Research Protocol (Full Proposal)

Human Research Ethics Committee (HREC)

Faculty of Medicine, Prince of Songkla University

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1. **Title of the study in Thai and English**

Thai: การศึกษาความแม่นยำของค่าผลต่างของคาร์บอนไดออกไซด์ในหลอดเลือดดำใหญ่และหลอดเลือดแดงและค่าการอักเสบต่อการทำนายผลการรักษาหลังผ่าตัดหัวใจ

English: Predictability of central venous to arterial CO2 difference ($AVCO_2$) and inflammatory markers in children with cardiac surgery to poor outcomes

2. **Principal Investigator**

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Responsibility in the project: drafting protocol, data analysis and manuscript writing

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Responsibility in the project: data collection and data analysis

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Responsibility in the project: data collection and review manuscript

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Responsibility in the project: data collection and review manuscript

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Responsibility in the project: methodology, data analysis, and review manuscript

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Responsibility in the project: blood sample analysis for inflammatory markers

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Responsibility in the project: recruit participant, review manuscript

4. Student support

Check ✓ in ( ) that apply

( ) Not associated

(✓) Undergrad./post grad. Pornnicha Chaiwiriyawong, M.D.

5. Keywords: $AVCO_2$, inflammatory markers, post cardiac surgery, cardiopulmonary bypass

6. Background and rationale

Cardiopulmonary bypass (CPB) is a potent stimulus causing systemic inflammatory response in children with cardiac disease who undergone open heart surgery. Mechanisms of inflammatory generator during CPB

include blood contact with CPB circuit, ischemia-reperfusion injury, heparin-protamine interactions, and surgical trauma. These mechanisms aggravate the activation of complement cascade, release of endotoxin, and altered cytokine production which led to low cardiac output syndrome (LCOS)[1]

The LCOS refers to declining cardiac function in the face of an elevated demand of cardiac output which may lead to multiorgan failure and death. Incidence of the LCOS is approximately 25% of children after cardiac surgery[1] and commonly occurs 6-18 hours following admission to intensive care unit[2]. Currently, there is no consensus for diagnostic criteria of the LCOS and accurate detection of early cardiac output (CO) insufficiency is a challenging task especially in post-operative children. Measurement CO by using thermodilution method via Swan-Ganz catheterization or transesophageal echocardiography are not applicable in pediatrics setting meanwhile transthoracic echocardiography immediately after post-cardiac operation is limited due to surgical wound and tissue swelling. In Songklanagarind Hospital, clinicians practically use parameters such as serum lactate, central venous oxygen saturations ($ScvO_2$), and arteriovenous oxygen difference (AVO_2) as bedside surrogates of CO. However, these parameters can be influenced by multiple factors unrelated to LCOS[3]. Hyperlactatemia in cardiac surgery could be elevated due to post-operative stress response, use of beta-adrenergic agonist, hyperglycemia, or reduce lactate clearance due to transaminitis while normal values of $ScvO_2$ can be found despite microcirculatory dysfunction and localized tissue hypoxia[4].

Central venous to arterial CO_2 partial pressure difference ($AVCO_2$) measures the circulatory clearance of tissue CO_2 and has been proven to be correlated with CO in critically ill adult patients[5]. Widening of $AVCO_2$ represents an imbalance between CO_2 clearance by CO and tissue CO_2 production. With limited data available, it remains unknown whether $AVCO_2$ is a useful adjunctive marker for assessment CO adequacy in pediatric patients after cardiac surgery with CPB.

Additionally, increase cytokine production both pro- (interleukin (IL)-6, IL-8, and tumor necrotic factor (TNF)-α) and anti-inflammatory cytokines (IL-4, IL-10) caused by CPB-induced inflammatory activation can alter multiorgan function and vascular permeability. The relationship between level of these cytokines and clinical outcomes also remains uncertainty in children population.

We aimed to evaluate the association between $AVCO_2$ and inflammatory markers at 4 different time points post operatively with LCOS-related poor outcome and calculate predictability of these markers to facilitate earlier recognition and management LCOS in children undergone cardiac surgery with CPB.

7. Objective(s) of the study

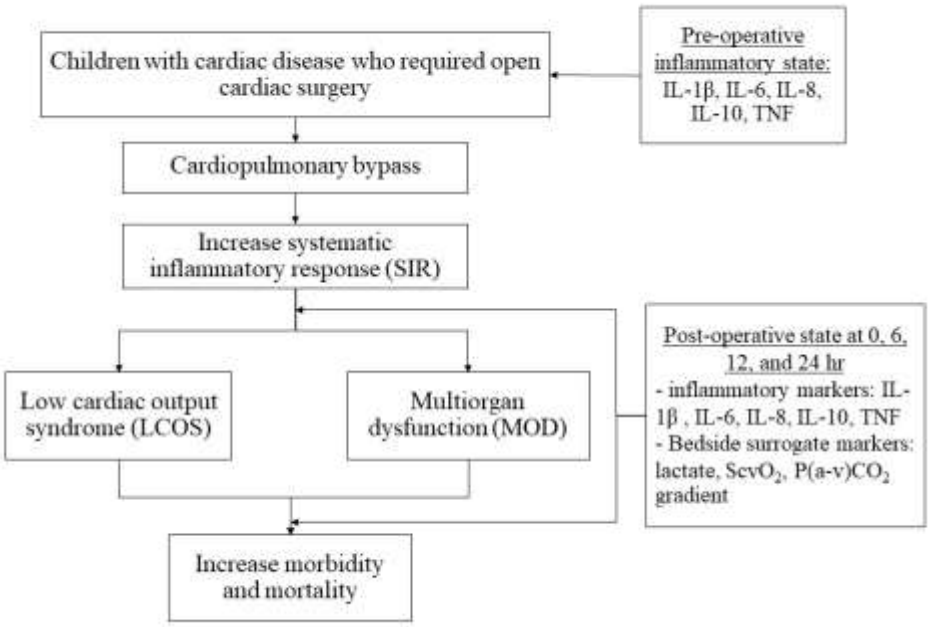
Main or Primary objective: To compare accuracy of prediction of LCOS-related poor outcome between $AVCO_2$ and traditional bedside surrogates (lactate, $ScvO_2$ and AVO_2) in children with cardiac disease who undergone CPB.

Secondary objectives:

7.1 To evaluate association between $AVCO_2$ in children with cardiac disease who undergone CPB and other morbidities: 28-ventilator free day, 28-ICU free day, acute kidney injury, and deaths from any cause during hospitalization.

7.2 To evaluate association between level of pro- and anti-inflammatory cytokine in children who undergone CPB and LCOS-related poor outcome.

8. Conceptual framework



9. Literature review

$AVCO_2$ difference

A retrospective study done by Rhodes LA, et al[3] evaluated correlation of $AVCO_2$ with other cardiac output surrogates (lactate, $ScvO_2$ and AVO_2) and its capacity to predict poor outcomes associated with LCOS in 139 infants who undergone CPB. There were only 24.5% of poor outcome in this study. The results showed moderate correlation between $AVCO_2$ difference with AVO_2 (R^2 = 0.53; p< 0.01), and $ScvO_2$ (R^2 = -0.43; p < 0.01), but not lactate. Unadjusted ROC analysis demonstrated that $AVCO_2$ had lower AUC than $ScvO_2$, but higher AUC than serum lactate (AUC of $AVCO_2$ 0.69 vs $ScvO_2$ 0.74 vs lactate 0.64). After multivariable logistic regression analysis, admission $AVCO_2$ remained significantly associated with poor outcome (OR, 1.3; 95% CI, 1.1-1.45), including independent association with mortality (OR, 1.2; 95% CI, 1.07-1.31). Limitation of this study include missing data of blood gas analysis and lack of simultaneous hemodynamic variables and concurrent echocardiogram. The population in this study also limited only to infant age less than 90 days. Another retrospective cohort study done in 54 Pakistan's children also showed moderate correlation between $AVCO_2$ and $ScvO_2$ (R2 = 0.34), but the participants were still limited in complete repair of congenital heart defect. As accuracy of ScvO2 depends on intracardiac structure, the correlation between $AVCO_2$ and $ScvO_2$ in incomplete repair is unknown[6].

A retrospective study done by Arkamatsu T, et al[7] evaluated the association of $AVCO_2$ with morbidities in 114 pediatrics patients who undergone CPB. The result reported poor correlation of $AVCO_2$ with other parameters (lactate, $ScvO_2$). Additionally, comparing patients who had high ($VACO_2$ > 6 mmHg) and low gap ($VACO_2$ < 6 mmHg) of $VACO_2$, there was no difference of intubation time nor length of ICU stay. However, the outcome in this study did not directly relate to LCOS.

There were 2 prospective and 4 retrospective studies done in adult who undergone CPB[4, 8-12]. Some reported the significant association between $AVCO_2$ and poor outcome[4, 9-11] but some did not[8, 12]. However, poor outcome was defined differently in each study which might not be referred to LCOS-related complications.

Inflammatory cytokines

Unlike the $AVCO_2$ studies, most studies on inflammatory cytokine in pediatric cardiopulmonary bypass surgery were prospective analysis. Allan CK, et al[13] assessed the relationship between level of inflammatory cytokines (IL-6, IL-8, IL-10, TNF α, IL-1β, and CPR) and clinical outcome at different time points (pre CPB, post CPB at 0, 6 12, and 24 hours) in 93 pediatric patients. The result demonstrated that level of postoperative IL-6 and IL-8 at 0 and 24 hours correlated significantly with length of ICU stay and postoperative blood product administration. However, this study did not report outcome related with LCOS. Another study measured inflammatory mediators (IL-6, IL-8, IL-10, and TNF α) and cardiac markers (N-terminal pro-B-type natriuretic peptide and troponin I) in 46 cardiac pediatrics patients[14]. Multivariate logistic regression identified pre-operative N-terminal pro-B-type natriuretic peptide ≥ 470 fmol/ml and post-operative IL-8 at 4 hour ≥ 128 pg/ml were significantly related with LCOS events.

The most recent study published in 2021[15] evaluated inflammatory mediators in 31 patients with congenital heart disease. It showed higher preoperative IL-10 and 24-hour postoperative IL-8 level were associated significantly with LCOS.

Research methodology

9.1. Study design: prospective diagnostic study

9.2. Setting of the study/Trial site: 8-bed pediatric intensive care unit, Songklanagarind Hospital, Hat Yai, Songkhla

9.3. Target population: Children with cardiac disease in Songklanagarind hospital who undergone open cardiac surgery with cardiopulmonary bypass (age < 18 years old)

9.4. Study population

9.4.1. Inclusion criteria:

- Children aged 0-18 years old with either congenital or acquired cardiac disease
- Undergone cardiopulmonary bypass for cardiac surgery
- Be admitted in PICU for post-operative care

9.4.2. Exclusion criteria

- Preterm infant (GA < 37 weeks) or weight less than 2 kg

- Patient who weaned off cardiopulmoanry bypass and required extracorporeal membrane oxygenator (ECMO) before leaving the operating room
- Patient who required emergency cardiac operation within 24 hour after hospitalization
- Patient who had significant residual left side outflow tract obstruction (e.g., coarctation of aorta, interrupted aortic arch) which defined by difference of SBP between upper and lower extremities more than 10 mmHg
- Patient who had already participated in another research project
- Patient who does not have both arterial line and central line catheter back from operating room
- Parents or legal guardian refuse to inform consent.

9.4.3. Withdrawal criteria:

- Parents or legal guardian request to withdraw from research

9.4.4. Termination criteria: none

9.5. Sample size calculation

For the 1st objective, we apply diagnostic study as follows:

$$n = n_0 / \text{prevalence or incidence of disease}$$

$$n_0 = \frac{(Z_{\alpha/2})^2 P (1-P)}{d^2}$$

Where: Sensitivity (p) = 0.85

Precision (d); not more than 10% of p = 0.09

Alpha = 0.05

We applied the sensitivity of lactate > 3 mmol/L for major morbidity, which defined as at least one of complications (i.e., neurological, pulmonary, gastroenteric, and need for extracorporeal membranes oxygenation or ventricular-assist device, or sepsis)[16].

Estimated patient (n_0) is 61 cases. Based on 48% of incidence of LCOS-related outcome last year in our study setting, the required total sample size including non-disease and disease participants will be 128 (=61/0.48).

Regarding with our sample size calculation for two objectives and an estimate of 20% of unusable data from a sample of 128 participants has been considered. The final sample size is 154 participants for sensitivity of 0.85. However, we will collect blood sample for inflammatory markers in 32 patients for preliminary analysis.

9.6. Variables of the study

9.6.1. Dependent variable: poor outcomes indirectly represented from low cardio output syndrome

- LCOS-related poor outcome is modified from the study of Drennan SE, et al and Carmona, et al[14, 15] which consisting of at least two of the following criteria within 48 hour post operatively:

a. Clinical findings suggestive of LCOS: prolonged capillary refill > 3 s, systolic blood pressure < 5th percentile for age and gender, persistently elevate of LA pressure above 10 mmHg for at least 6 hr , low urine output (< 1mL/kg/hr) for at least 6 hour despite diuretic use

b. Laboratory findings suggestive of LCOS: persistently elevated lactate (>2 mmol/L), metabolic acidosis with an increase in the base deficit (>4 mmol/L) for at least 6 hr consecutively.

c. Vasoactive-inotropic score (VIS) ☐ 20

d. Any unplanned surgical reinterventions, cardiac arrest, or utilization of extracorporeal membrane oxygenator (ECMO) within 48 hours

e. Left ventricular fraction less than 50% by echocardiography

The LCOS-related outcome will include both anatomical LCOS and physiological LCOS that caused by CPB

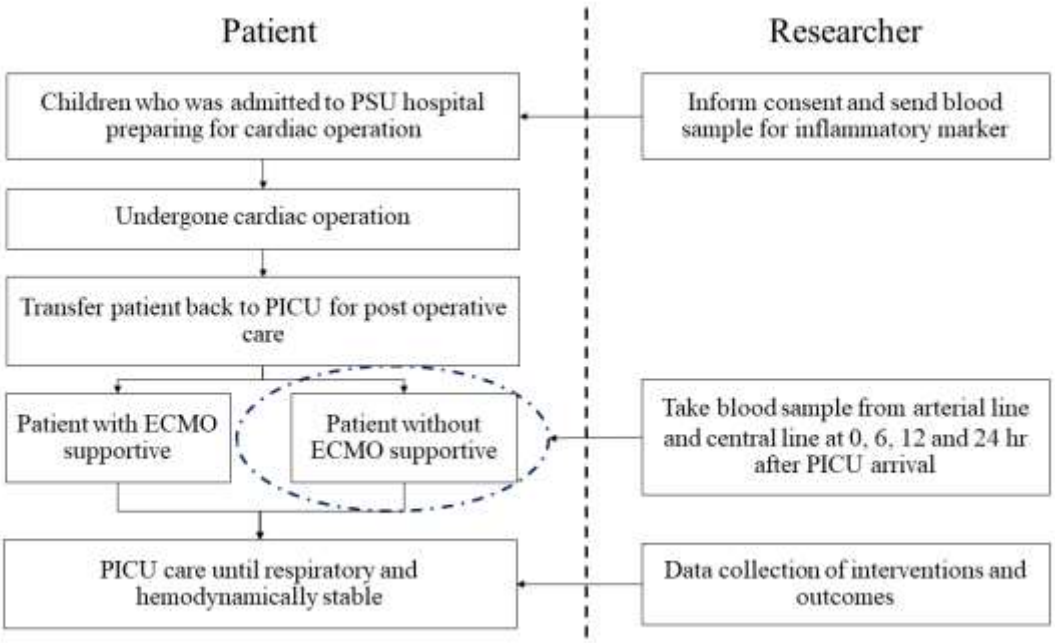
- Vasoactive-Inotropic Score (VIS) based on Belleti A, et al[17] which is summation of dopamine dose (mcg/kg/min) + dobutamine dose (mcg/kg/min) + 100 x epinephrine dose (mcg/kg/min) + 10 x milrinone dose (mcg/kg/min) + 100 x norepinephrine dose (mcg/kg/min) + 10000 x vasopressin dose (U/kg/min) + 50 x levosimendan dose (mcg/kg/min) + 20 x methylene blue dose (mg/kg/h) + 10 x phehylephrine dose (mcg/kg/min) + 10 x terlipressin dose (mcg/min) + 0.25 x angiotensin II dose (ng/kg/min)
- Acute kidney injury (AKI) based on KDIGO guideline 2012[18] defined AKI as any of following: increased in SCr by ☐ 0.3 mg/dL within 48 hours, or increased in SCr to ☐ 1.5 times baseline, which is known or presumed to have occurred within the prior 7 days, or urine volume < 0.5 mL/kg/h for 6 hours
- 28 ventilator free day (VFDs) and 28 ICU free day (IFDs): the number of days those patients were alive and free from invasive ventilator or ICU stay within 28 days by counting the day of first operative night as the first day. VFDs and IFDs will be counted as zero in non-survivor patients.

9.6.2. Independent variables: $AVCO_2$, $ScvO_2$, lactate, inflammatory markers at 0, 6, 12, and 24 hour after operation with additional inflammatory markers at pre-operative stage

9.6.3. Potential confounder variables: age, gender, risk stratification for cardiac surgery (RACHS score), nutritional status, duration of bypass time or aortic clamp time

9.6.4. Methods to minimize bias during study: we will blind the result of inflammatory markers to attendant physician by sending blood sample to research lab which will not be reported in HIS system. Blood sample collection and processing time will be fixed to ensure laboratory quality of the specimen and its interpretation.

9.7. Study procedure(s)/stage(s)



1. Researcher will introduce research project verbally to cardiac pediatrics patient or their legal guardians at cardiac outpatient clinic when he/she are scheduled to have cardiopulmonary bypass surgery at Songklanagarind Hospital. Researcher will later request patient or legal guardians to sign the inform consent when he/she is admitted in hospital 24 hours in advance.

2. If patient has already been admitted in hospital for any reason and have schedule for surgery, researcher will inform patient or legal guardians 48 hours in advance.

3. Preoperative blood sample 1.5 mL will be collected for inflammatory markers level at the same time when patient has intravenous access preparing for NPO or other preoperative blood sample.

4. Patient goes on open heart cardiac surgery and will be admitted directly to pediatric intensive care unit (PICU) for postoperative care.

5. Blood sample for blood gas 0.3 mL for each central line catheter and arterial line catheter and inflammatory markers 1.5 mL drawn from arterial line will be collected at 0, 6, 12, and 24 hours after PICU arrival. Arterial and venous blood gas are accepted if samples are collected within 5 minutes of each other. We allow time gap for collecting blood +/- 1 hr from schedule due to unpredictable ICU occupy.

6. Attending physician provides standard care for post cardiac operation. The decision of treatment by attending physician will not be involved by researchers. Inflammatory markers results will be reported after patient is discharged from hospital to minimize influence of result to physician's decision on treatment.

7. Cardiologist will perform echocardiography to evaluate left ventricular function and anatomical defect that could mimic signs of LCOS within 48 hours postoperatively.
8. Researcher prospectively collects postoperative variables including hemodynamic variables and therapeutic interventions and patient's outcome.
9. The primary study endpoint is poor outcome related to LCOS. The secondary endpoints are 28-day ventilator free day, ICU free day, deaths and correlation of $AVCO_2$ with other cardiac output surrogates (AVO_2, $ScvO_2$ and arterial blood lactate).
10. If either central line or arterial line catheter are displaced before 24-hour, remaining data will be recorded as missing data.

Describe procedures/process of the study. Show how research methodology in the study is different from the routine practice?

Processes which are routine practice	Processes which are research
1. Pre- operative lab sampling; CBC, Coagulogram, electrolyte, group match for blood component	1. Adding inflammatory markers to pre-operative lab
2. Serial ABG sampling q 2-6 hr depend on severity of patient 3. Serial VBG sampling q 24 hr	2. Extra 2 blood sampling for VBG (0.3 mL each) 3. Mandatory blood sampling q 6 hr x 5 times for inflammatory markers (1.5 mL each)

9.8. Study instrument(s) and outcome measurement(s)

- Blood sample for blood gas analysis 0.3 mL will be draw from arterial line and central venous catheter each with different time less than 5 minutes. The arterial and venous gas will be analyzed by ABL800 Basic (Radiometer, Copenhegen, Denmark) which located in PICU
- Blood sample for inflammatory markers 1.5 mL will be collected into 3 mL vacutainers without anticoagulant. Collected blood will be kept on room temperature and sent to division of immunology and virology within 3 hours after collecting. Samples will be centrifuged at 500 x g for 15 minutes at 4˚C. Supernatant will be transferred to a clean polypropylene tube. Separated serum will be aliquot into cryogenic vial and stored at -80˚C until analysis.
- Serum cytokines (TNFα, IL-1β, IL-6, IL-8, IL-10) will be quantified in serum using multiplex immunoassays (Bio-Plex Pro Humam Screening Panel 5plex XPLEX, Bio-Rad, California, USA). The plate will be read on Bio-Plex®200 array reader (Bio-Rad, California, USA) and analyzed using Bio-Plex Manager™ software. Sample will be run in duplicated and cytokine level will be quantified via comparison to standard curve.

9.9. Data collection methodology

- Data record form consists of 5 parts: patient's profile, characteristic of cardiac disease, intra- and post-operative intervention, laboratory values at 4 different time points, and PICU outcome. Researcher will collect data via electronic medical records, nursing record form, and blood gas record sheet of each participant.

9.10. Data management

- All of data will be collected in record form before putting in Epidata program. Study codes will be used on data collection and data document instead of hospital number of patients.

9.11. Statistical analysis

Descriptive part:

- For continuous data (i.e., $AVCO_2$, $ScvO_2$, lactate, inflammatory markers, and others), we will use mean (±SD) in normal distribution and median (±IQR) in non-normal distribution. We will use percentages for categorical data (i.e., LCOS-related poor outcome).

Analytic part:

- Two means of the continuous outcomes will be compared using t-test or Mann Whitney U test. Two or more proportions of the categorical outcomes will be compared using Chi-square or Fisher-exact test.
- We will apply the pairwise correlation analysis among independent variables: $AVCO_2$, lactate, $ScvO_2$, inflammatory markers with time lagged factor.
- For primary objective, we will calculate and compare the accuracy of prediction of LCOS-related poor outcome between AVCO2 and traditional bedside surrogates, using diagnostic test indices (i.e., sensitivity, specificity, predictive values).
- According to secondary objectives, Cox proportional hazard model will be conducted to examine the association between LCOS-related outcomes and other variables. Potential confounders (i.e., age, gender, RACHS score, nutritional status, duration of bypass time or aortic clamp time) were included in the initial model. Then, backward stepwise refinement was performed. Statistical significance was considered when the p-value is less than 0.05.
- Area under the Receiver Operating Characteristic (ROC) to evaluate predictability of $AVCO_2$ compare with other parameters.
- Sensitivity analysis in patient who has physiological LCOS-related outcome.
- Subgroup analysis between children who undergone univentricular repair and biventricular repair

10. Ethical consideration

10.1. Possible risks/effects in the study, including preventive and alleviation measures

- According to protocol of this study, the patients might be at risk of infection from collecting blood from arterial line and central venous catheter. However, expertise ICU nurses practically perform blood

collection in sterile technique. If patient develops catheter-related blood stream infection, empirical antibiotics will be given immediately.

- Patient will be at risk of anemia. According to Ethics committee guideline, amount of blood drawn from patient will not be over the maximum volume for patient's body weight and patient's Hb must be more than 10 g/dL at the time of blood drawing. Packed red cell will be given if patient's Hb is less than 10 g/dL in standard care

10.2. Describe the process/ system for assuring confidentiality and the privacy of the research participants/communities

Study codes will be used on data collection and data document instead of hospital number. Subject information will be stored securely in the computer and only researcher can access through data.

10.3. Benefits of the study for participants and the community/ country including how findings of the study use for strengthening community.

Since there are limitation of using traditional bedside surrogates (serum lactate, ScvO2 and AVO_2) to predict LCOS in children who undergone CPB, this new bedside surrogate ($AVCO_2$) might be another bedside tool to detect post-operative LCOS earlier in order to improve outcome.

10.4. Informed consent process: Process/ method of invitation the participants to participate in the research, such as personal contact, referral from other(s), brochure, and announcement, etc.

- Researcher will introduce research project verbally to cardiac pediatrics patient or their legal guardians at cardiac outpatient clinic when he/she are scheduled to have cardiopulmonary bypass surgery at Songklanagarind Hospital and give consent form to legal guardians to sign. Researcher will later request collect the inform consent when he/ she is admitted in hospital 24 hours in advance.

- If patient has already been admitted in hospital for any reason and have schedule for surgery, researcher will inform patient or legal guardians 48 hours in advance.

10.5. Procedure specifying for research participant withdrawal from the study

- Blood sample taking will be ceases immediately after participant/ legal guardians withdrawn from the study
- Data collected from a withdrawn patient will be used in the study analysis. Data after patient withdraw will be inserted as missing data.

10.6. Clearly indicate person(s) responsible for payment for treatment of complications and adverse effects

There is no any intervention or therapeutic trial in this study.

10.7. Compensation for research participant

☐ Yes, please provide detail:.....

✓ No, please provide reasons: patient does not have to spend extra time or any effort during the research, so there is no compensation will be provided in this study

10.8. Does the study involve biological specimen collection? If yes, also explain how the investigator manages the leftover specimen.

The study will collect additional total 8.1 mL of blood for inflammatory mediators (1.5 mL each x 5 times for inflammatory markers and extra blood sampling 0.3 mL x 2 times for VBG analysis during study period). After complete analysis, leftover specimen will be discarded immediately.

10.9. Research project with special ethical consideration *(if applicable)*

11. Limitation(s) and barrier(s) of the study *(If applicable)* and plans for mitigation

- The time of blood sampling might be inaccurate as schedule. The protocol allows the time gap 1 hour from schedule to be more flexible for ICU nurse.

12. Time schedule of the study

Duration of the study: From August 2021 to April 2023 Total time: 21 months

Activity	**Jun-Dec 2021**	**Jan-Dec 2022**	**Jan-Sep 2023**
1. Proposal submission for EC	Jun-Aug 2021 →		
2. Data collection	Aug 2021 – Apr 2023 →		
3. Data analysis		Jan-Apr 2023 →	
4. Manuscript writing		Apr-Jun 2023 →	
5. Presentation to faculty			Sep 2023 →

13. Budget detail of the study

✓ Funded by: Research fund, Faculty of Medicine, Prince of Songkla University

Budget amount: .....

☐ Expecting funded from: .....

Budget amount: .....

☐ Private fund

Budget amount: .....

Cost category	**Cost**
Payment for laboratory technician who centrifuge blood sample as protocol 20 baht/1 blood sample	3,200 baht

= 20 baht x 5 times x 20 participants	
Equipment Multiplex immunoassays (Bio-Plex Pro Humam Screening Panel 5plex XPLEX, Bio-Rad, Californias, USA) = 87,205 x 2 plate	174, 410 baht
Grand total	177,610 baht

14. Expected outcomes of the study

Publication in international journal (Scopus, pubmed) and oral/poster presentation in international conference.

15. References

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We, the principal investigator and co-investigators listed and signed below, certify that we will adhere strictly to the information provided in the research protocol. We hereby certify that we will start our study only after the certification of approval by Human Research Ethics Committee, Faculty of Medicine, Prince of Songkla University.

(.....)

Date.....

Adviser

(Pharsai Prasertsan M.D.)

Date 5 Nov 2023

Principal Investigator

(Pornnicha Chaiwiriyawong, M.D.)

Date…5 Nov 2023

Sub-investigator

(Jirayut Jarutach, M.D.)

Date5 Nov 2023

Sub-investigator

(Pongsanae Duangpakdee, M.D.)

Date5 Nov 2023

Sub-investigator

(Polathep Vichitkunakorn, M.D., Ph.D.)

Date…5 Nov 2023

Sub-investigator

(Smonrapat Surasombatpattana, Ph.D.)

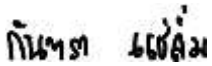
Date5 Nov 2023

Sub-investigator

(Kanokpan Ruangnapa, M.D.)

Date…5 Nov 2023

Sub-investigator



(Kantara Saelim, M.D.)

Date…5 Nov 2023

Sub-investigator

Signature of authorized person/institution

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(.....)

Date.....

Head of Department /Unit

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