missing blood copeptin and/or blood pressure data is expected to occur at time points after baseline assessment because of the following reasons:
- Transfer to another unit (mainly associated with improvement of patient condition)
- Death (associated with worsening of patient condition)

Missing copeptin and/or blood pressure data may occur at any time point because of the following reasons:
- Measurement below the limit of quantification of the analytical method (only copeptin)
- Technical difficulties in blood sampling and/or blood pressure measurement
- Consent withdrawal

The remaining covariates, including age and medical condition (including septic shock, heart failure, traumatic head injury, brain tumor and/or intracranial operation, perinatal asphyxia and severity of disease) are unlikely to be missing since they are assessed at ICU admission and included in the electronic patient record.

For the initial assessment of blood copeptin kinetics, all individual data independent of later missing blood copeptin measurements will be included in the analysis ("full analysis", i.e. according to the intention-to-treat principle). One advantage of the planned mixed-effect modelling is that it can indeed handle unbalanced individual data. Measurements below the limit of quantification will not be omitted but included as censored measurements. Missing covariates at specific time points (age, blood pressure) will be imputed or interpolated based on other observed individual data (e.g. based on previous and/or subsequent assessments).
In a sensitivity analysis, the time-course of only complete patient data over 7 days ("complete case analysis", i.e. per protocol) will be compared with the "full analysis set" and model parameters will be assessed for a bias induced by potentially copeptin-dependent (i.e. non-random) missingness reasons.
If a bias is suggested we will furthermore investigate the time-course of copeptin in patients with missing data, stratified for different reasons of missingness ("stratified sub-analyses").

Secondary outcome:
Missing secondary outcomes (date of discharge from ICU, date of end of respiratory support, date of death) can occur because the observation period may not be long enough to observe these events. Therefore, these unobserved outcomes will be right-censored in the time-to-event analysis (i.e. censored at random).
Catecholamine prescription is part of the electronic patient record. A non-reported dose of catecholamines will therefore be handled as such.

8.2.3   Ancillary analysis
If sample serum volume permits measurement of additional biomarkers, the two following biomarkers would be measured none-mandatorily in decreasing priority: Procalcitonin to contribute to the creation of an international agreed-on threshold for procalcitonin-guided antibiotic treatment of intensive care patients; and MR-proADM to evaluate the so far in critically ill children little-explored predictive value for organ failure and mortality. Rests of serum samples may be used for future biomarkers unknown today.

8.2.4   Deviations from the original statistical plan
There are no likely deviations from the research plan. All unforeseen deviations from the original research plan will be reported to the local ethics committee by the project leader.

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9. DATA AND QUALITY MANAGEMENT

9.1 Data handling and record keeping / archiving

Demographic baseline data, diagnosis, vital parameters, laboratory values, respiratory parameters, vasoactive and other drugs, fluids administered and neurological data are noted at the time of blood sampling on bedside case report forms. Copeptin values will be analysed later in complete batches at BRAHMS, Henningsdorf, Berlin. All blood samples will leave the University Children`s Hospital Zurich strictly coded by study number without patients names or date of birth. For analysis, coded electronic storage of all data including copeptin values with study number and age in years and months (if neonatal (<28 days) in days) in a SPSS database will be used. To allow tracking of database changes every day changes are made to the database a PDF copy of the SPSS database (SPPS->export to Microsoft Excel-> save as PDF) will be electronically stored in a password-protected electronic folder inside the University Children`s Hospital Zurich. The respective password is known only to V. Cannizzaro, MD, PhD and to P. Baumann, MD. The folder will be available to legal authorities, monitors and audits for review.

9.2 Confidentiality, Data Protection

All individual participant medical information obtained in this study is considered confidential and disclosure to third parties is prohibited. Data will be initially noted on bedside paper case report forms, which then will be stored in especially marked folders on the intensive care ward. All data transferred to the SPSS study data base will be stored inside the University Children`s Hospital Zurich.

For data verification purposes, direct access to parts of the medical records relevant to the project, including participants' medical history, will only be given to a competent authority or an ethics committee.

As mentioned in 8.2.3 rests of serum samples may be used for procalcitonin and pro-ADM analyses as well as future biomarkers unknown today. The patients and their care takers have to give informed consent for this separately. If an adolescent ≥ 14 years of age is sedated or for other reasons not able to give informed consent, this has to be obtained for the use of serum samples rests in any case from her-/himself as soon as the adolescent is able to give consent. The legal care takers cannot give informed consent in place of the adolescent for the use of serum sample rests.

9.3 Coding

Except blood copeptin values all obtained medical information is open-label and used for clinical routine. Therefore no initial coding of primary data is necessary. When primary data are transferred to the SPSS database, data will be coded by study number and age in years and months (if neonatal (<28 days) in days). The blood samples will be sent to the laboratory of the University Children`s Hospital Zurich labelled with names and date of birth. The staff at the internal laboratory of the Children`s Hospital Zurich is not blinded to the names, but as this is daily routine in the laboratory, patients` data are protected by professional discretion as this applies also to all other patients of the University Children`s Hospital Zurich. Further, besides from pre-processing no analysis will be performed in the internal laboratory. Before aliquots leave the University Children`s Hospital Zurich, they will be re-labelled by study staff with study number only. Only coded blood samples will leave the University Children`s Hospital Zurich.

9.4 Archiving and Destruction

Paper bedside case report forms contain only information of the medical file. Paper folders will be stored inside the University Children`s Hospital Zurich, room H-1.C13. Data will be electronically stored on hard drives inside the University Children`s Hospital Zurich.

Blood samples will be stored inside the laboratories of the University Children`s Hospital Zurich at -80°C. Aliquots batches sent to BRAHMS in Germany will be entirely used for copeptin analyses. If further aliquots remain at the University Children`s Hospital Zurich, these will be stored for potential additional analyses later.

10. PUBLICATION AND DISSEMINATION POLICY

10.1 Publication of results

Results will be presented on national and/or international conferences for discussion.
Further, results will be published in a peer-reviewed medical journal.

Criteria for authorship

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Each author should have participated sufficiently in the work to take public responsibility for the content. Authorship credit should be based on substantial contributions to 1) the intellectual content (i.e., conception and design, acquisition, analysis, and interpretation of data); and to 2) drafting of the article or revising it critically and prompt for important intellectual content; and 3) final approval of the version to be published. Potential conflicts of interest will be listed. Additional journal-specific regulations may apply.

10.2 Data sharing
No sharing planned.

11. FUNDING AND SUPPORT

12. INSURANCE

As this study places the included patients at only minimal risk (risk category A), no specific study insurance is necessary. Any kind of relevant harm to the patients related to the study is covered by the general liability insurance of the University Children`s Hospital. Costs for the insurance are covered by the University Children`s Hospital Zurich.

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