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Bioactive oxylipins in infants and children with congenital heart disease undergoing pediatric cardiopulmonary bypass

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Abstract

Objective: To determine the production of 9-hydroxyoctadecadienoic acid (9-HODE) and 13-hydroxyoctadecadienoic acid (13-HODE) during cardiopulmonary bypass (CPB) in infants and children undergoing cardiac surgery, evaluate their relationship with increase in cell-free plasma hemoglobin (PHb), provide evidence of bioactivity through markers of inflammation and vasoactivity (WBC count, milrinone use, vasoactive inotropic score [VIS]), and examine their association with overall clinical burden (ICU/hospital LOS and mechanical ventilation duration)

Design: Prospective observational study

Setting: 12-bed cardiac ICU in a university-affiliated children's hospital

Patients: Children were prospectively enrolled during their pre-operative clinic appointments with the following criteria: >1month to <18 years old, procedures requiring CPB

Interventions: None

Measurements and Main Results: Plasma was collected at the start (StartCPB) and end of CPB (EndCPB) in 34 patients. 9-HODE, 13-HODE, PHb, and WBC increased. 9:13-HODE at StartCPB was associated with VIS at 2–24h post-CPB ($R^2=0.25$, $p<.01$), milrinone use ($R^2=0.17$, $p<.05$), and WBC ($R^2=0.12$, $p<.05$). 9:13-HODE at EndCPB was associated with VIS at 2–24h ($R^2=0.17$, $p<.05$) and 24–48h post-CPB ($R^2=0.12$, $p<.05$) and milrinone use ($R^2=0.19$, $p<.05$). 9:13-HODE at StartCPB and EndCPB were associated with the PHb ($R^2=0.21$ and $R^2=0.23$,

$p < .01$). The PHb was associated with milrinone use ($R^2 = 0.36$, $p < .001$) and VIS < 2 h ($R^2 = 0.22$, $p < .01$), 2–24h ($R^2 = 0.24$, $p < .01$), and 24–48h ($R^2 = 0.48$, $p < .001$) post-CPB. CPB duration, 9:13-HODE at StartCPB, and PHb may be risk factors for high VIS. CPB duration, PHb, 9:13-HODE, and VIS correlate with ICU and hospital LOS and/ mechanical ventilation days.

Conclusions: In low-risk pediatric patients undergoing CPB, 9:13-HODE was associated with PHb, VIS, and WBC count, and may be a risk factor for high VIS, indicating possible inflammatory and vasoactive effects. Further studies are warranted to delineate the role of HODEs and PHb in CPB-related dysfunction and to explore HODE production as a potential therapeutic target.

Keywords

oxidative lipidomics; hydroxyoctadecadienoic acid; cell-free plasma hemoglobin; cardiopulmonary bypass; vasoactive inotropic score; pediatrics

INTRODUCTION

Cardiopulmonary bypass (CPB) represents a life-sustaining modality imperative to the success of modern day cardiac surgery. However, it is also associated with organ dysfunction related to causes such as hypoperfusion, ischemia/reperfusion, inflammation, and oxidative injury [1, 2]. Oxidative responses have been found in both pediatric and adult patients undergoing CPB [3, 4].

The bioactive oxylipins, 9-hydroxyoctadecadienoic acid (9-HODE) and 13-hydroxyoctadecadienoic acid (13-HODE), are oxidation products of linoleic acid (LA), which is a predominant polyunsaturated essential fatty acid. LA can be oxidized by intracellular enzymes as well as unusual catalysts such as cell-free plasma hemoglobin (PHb). HODEs are good indicators of lipid peroxidation and oxidative injury. Enzymatically, LA is oxidized by lipoxygenases to form 9-hydroperoxyoctadecadienoic acid and 13-hydroperoxyoctadecadienoic acid which are then rapidly reduced to form the more stable 9-HODE and 13-HODE [5–7]. In addition, HODEs can be produced from LA by “aberrant” catalysts appearing in tissues within pro-oxidant environments such as conditions of inadequate blood flow, inflammation, or injury [8].

HODEs are also bioactive [9–13] and may affect vascular regulation after CPB; 9-HODE and 13-HODE levels were significantly increased in a cohort of patients with pulmonary artery hypertension (PAH) [9]. Specifically, 9-HODE has vasoconstrictive properties while 13-HODE is vasodilatory [14–17]. Thus, because of their common precursor, their overall effect may depend on their ratio. Accordingly, the ratio of 9:13-HODE was identified as a potential biomarker of immune status during infection [18]. However, 9-HODE and 13-HODE levels during CPB have yet to be reported. CPB is a therapy thought to induce oxidative injury, so one can speculate that the levels of these oxylipins will be elevated and can be related to differential inflammatory and vascular effects.

Recent data also suggest that PHb, which results from hemolysis during CPB, increases nitric oxide (NO) consumption, augments oxidative damage, and causes vascular

dysfunction [4, 19–21]. PHb can act as a peroxidase with quasi-lipoxygenase (LOX) activity [22, 23] and is able to oxidize lipids and induce oxidative injury [4, 24, 25]. Thus, we anticipated there would be a relationship between the HODEs and the production of PHb during CPB.

The potential bioactivity of HODEs and PHb make them clinically relevant products of CPB that perhaps should be viewed not only as indications of CPB-related complications but also potential therapeutic targets. We hypothesized that there would be an increase in 9-HODE and 13-HODE and ratio of 9:13-HODE during pediatric CPB and an association with increased PHb, markers of inflammation (WBC) and vasoactive medication use (milrinone use, vasoactive inotropic score [VIS]), and indications of overall clinical burden such as hospital and ICU length of stay (LOS) and duration of mechanical ventilation.

MATERIALS AND METHODS

This prospective study was approved by the Institutional Review Board at the University of Pittsburgh. Informed consent was obtained during outpatient pre-surgery clinic visits at the UPMC Children's Hospital of Pittsburgh between May 2012-January 2016. Inclusion criteria were age <18yrs and cardiac surgery requiring CPB. Exclusion criteria were neonatal age, preexisting renal dysfunction, and pregnancy.

CPB involved the use of a roller pump (Stockart SIII; Sorin Group, Arvada, CO) with blood flow based on cardiac index of 2.5–3 L/min/m² and maintenance of core temperatures 32–35 °C. A blood-primed CPB circuit was used for patients <25kg or when expected diluted hematocrit was <25%.

Blood samples were collected at the start (StartCPB) and end of CPB (EndCPB). Additional data included: age, weight, gender, surgical procedure, the Risk Adjusted classification for Congenital Heart Surgery (RACHS-1) score [26], CPB duration, cross-clamp duration, blood prime, mechanical ventilation days, and ICU (ICU LOS) and hospital length of stay (Hosp LOS).

PHb levels were determined in the CHP clinical labs and PHb was defined as EndCPB-StartCPB levels. PHb data from some patients in this population were previously published [27]. VIS [28] was calculated from three periods: <2h post-CPB, 2–24h post-CPB, 24–48h post-CPB. SvO₂ was obtained from medical records on ICU admission <2h post-CPB and within the first postoperative day 2–24h post-CPB. WBC count was obtained from medical records at baseline, ICU admission, and post-operative days #1 and #2. Milrinone use was quantified in days. Milrinone is routinely started in the operating room and titrated during the first postoperative day based on surrogate markers of cardiac output, including SVO₂ and urine output, as well as clinical factors such as blood pressure and peripheral perfusion. It is standard practice to reduce the infusion over the first postoperative night as tolerated and minimize the use of exogenous catecholamines.

HODEs were assayed from plasma lipids. Ongoing oxidation was stabilized upon sample collection with EDTA tubes and treatment of plasma with butylated hydroxytoluene prior to storage at –80 degrees. Total plasma lipids were extracted using the Bligh and Dyer method

[29]. Lipid extracts spiked with 3-hydroxyheptadecanoic acid and heptadecanoic acid (internal standards) were then hydrolyzed (saponified) with KOH. The mixture of free fatty acids was extracted and methylated with diazomethane obtained with the System 45 Aldrich diazomethane generator [30]. The fatty acid methyl esters mixture was dissolved in cyclohexane and then deuterated with platinum catalyst [30, 31]. Plasma was aliquoted for quantitation of LA with gas chromatography-mass spectrometry (GC/MS) and further processing with silica solid phase extraction cartridges to isolate saturated methyl esters of hydroxy fatty acids [30]. These isolated fractions were derivatized with tert-butyldimethylsilylimidazole [32]. Prepared tert-butyldimethylsilyl ethers of methyl esters of hydroxy fatty acids were dissolved in ethyl acetate and then analyzed with GC/MS performed with the Shimadzu GC-MS QP-2010 system equipped with a SHRXI-5MS 0.25mmx30m fused-silica capillary column. Signals from ion fragments 371, 339, 271 m/z, and 371, 339, 215 m/z and corresponding peak retention time were monitored to identify peaks of 9-HODE and 13-HODE, which were then normalized to levels of LA.

Statistical analysis

Categorical variables are presented as frequencies, compared with chi-squared or Fisher's exact tests. Continuous variables are presented as median, interquartile range (IQR) and compared with the Mann-Whitney U test. To account for repeated measures and clustering of data within subjects across time points for HODEs, WBC, and PHb, we used random effects models with generalized linear models and the longitudinal data modules of STATA (xt). The highest VIS within each of the time periods was calculated. Simple linear regression methods evaluated relationships between ratios of 9:13-HODE and milrinone use, VIS, SvO₂, WBC count, and PHb as well as between PHb and milrinone use, VIS, and SvO₂. Outliers (3*IQR below the 25th or above the 75th percentile) were removed (1 value for milrinone use, 2 values for 9-HODE and 13-HODE at Start and EndCPB, 1 value for mechanical ventilation and ICULOS). Data were transformed for normalization.

The VIS outcome was dichotomized into those with VIS ≥ 10 or VIS <10 within 48h post-CPB [28, 33–35]. Backward stepwise logistic regression identified risk factors associated with VIS ≥ 10 . Variables with the weakest adjusted associations with VIS ≥ 10 (by Wald test and with a $p > 0.1$) were removed from the multivariable model if their elimination did not significantly reduce the goodness of fit. Model fit was assessed using the area under the receiver operating characteristic curve (AUC) and the Hosmer-Lemeshow (H-L) goodness of fit test.

Spearman's rank order correlations were completed between LOS and CPB, PHb, HODE, and VIS data.

Alpha of 0.05 was selected for analyses using STATA 14.0 (StataCorp, College Park, TX).

RESULTS

Demographic, CPB, RACHS-1, mechanical ventilation, and LOS data as well as differences between dichotomized VIS groups within the three time periods are listed in Table 1. There

was no mortality. Pearson correlation coefficients for the linear relationships and medium to large effect sizes of the associations are provided in Supplemental Table 1.

Plasma HODE and association with CPB duration, milrinone use, VIS, and SvO₂

LA levels were unchanged (median 69.6 μ mol/dL [IQR 59.0–79.0] at Start CPB and 64.4 [54.4–74.0] at EndCPB). 9-HODE and 13-HODE (normalized to LA) increased during CPB (Figure 1A and 1B). Individual levels of 9-HODE and 13-HODE at StartCPB and EndCPB as well as the change in 9-HODE and 13-HODE from Start to EndCPB were not associated with CPB duration, milrinone use, or VIS.

The ratio of 9:13-HODE decreased from StartCPB to EndCPB ($p<.05$) (Fig 1A inset). 9:13-HODE at EndCPB ($R^2=0.12$, $p<.05$) was associated with CPB duration.

9:13-HODE at StartCPB (Fig 1C) was associated with VIS at 2–24h post-CPB ($R^2=0.25$, $p<.01$) and milrinone use ($R^2=0.17$, $p<.05$) whereas 9:13-HODE at EndCPB (Fig 1D) was associated with VIS at 2–24h and 24–48h post-CPB ($R^2=0.17$ and $R^2=0.12$, $p<.05$) and milrinone use ($R^2=0.19$, $p<.05$).

9:13 HODE at StartCPB was negatively associated with SvO₂ 2h-24h post-CPB ($R^2=0.13$, $p<.05$). 9:13 HODE at EndCPB was negatively associated with SvO₂ <2h ($R^2=0.16$, $p<.05$) and 2h-24h ($R^2=0.13$, $p<.05$) post-CPB.

White blood cell count and association with 9:13-HODE

WBC count increased during CPB and remained elevated through the collected 2 postoperative days (Fig 2A). Foldchange in WBC count (at ICU admission from baseline) was associated with 9:13-HODE at StartCPB ($R^2=0.12$, $p<.05$) (Fig 2B).

Cell-free plasma hemoglobin and associations with 9:13-HODE, milrinone use, VIS, and SvO₂

PHb levels increased during CPB (Fig 3A).

Individual levels of 9-HODE and 13-HODE at StartCPB and EndCPB as well as the change in 9-HODE and 13-HODE from Start to EndCPB were not associated with the PHb but 9:13-HODE at both StartCPB and EndCPB ($R^2=0.21$ and $R^2=0.23$, $p<.01$) were (Figure 3B).

The PHb was associated with milrinone use ($R^2=0.36$, $p<.001$) (Figure 3C) and VIS <2h ($R^2=0.22$, $p<.01$), 2–24h ($R^2=0.24$, $p<.01$), and 24–48h ($R^2=0.48$, $p<.001$) post-CPB (Figure 3D).

PHb was negatively associated with SvO₂ <2h ($R^2=0.22$, $p<.05$) and 2h-24h ($R^2=0.21$, $p<.01$) post-CPB.

Risk factors for an elevated VIS

<2h post-CPB, CPB duration was a risk factor for an elevated VIS. 9:13-HODE at StartCPB and PHb were identified between 2h-24h post-CPB and 24–48h post-CPB, respectively (Table 2).

Length of stay and mechanical ventilation days

All the variables in Table 3 were correlated with ICU and Hosp LOS. Similarly, these same variables except 9:13-HODE at StartCPB and VIS<2h post-CPB were correlated with mechanical ventilation days.

DISCUSSION

In this patient population, i) 9-HODE and 13-HODE, PHb, and WBC increased and 9:13-HODE decreased; ii) 9:13-HODE at StartCPB and EndCPB was associated with milrinone use and VIS iii) 9:13-HODE at StartCPB and EndCPB was associated with the PHb; iv) the PHb was associated with VIS at all time periods (<2h, 2–24h, and 24–48h post-CPB); v) 9:13-HODE at StartCPB was associated with the fold change in WBC; vi) CPB duration, PHb, and 9:13-HODE at StartCPB were identified as time dependent risk factors for an elevated VIS; vii) CPB duration, PHb, 9:13-HODE, and VIS correlated with ICU and HospLOS and/ mechanical ventilation days.

To our knowledge, this is the first report of increased generation of HODEs in CPB using GC-MS. Plausible mechanisms include PHb's quasi-lipoxygenase activity, CPB-related inflammatory cell activation resulting in lipoxygenase activation, random free radical mediated oxidation, or possibly, a combination of all three.

It was not the individual levels but rather the ratio of 9:13-HODE that seemed to have the most significant relationship with PHb and VIS. Interestingly, it has been suggested that 9-HODE and 13-HODE have opposing effects and thus the level of 9-HODE relative to that of 13-HODE may have biological significance.

HODEs may have effects on vascular tone. Activation of the nuclear receptor, peroxisome proliferator-activated receptor gamma (PPAR γ), by 13-HODE was shown to inhibit thromboxane formation, which is a vasoconstrictor and potent inducer of platelet aggregation [14]. 13-HODE stimulated prostacyclin synthesis in fetal bovine aortic endothelial cells and canine coronary arteries where it induced vasodilation [15, 16]. The addition of 9-HODE effectively decreased production of the vasodilator, prostaglandin I₂, in human endothelial cultures [17]. Thus, we expected that the vasoconstrictive effects of 9-HODE vs. the vasodilatory effects of 13-HODE may be detectable via VIS. Because milrinone is a significant part of VIS [28], we also analyzed its use separately. We did this because in the pediatric cardiac surgery population, milrinone acts as a vasodilator by decreasing systemic vascular resistance and is commonly used to treat post-CPB low cardiac output. We found an association between higher 9:13-HODE ratios with VIS and milrinone use, suggesting that higher levels of 9-HODE relative to 13-HODE may lead to increased systemic vascular resistance necessitating milrinone use, resulting in a higher VIS.

HODEs are lipid mediators of inflammation and cell proliferation processes with both pro- and anti-inflammatory effects. HODE levels were significantly increased in patients with PAH and may contribute to the proinflammatory dysfunction seen in this disease [9]. 13-HODE is a known ligand for the anti-inflammatory effects of PPAR γ [11]. 9-HODE, on the other hand, has been shown to induce IL-1 β release from human monocyte-derived macrophage, suggesting a role in vascular smooth muscle cell proliferation [12], and exhibits pro-inflammatory effects through G2A (a stress-inducible G protein-coupled receptor) signaling [13]. Our data suggests some level of inflammation as evidenced by increased WBC count. 9:13-HODE at StartCPB was related to WBC count, suggesting that increased baseline levels of 9-HODE may magnify the inflammatory response on CPB. Congenital heart disease itself is a stressful condition and there may be a difference between subjects in their baseline level of inflammation or potential inflammatory response to stressful situations such as CPB. In addition, there may be an effect of the ratio of HODE production during stress or a threshold effect where higher concentrations of 9-HODE may prove to be more detrimental.

PHb in our study was associated with 9:13-HODE, milrinone use, VIS, and SvO₂. It is not possible to determine with certainty from this study whether PHb led to increased production of 9-HODE relative to 13-HODE versus 9:13-HODE predisposing to increased

PHb. We expected that the non-enzymatic quasi-LOX activity of PHb [22, 23] may result in higher HODE levels but thought it would do so indiscriminately. However, as higher ratios of 9:13-HODE and not absolute levels were associated with higher PHb, this suggested instead that baseline ratios of 9:13-HODE may confer increased susceptibility to hemolysis through the action of these lipid mediators. In rats, the injection of linoleic acid causes oxidative damage to erythrocytes leading to acute anemia [36]. Thus, the potential relationship between PHb and HODE levels merit further study.

We used VIS as an objective and established outcome measure of vascular dysfunction. It is based on the type and amount of vasoactive medications given which, in our institution, are titrated to achieve adequate cardiac output. In a single center such as ours where practitioners adopt similar management styles, VIS can thus serve as a surrogate measure of adequate cardiac output end-organ perfusion. Higher VIS is also associated with increased morbidity and mortality after pediatric cardiac surgery and has been validated in this population [28, 33–35].

We dichotomized VIS based on previous studies which used cutoffs between 10 and 20 [28, 33–35]. We used the lower cutoff of 10 because our population represents a relatively healthy cohort of patients undergoing CPB and generally have good outcomes. Regardless, a higher VIS even in this population was correlated with LOS and mechanical ventilation duration. It is likely there would be a much greater impact in sicker or more complex patients undergoing CPB.

The initial time frame <2h post-CPB was separated from the subsequent time points because this reflected a period where patients were often still in the operating room. It also coincided with recent arrival to the cardiac ICU after transport. Thus, we thought that VIS did not fully reflect the patient's daily vasoactive medication requirements and more likely represented

the immediate postoperative period where patients were recovering from anesthesia and requiring blood product or fluid resuscitation. Our findings suggested that although operative conditions such as CPB duration may be more important in the immediate postoperative period, 9:13-HODE at StartCPB and the PHb also seemed to have relevance as time progresses.

Lipid peroxidation products are often difficult to analyze as they are present in low abundance, relatively unstable, and break down into secondary metabolites. We enhanced the accuracy of our results by using a sensitive MS technique and pretreating samples to stabilize ongoing *ex vivo* oxidation. Despite the relatively modest increase in HODEs, we found associations with physiologically important parameters that suggested that HODEs may be highly potent lipid mediators.

Many of our patients did not have SvO₂ measured after the first 24 postoperative hours, but we found that 9:13 HODE at both StartCPB and EndCPB and PHb were negatively associated with SvO₂ during this time period. This suggests that HODEs and PHb can have clinically relevant impacts and would need to be explored further in a larger, sicker patient population.

There are several other limitations to this study. The results are not generalizable to all pediatric CPB as our study population represents a fraction of the type of patients that undergo CPB. Our sample size was limited and additional studies in larger, more heterogeneous populations are warranted to explore the potential unfavorable effects of these oxylipins and their relationship with other potential risk factors for CPB-related injury. This is evident in the multivariable VIS models where the confidence intervals of the 9:13-HODE ratios were very large. Although the VIS has been validated in this population, we recognize that it is an imperfect outcome measure. In our relatively healthy patient population where vasoactive medications are titrated to clinical markers such as SvO₂ and peripheral perfusion and more sophisticated measurements of post-operative cardiac output are not routinely obtained, the VIS gave us the most consistently available measure of hemodynamic status. However, assumptions had to be made about the indications for the use of vasoactive medications, especially that of milrinone, and the uniformity of clinical practice within our institution and is a limitation of using VIS as an outcome measure.

Nevertheless, we showed that lipid oxidation products, such as HODEs, are not just end products of CPB-related inflammation or oxidative injury but can also have detrimental bioactive properties of their own. Our observations were detectable very early at the end of CPB in a single center, relatively healthy sample and were also correlated with indicators of health care utilization and cost (ICU and HospLOS and mechanical ventilation days). These observations may be even more meaningful for sicker, more complex patients. More importantly, our results suggest that HODEs can be biologically active with implications for critical care management and could possibly be considered a therapeutic target.

CONCLUSIONS

In low-risk pediatric patients undergoing CPB for cardiac surgery, 9-HODE, 13-HODE, PHb, and WBC count increased and 9:13-HODE was associated with the PHb, milrinone use, VIS, and WBC count. HODE and PHb may represent risk factors for CPB-related dysfunction. Future studies in larger, more heterogeneous pediatric patient populations are warranted to further delineate the role of HODEs and PHb in CPB-related dysfunction and explore HODE production as a potential therapeutic target.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

(A) 9-HODE and 13-HODE levels (normalized to LA) increased during cardiopulmonary bypass and the ratio of 9:13-HODE decreased from Start to EndCPB (* $p < .01$). (B) Single ion GC/MS chromatograms of 9-HODE (Rt=28.77min) and 13-HODE (Rt=29.35 min). Signals from ion fragment with $m/z = 339.0$ ($[M-57-32]^+$). Y-axis -relative abundance (au); X-axis – time (min.). Chromatograms were normalized on the content of LA in the samples. (C) 9:13-HODE at StartCPB was associated with VIS at 2–24h post-CPB ($R^2=0.25$, $p < .01$) and milrinone use ($R^2=0.17$, $p < .05$) (D) 9:13-HODE at EndCPB was associated with VIS at 2–24h ($R^2=0.17$, $p < .05$) and 24–48h post-CPB ($R^2=0.12$, $p < .05$) as well as milrinone use ($R^2=0.19$, $p < .05$).

Figure 2.

(A) The WBC count increased during CPB and remained elevated for 2 days post-CPB

(* $p < .05$, ** $p < .01$). (B) There is an association between the fold change in WBC count and the ratio of 9:13-HODE at StartCPB ($R^2 = 0.12$, $p < .05$).

Figure 3.

(A) Cell-free plasma hemoglobin levels increased during cardiopulmonary bypass ($*p < .001$). (B) The ratio of 9:13-HODE at both StartCPB ($R^2 = 0.21$, $p < .01$) and EndCPB ($R^2 = 0.23$, $p < .01$) was associated with the change in cell-free plasma hemoglobin. (C) The change in cell-free plasma hemoglobin was associated with milrinone use ($R^2 = 0.36$, $p < .001$). (D) The change in cell-free plasma hemoglobin was associated with VIS <2h ($R^2 = 0.22$, $p < .01$), 2–24h ($R^2 = 0.24$, $p < .01$), and 24–48h ($R^2 = 0.48$, $p < .001$) post-CPB.

Table 1:

Demographic and clinical characteristics

Characteristic	All patients (n=34)	VIS<10 (<2h post-CPB) (15/34)	VIS 10 (<2h post-CPB) (19/34)	VIS<10 (2-24h post-CPB) (17/34)	VIS 10 (2-24h post-CPB) (17/34)	VIS<10 (24-48h post-CPB) (29/34)	VIS 10 (24-48h post-CPB) (5/34)
Age (in Years)	2.5 (0.6–12.0)	6.3 (2.1–12.6)	1.8 (0.4–12.0)	6.8 (2.5–12.1)	1.75 (0.4–3.4) [#]	2.6 (0.6–12.1)	0.7 (0.4–3.4)
Weight (kg)	12.1 (7.0–38.9)	17.3 (9.2–46.9)	10.5 (6–25.1)	18.5 (9.9–46.9)	10.5 (6.0–12.5) [#]	12.4 (8.4–40.4)	6.2 (5–12.5)
Gender - Male	18/34 (53%)	8/15 (53%)	10/19 (53%)	9/17 (53%)	9/17 (53%)	17/29 (59%)	1/5 (20%)
CPB duration (min)	74.5 (60–109)	62 (45–78)	106 (72–134) [*]	69 (59–78)	106 (72–128) [#]	73 (60–109)	101 (78–128)
Cross-clamp duration (min)	37 (21–63)	29 (21–40)	50 (18–75)	32 (22–50)	52 (18–72)	40 (23–63)	10(0–28)
Blood prime	25/34 (74%)	10/15 (67%)	15/19 (79%)	11/17 (65%)	14/17 (82%)	20/29 (69%)	5/5 (100%)
RACHS-1							
Risk Category 1	3/34 (9%)	2/15 (13%)	1/19 (5%)	2/17 (12%)	1/17 (6%)	3/29 (10%)	0/5 (0%)
Risk Category 2	15/34 (44%)	7/15 (47%)	8/19 (42%)	8/17 (47%)	7/17 (41%)	15/29 (52%)	0/5 (0%)
Risk Category 3	13/34 (38%)	4/15 (27%)	9/19 (47%)	5/17 (29%)	8/17 (47%)	9/29 (31%)	4/5 (80%)
Risk Category 4	3/34 (9%)	2/15 (13%)	1/19 (5%)	2/17 (12%)	1/17 (6%)	2/29 (7%)	1/5 (20%)
Mechanical ventilation (days)	0 (0–1)	0 (0–0)	0 (0–1)	0 (0–0)	0 (0–1)	0 (0–0)	2 (2–2) [†]
ICU LOS (days)	2 (1–7)	1 (1–3)	3 (2–7) [*]	1 (1–3)	4 (2–9) [#]	2 (1–4)	7 (7–10) [†]
Hospital LOS (days)	5 (3–10)	4 (3–10)	8 (4–14)	4 (3–8)	8 (4–14)	4 (3–9)	10 (8–14) [†]

Continuous data described as median (IQR). All other data described as proportion (percentage).

^{*} p<.05 between VIS groups when <2h post-CPB,[#] p<.05 when 2–24h post-CPB,[†] p<.05 when 24–48h post-CPB

Table 2.

Factors associated with elevated VIS scores

VIS score 10 within 2h after CPB (19/34 patients)		
	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Ratio of 9:13-HODE at Start CPB	7.82 (0.52–117.05), p=0.14	
Ratio of 9:13-HODE at End CPB	20.33 (0.36–1142.48), p=0.14	
Change in cell-free plasma hemoglobin	1.02 (1.00–1.04), p<.05	
CPB duration	1.04 (1.01–1.07), p<.05	1.04 (1.01–1.07), p<.05
		AUC=0.80, H-L p=0.46

VIS score 10 between 2h-24h after CPB (17/34 patients)		
	Unadjusted	Adjusted
Ratio of 9:13-HODE at Start CPB	19.23 (1.11–333.16), p<.05	17.86 (1.03–309.07, p<.05)
Ratio of 9:13-HODE at End CPB	42.44 (0.67–2680.15), p=0.08	
Change in cell-free plasma hemoglobin	1.02 (1.00–1.03), p=.06	
CPB duration	1.02 (1.00–1.04), p=.08	
		AUC = 0.70, H-L 0.37

VIS score 10 between 24h-48h after CPB (5/34 patients)		
	Unadjusted	Adjusted
Ratio of 9:13-HODE at Start CPB	14.22 (0.43–466.58), p=0.14	
Ratio of 9:13-HODE at End CPB	129.05 (0.30–55900), p=0.12	
Change in cell-free plasma hemoglobin	1.02 (1.00–1.05), p<.05	1.02 (1.00–1.05, p<.05)
CPB duration	1.01 (0.98–1.03), p=0.58	
		AUC = 0.84, H-L p=0.69

Table 3.

Correlation of CPB duration, change in cell-free plasma hemoglobin, 9:13 HODE, and VIS score with ICU LOS, Hospital LOS, and mechanical ventilation days

Variable	ICU LOS (days)		Hospital LOS (days)		Mechanical ventilation days	
	rho (ρ)	p	rho (ρ)	p	rho (ρ)	p
CPB duration	0.70	<.0001	0.60	<.001	0.61	<.001
Change in cell-free plasma hemoglobin	0.73	<.0001	0.66	<.0001	0.55	<.01
9:13 HODE at Start CPB	0.42	<.05	0.37	<.05	0.33	0.06
9:13-HODE at End CPB	0.58	<.001	0.52	<.01	0.44	<.05
VIS score within 2h after CPB	0.44	<.05	0.49	<.01	0.32	0.07
VIS score 2h-24h after CPB	0.60	<.001	0.62	<.001	0.41	<.05
VIS score 24h-48h after CPB	0.83	<.0001	0.78	<.0001	0.60	<.001