STUDY PROTOCOL AND STATISTICAL ANALYSIS PLAN

Official title: Predictive Value of Ventricular-Arterial Coupling for Post-Induction Hypotension: A Prospective Observational Cohort Study

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STUDY PROTOCOL

1. Study design and participants

This prospective observational cohort study (ClinicalTrials.gov: NCT05969886) enrolled adult patients (≥18 years) undergoing elective surgery under general anesthesia with endotracheal intubation between July 2023 and November 2024. The present study was approved by the Institutional Ethics Committee of the University Medical Center Ho Chi Minh City (approval number: 32/GCN-HDDD).

Inclusion criteria were adults (≥18 years) undergoing non-cardiac, non-obstetric, and non-gynaecologic elective surgery under general anaesthesia with endotracheal intubation. The patients were classified as American Society of Anaesthesiologists (ASA) physical status I–IV and underwent transthoracic echocardiography within 48 hours prior to induction.

Exclusion criteria included uncorrected congenital heart disease, moderate-to-severe valvular disease or pulmonary hypertension, arrhythmias, anticipated difficult airway, and known allergy to anaesthetic agents.

2. Preoperative data collection

Demographics, comorbidities, current smoking, and medications were obtained from clinical exams and records. General health was classified by ASA physical status. Laboratory tests such as electrolytes, hemoglobin, creatinine, fasting glucose, and other relevant exams were retrieved from electronic medical records.

Preoperative transthoracic echocardiography (TTE) was performed in accordance with the American Society of Echocardiography (ASE) guidelines by experienced operators. A PHILIPS EPIQ 7C system with X5-1 (2.5–5 MHz) and eL18-4 PureWave (2–22 MHz) probes was used. ECG monitoring was applied during the exam. Left ventricular ejection fraction (LVEF) was measured by Simpson’s biplane method. Left ventricular outflow tract diameter (LVOTd) was measured at mid-systole from the parasternal long-axis (PLAX) view. Pulse wave

Doppler (PWD) in the apical five-chamber (A5C) view measured blood flow velocity 5 mm below the aortic valve at 150 mm/s sweep speed to calculate velocity-time integral (VTI). Pre-ejection period (PEP) and total systolic period (TSP) were measured from QRS onset to ejection start and end, respectively. Stroke volume (SV) was calculated using the formula (1). Early (E) and late (A) mitral inflow velocities were measured by pulse wave Doppler (PWD) from the apical four-chamber (A4C) view. Septal and lateral mitral annular early diastolic velocities (e′) were assessed by tissue Doppler imaging (TDI) and PWD, respectively. Tricuspid annular plane systolic excursion (TAPSE) was measured by M-mode, and lateral tricuspid annular systolic velocity (s′) by TDI. Valvular heart disease, regional wall motion abnormalities (RWMA), diastolic dysfunction, and left ventricular hypertrophy were evaluated and graded according to ASE guidelines. Left ventricular global longitudinal strain (LVGLS) was measured using two-dimensional speckle-tracking echocardiography from apical views. Additional parameters included interventricular septal thickness in diastole and systole (IVSd, IVSs), left ventricular end-diastolic and end-systolic diameters (LVDd, LVDs), posterior wall thickness in diastole and systole (LVPWd, LVPWs), ascending aortic diameters in diastole and systole (DD, SD) measured 3–4 cm above the aortic valve, and left atrial volume (LAV).

$$SV = VTI \times \pi \times \left(\frac{LVOTd}{2}\right)^2 \quad (1)$$

Systolic arterial pressure (SAP) and diastolic arterial pressure (DAP) were measured at the time of echocardiography using an automated blood pressure monitor (JPN600 model, Omron, Japan). Before data processing, E_{es}, E_a, and the E_a/E_{es} ratio were calculated using iElastance software by inputting SAP, DAP, LVEF, SV, PEP, and TSP.

E_a is calculated as end-systolic pressure divided by left ventricular stroke volume: E_a = 0.9 x (SAP / SV). E_{es} is defined as the change in left ventricular pressure over the change in volume at end-systole: E_{es} = [DBP - ($End_{(est)}$ x SBP x

0.9)] / ($End_{(est)}$ x SV), where $End_{(est)}$ is the estimated normalised ventricular elastance at the onset of ejection.

Regional arterial stiffness indices will be calculated from SD, DD, SAP, and DAP parameters using the following formulas:

1. Arterial diameter change (mm) = SD – DD
2. Arterial strain = (SD – DD)/DD
3. Elastic modulus E (p) = (SAP – DAP)/strain
4. Arterial stiffness index β = ln (SAP/DAP)/strain (ln: natural logarithm)
5. Arterial distensibility = (2 x strain)/(SAP – DAP).

3. Anesthesia induction and monitoring

All patients were admitted and anesthetized following the protocol of the Department of Anesthesia, University Medical Center Ho Chi Minh City. Before induction, an 18G or 20G intravenous line was established. Crystalloid fluids were infused at a rate of 1–2 ml/kg/h. In the operating room, intraoperative monitoring included electrocardiography (ECG), non-invasive blood pressure (NIBP), oxygen saturation (SpO_2), and bispectral index (BIS). NIBP was set to measure every 2 minutes during the pre-incision period, immediately before and after endotracheal intubation. Hypotension was defined as any occurrence of MAP <65 mmHg during this period. Patients received 100% oxygen for 3 minutes. Subsequently, fentanyl 1–3 µg/kg was administered. Two minutes after fentanyl injection, 1% propofol was injected at 40 mg every 10–20 seconds until the BIS reached 50. Rocuronium bromide 0.6 mg/kg was given, and endotracheal intubation was performed 2 minutes later. An additional 20 mg of propofol was administered if the BIS was >50 before intubation and/or >60 during intubation, repeated after 20 seconds if needed. Initial ventilator settings included tidal volume (Vt) of 6–8 ml/kg, positive end-expiratory pressure (PEEP) of 5 cm H_2O, fraction of inspired oxygen (FiO_2) 40–50%, and an inspiratory-to-expiratory ratio (I:E) of 1:2. Respiratory rate was adjusted to maintain end-tidal CO_2 ($EtCO_2$)

between 36–40 mmHg. Anesthesia was maintained with sevoflurane to keep BIS between 40 and 60.

We record the following variables HR (heart rate), SAP, MAP, DAP, SpO_2, BIS, MAC (minimum alveolar concentration), $EtCO_2$, Pplat (plateau pressure), Vt, RR (respiratory rate), PEEP at the following time points:

- T0: after conditioning of the patient in the operating room.
- T1: time immediately before injection of fentanyl.
- T2: the time at lowest MAP during manual mask ventilation (100% O_2).
- T3: time immediately before intubation.
- T4: time immediately after successful intubation and manual ventilation.
- T5: the time at lowest MAP during mechanical ventilation and before skin incision.
- T6: time immediately before skin incision.

(note: treatment of eventual hypotension is at the discretion of the attending anesthesiologist).

STATISTICAL ANALYSIS PLAN

1. Sample size calculation

The sample size was calculated to detect the risk of post-induction hypotension (PIH), defined as mean arterial pressure (MAP) <65 mmHg, with an expected area under the receiver operating characteristic curve (AUC) of 0.70.

This assumed an improvement in model performance after the addition of ventricular–arterial coupling (VAC)-related variables to the base model. With a significance level (α) of 0.05, statistical power of 0.90, and an expected 2:1 ratio of non-PIH to PIH patients, a minimum of 93 patients was required, including 62 non-PIH and 31 PIH cases.

For estimating PIH incidence ($p = 0.53$) with a margin of error (d) of 0.05, the minimum required sample size was 383. Accounting for a 5% potential loss to follow-up or data exclusion, the final required sample size was at least 403 patients.

2. Statistical analysis plan

All statistical analyses were conducted using R version 4.4.2. A two-sided p-value <0.05 was considered statistically significant.

Data distribution was assessed using the Shapiro–Wilk test. Continuous variables with a normal distribution were summarized as mean ± standard deviation (SD) and compared using Welch's t-test. Non-normally distributed variables were presented as median [interquartile range, IQR] and compared using the Wilcoxon rank-sum test. Categorical variables were compared using the χ^2 test or Fisher's exact test when expected cell counts were <5. Binary variables with fewer than five observations in any group were excluded from regression analyses. Missing values for global longitudinal strain (GLS), accounting for <5% of the data, were imputed using the median.

A generalised additive model (GAM) was used to assess associations between risk factors and PIH, with categorical variables expressed as odds ratios and continuous variables modelled non-linearly.

The predictive performance for PIH was evaluated using three models: (1) the first model, M_0 includes clinical variables selected using least absolute shrinkage and selection operator (LASSO); (2) the second model, M_{echo}, adds echocardiographic measurements; and (3) the third model, M_{full}, builds on M_{echo} by adding three specific measures: E_a, E_{es}, and the E_a/E_{es} ratio. Incremental predictive value was assessed as fraction of new information (likelihood ratio test with Holm adjustment) and improvement in AUC (DeLong test). Variable contributions in M_{full} were estimated by percentage of total explained variance in PIH risk, with 95% confidence intervals from 2,000 bootstrap samples.