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Cell-free plasma hemoglobin and male gender are risk factors for AKI in low risk children undergoing cardiopulmonary bypass

Nahmah Kim-Campbell, MD¹, Catherine Gretchen, MD¹, Clifton Callaway, MD², Kathryn Felmet, MD¹, Patrick M. Kochanek, MD^{1,5}, Timothy Maul, CCP, PhD⁶, Peter Wearden, MD, PhD^{6,7}, Mahesh Sharma, MD⁶, Melita Viegas, MD⁶, Ricardo Munoz, MD, FAAP, FCCM, FACC¹, Mark T. Gladwin, MD^{3,4}, and Hülya Bayir, MD^{1,5}

¹Department of Critical Care Medicine, UPMC and University of Pittsburgh, Pittsburgh, PA

²Department of Emergency Medicine, UPMC and University of Pittsburgh, Pittsburgh, PA

³Pittsburgh Heart, Lung, Blood and Vascular Medicine Institute, University of Pittsburgh, Pittsburgh, PA

⁴Division of Pulmonary, Allergy and Critical Care Medicine, UPMC and University of Pittsburgh, Pittsburgh, PA

⁵Safar Center for Resuscitation Research, University of Pittsburgh, Pittsburgh, PA

⁶Department of Cardiothoracic Surgery, UPMC and University of Pittsburgh, Pittsburgh, PA

⁷Nemours Cardiac Center –Florida, Orlando, FL

Abstract

Objective—To determine the relationship between the production of cell-free plasma hemoglobin (PHb) and acute kidney injury (AKI) in infants and children undergoing cardiopulmonary bypass (CPB) for cardiac surgery.

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 outpatient appointment with the following criteria: >1month to <18 years old, procedures requiring CPB, no preexisting renal dysfunction.

Interventions—None

Measurements and Main Results—Plasma and urine were collected at baseline (in a subset), the beginning and end of CPB, and 2h and 24h after CPB in 60 subjects. Levels of PHb increased

Correspondence and reprints to: Nahmah Kim-Campbell, MD, 4401 Penn Ave. Faculty Pavilion Suite 2000, Pittsburgh, PA 15224, 412-692-5164, nahmah.kimcampbell@chp.edu.

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during CPB and were associated ($p<0.01$) with CPB duration ($R^2=0.22$), depletion of Hp at end and 24h after CPB ($R^2=0.12$ and 0.15 , respectively), LDH levels at end CPB ($R^2=0.27$), and change in creatinine ($R^2=0.12$). 43% of patients developed AKI. There was an association between PHb level and change in creatinine that varied by age (overall [$R^2=0.12$, $p<0.01$], in age >2 yrs [$R^2=0.22$, $p<0.01$], and in <2 yrs [$R^2=0.03$, $p=0.42$]). Change in PHb and male gender were found to be risk factors for AKI (OR 1.02 and OR 3.78, $p<0.05$).

Conclusions—Generation of PHb during CPB and male gender are associated with subsequent renal dysfunction in low risk pediatric patients, especially in those >2 yrs of age. Further studies are needed to determine whether specific subgroups of pediatric patients undergoing CPB would benefit from potential treatments for hemolysis and PHb-associated renal dysfunction.

Keywords

hemolysis; cell-free plasma hemoglobin; creatinine; acute kidney injury; cardiopulmonary bypass; pediatrics

INTRODUCTION

Cardiopulmonary bypass (CPB) during pediatric cardiac surgery facilitates the palliation or correction of congenital heart defects. However, the unfavorable sequelae of CPB-supported cardiac surgery remain complex and incompletely understood. Post-operative acute kidney injury (AKI) is a common complication of CPB reported in up to 52% of cardiac surgeries in pediatric studies, which usually include neonates and cyanotic lesions [1–3]. AKI independently predicts mortality and is associated with longer length of stays in critically ill pediatric patients [4–7]. The long-term sequelae of CPB-mediated AKI and its impact in the setting of multiple surgeries also remain to be defined.

The pathophysiology of AKI after CPB is likely multifactorial. Possible contributors include hypoperfusion or ischemia-reperfusion induced inflammation. Age, particularly neonates, and CPB duration are associated with increased risk of AKI [3, 8, 9]. Because AKI can occur without measurable hypoperfusion, its association with longer CPB durations suggest that CPB can directly injure the kidney. One potential mechanism for this is increased hemolysis resulting from longer CPB durations. Recent data suggest that cell-free plasma hemoglobin (PHb) increases nitric oxide (NO) consumption, augments oxidative damage, and causes vascular dysfunction [10–12].

The relative contribution of PHb to CPB-associated AKI in pediatric patients is unclear. Many patients with congenital heart disease have pre-surgical hemodynamic compromise, cyanosis, or other complications that can lead to AKI. In order to better understand the specific effects of CPB, we studied a group of pediatric patients undergoing semi-elective cardiac surgery. The primary objective of this study was to determine the relationship between the production of PHb and AKI, while accounting for other risk factors, in this relatively healthy pediatric population.

MATERIALS AND METHODS

This was a prospective study approved by the Institutional Review Board at the University of Pittsburgh. Patients were enrolled during their outpatient pre-surgery clinic visits at the Children's Hospital of Pittsburgh (CHP) between May 2012 and September 2016. Inclusion criteria were age <18yrs and a scheduled procedure requiring CPB. Exclusion criteria were neonatal age, preexisting renal dysfunction, and pregnancy.

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

Blood and urine were collected at the start (StartCPB) and end of CPB (EndCPB) and 2h (2hREP) and 24h after reperfusion (24hREP). Blood samples were collected from the venous side of the CPB circuit during surgery or from a central venous or arterial catheter after reperfusion. In a subset of 40 subjects, baseline samples were collected upon insertion of a central venous catheter.

Demographic and clinical data collected included: age, gender, weight, surgical procedure, Kidney Disease: Improving Global Outcomes (KDIGO) score [13], the Risk Adjusted classification for Congenital Heart Surgery (RACHS-1) score [14], CPB duration, cross-clamp duration, mechanical ventilation days, and ICU (ICULOS) and hospital length of stay (HospLOS). Baseline creatinine levels were collected at the time of enrollment.

AKI was defined as an increase in serum creatinine (SCr) of ≥1.5 times baseline at any point during hospitalization as described in the KDIGO guidelines [13]. Urine output was intentionally not used in our definition due to variability in quantification (early removal of urinary catheters, use of absorbent diapers, and variable physician-dependent diuretic use).

PHb (normal <6mg/dL), haptoglobin (Hp), and LDH levels were determined in the CHP clinical labs. Lactate and SvO₂ were obtained from the medical record and were generally only available for 24hREP. The vasoactive-inotropic score (VIS score) was calculated as previously described [15].

Statistical analysis

Categorical variables are presented as frequencies and compared with a chi-squared test or Fisher's exact test when appropriate. Continuous variables are presented as median, interquartile range (IQR) and compared with the Mann-Whitney U test.

In order to account for the repeated measures and clustering of data within subjects across time points of PHb, Hp, and LDH, we used time-course analyses with general linear models and the longitudinal data modules of STATA (xt). Simple linear regressions were used to evaluate the association of changes in Hp and LDH with changes in PHb from StartCPB to EndCPB (ΔPHb).

Our primary outcome of AKI was analyzed as both a continuous (fold change in creatinine, fold Cr) and categorical variable (Stage 0 = no AKI, Stage 1 = AKI). We used a simple linear regression to explore the relationship between fold Cr and PHb. As renal maturity does not fully occur until 2yrs [16], we included an interaction between PHb and age. Backward stepwise regression analysis was used to identify risk factors associated with AKI. Risk factors with $p < 0.1$ in bivariate analysis were included in a multivariate logistic regression model. Keeping PHb in the model, variables with the weakest adjusted associations with AKI (by Wald test and with a p to remove of 0.1) were removed from the multivariate model if their elimination did not significantly reduce the goodness of fit.

Pearson product correlations were completed for LOS data and variables from Table 1 that were different between groups. Effect size was calculated by Cohen's d , the area under the receiver operating characteristic curve (AUC) and the Hosmer-Lemeshow (H-L) goodness of fit test.

An alpha-error rate of 0.05 was selected for analyses which were completed using STATA 14.0 (StataCorp, College Park, TX). Figures were created using GraphPad Prism 7.00 (GraphPad Software, San Diego, CA).

RESULTS

Demographic and clinical characteristics of 60 subjects are listed in Table 1. There was no mortality. Subjects with AKI were more often male, had higher PHb, longer CPB duration, higher VIS scores 24REP-48hREP, lower SvO₂ within 24hREP, and longer ICU and HospLOS.

Generation of cell-free plasma hemoglobin (PHb) during CPB and indicators of hemolysis

PHb levels increased during CPB and returned to StartCPB levels by 24hREP (Figure 1A). CPB duration was associated with PHb ($R^2=0.22$, $p<0.01$). In the subset of 40 subjects with baseline levels, there was no difference in PHb between baseline (median [IQR], 5.9mg/dL [2.9–13.0]) and StartCPB (7.8mg/dL [3.8–12.8]).

Median PHb in 45 subjects with a blood prime and 15 subjects without was 48.8mg/dL (35.6–86.4) and 33.9mg/dL (15–47.9), respectively, with no difference ($p>0.05$) between groups.

Haptoglobin (Hp) levels decreased during CPB and declined until 24hREP (Figure 1B). Change in Hp levels from StartCPB to EndCPB and to 24hREP was associated with PHb ($R^2=0.12$, $p<0.01$ and $R^2=0.15$, $p<0.01$, respectively).

LDH levels increased during CPB and rose until 24hREP (Figure 1C). Change in LDH levels from StartCPB to EndCPB was associated with PHb ($R^2=0.27$, $p<0.01$).

Evaluation of renal dysfunction

26/60 subjects (43%) met criteria for AKI [Stage 1 (18/26, 69%), Stage 2 (4/26, 15%), Stage 3 (4/26, 15%)] by change in SCr levels as defined by the KDIGO guidelines [13]. No subjects required renal replacement therapy.

Creatinine peaked within 48hREP in 92% of subjects (50 within 24hREP, 5 between 24hREP-48hREP, and 5 after 48hREP). Of the 26 patients with AKI, 17/26 (65%) developed AKI within 24hREP (65%), 4/26 (15%) between 24hREP-48hREP, and 5/26 (19%) after 48hREP.

Association of cell-free plasma hemoglobin with renal dysfunction

Overall, fold Cr was associated with PHb ($R^2=0.12$, $p<0.01$, Figure 2A) which was also demonstrated in subjects >2 yrs ($R^2=0.22$, $p<0.01$, Figure 2B) but not in subjects <2 yrs ($R^2=0.03$, $p=0.42$, Figure 2C).

Using AKI as a dichotomous outcome variable, age, male gender, PHb, CPB duration, RACHS-1 score, highest VIS score within 24hREP and between 24hREP-48hREP, and highest lactate and lowest SvO₂ within 24hREP were entered into a backward stepwise logistic regression analysis. PHb (OR 1.02, $p<0.05$) and male gender (OR 3.78, $p<0.05$) were identified as risk factors for AKI (Table 2).

When repeating this analysis in subjects >2 yrs, PHb (OR 1.04, $p<0.05$) and male gender (OR 26.01, $p<0.05$) were again identified as risk factors for AKI (Table 3). None of the variables showed a relationship with AKI in the subset of subjects <2 yrs (Supplemental Digital Content – Table 1).

There were no differences in the variables of interest between males and females (Supplemental Digital Content-Table 2).

Length of stay

Subjects with AKI had longer ICU LOS and Hosp LOS (median 4d vs. 1d and 9d vs. 4d, respectively). ICU LOS correlated with Hosp LOS in both non-AKI and AKI groups ($\rho=0.66$ and 0.79 , respectively). In the non-AKI group, ICU LOS correlated with CPB duration, RACHS-1 score, highest VIS score (within 24hREP and 24hREP-48hREP), and days of mechanical ventilation whereas Hosp LOS correlated with RACHS-1 score and highest VIS score within 24hREP. In the AKI group, ICU LOS correlated with lowest SvO₂ within 24hREP and days of mechanical ventilation whereas Hosp LOS was only correlated with days of mechanical ventilation (Supplemental Digital Content – Table 3).

Effect size

In this study, overall, PHb was associated with AKI as measured by fold Cr with a medium effect size as calculated by Cohen's $d = 0.50$ which improved to 0.79 in the subset of subjects who were >2 yrs of age. Likewise, the AUC=0.69 and the H-L test $p=0.42$ in our whole population improved to AUC=0.84 and the H-L test $p=0.87$ in the >2 yrs subset.

DISCUSSION

Our study in patients with few pre-existing comorbidities suggests that: i) PHb levels rise during CPB; ii) AKI is common in low risk CPB patients; iii) PHb and male gender are associated with AKI; and iv) PHb is likely more important a risk factor for AKI in patients >2yrs.

PHb levels at EndCPB were higher than the upper limit measured during vaso-occlusive crisis in sickle cell patients (20 μ M [32mg/dL]) and the level associated with an 80% reduction of forearm blood flow in response to nitroprusside (6 μ M [9.7mg/dL]) [17, 18]. Thus, PHb produced during CPB is likely biologically active.

Consistent with prior studies, PHb was associated with CPB duration [3, 8, 19], likely from the mechanical destruction of red cells. Release of PHb disrupts the biological effects of nitric oxide (NO) and contributes to oxidative injury [12, 20–22]. NO maintains normal vascular tone and is scavenged by PHb, which reduces its bioavailability and impairs vascular function. This has been shown in several hemolysis-associated conditions (sickle cell disease and renal replacement therapy) [17, 21–23] and in studies involving animal models [24] and stored blood [25]. PHb was associated with NO consumption and biomarkers of renal and intestinal injury in adult CPB [26]. Children who received NO during CPB had shorter durations of mechanical ventilation and ICULOS [12]. PHb's role in oxidative stress lies, partly, in its peroxidase activity [27] which is influenced by reducing agents such as NO. In the presence of oxidizing agents, Hp bound hemoglobin also has peroxidase activity [27, 28]. Supporting this, PHb correlated with increased lipid peroxidation after CPB in both adults and children [11, 20].

There is conflicting evidence that transfusion of stored blood can lead to hemolysis [29–31] and so far, there is no evidence that a blood prime causes higher PHb levels. Many of our subjects (45/60, 75%) received a circuit blood prime with no difference in PHb from those who did not. There was also no association between PHb and RACHS-1 score. Thus, blood prime and surgery complexity did not contribute to PHb generation.

Most pediatric CPB studies include neonates, high RACHS-1 scores (5 and 6), pre-surgical ICU admissions, and cyanotic lesions. The presence of these comorbidities makes it difficult to define the specific contribution of PHb to AKI. A pediatric study involving 311 patients, which did not include neonates or high RACHS-1 scores, found a 42% incidence of AKI that was associated with younger age and longer CPB duration; PHb was not measured [9]. We attempted to create a homogenous sample with limited confounders by recruiting subjects through the cardiothoracic surgery clinic who are not neonates, generally without cyanosis, and well enough to be home without baseline renal dysfunction. 43% of our patients met criteria for AKI. Consistent with prior studies, a majority met criteria within 48hREP [2, 3, 9]. Thus, AKI was likely CPB-related and not secondary to later post-surgical complications. Our population did not include patients previously implicated to be at higher risk for AKI (i.e. neonates), yet we found a similar incidence (40–51%) [2, 3, 9]. This is an unexpected yet important finding and suggests that this phenomenon affects even low risk patients and likely represents a substantial problem.

PHb is an additional plausible contributor to CPB-associated AKI. In guinea pigs, hemolysis, vascular injury, and kidney dysfunction was attenuated by the Hb scavenger, haptoglobin [32]. In adult sickle cell patients, hemolysis is associated with progression of chronic kidney disease [33]. The production of PHb has been associated with renal injury in both adult [11, 26, 34] and pediatric [3] cardiac surgery.

We found an overall association between AKI and PHb that was independent of risk factors such as age, CPB duration, surgical complexity, and markers of cardiac output and perfusion. However, unlike previous studies, we also evaluated whether this association changed in patients <2yrs with immature kidneys. In this age group, the relationship between AKI and PHb is lost. In the analysis of our whole study population, PHb was forced into the model because the relationship of PHb with AKI was the primary objective and evidence from prior studies suggested that PHb has clinical relevance. However, in patients >2yrs, PHb was significant on its own and was not forced into the model. Thus, PHb is likely more important as a risk factor in patients >2yrs.

Another explanation is that SCr may not be an accurate measure of kidney function in the younger patient [35–37]. Prior CPB studies that found associations between younger age and AKI [2, 3, 38] included neonates who are not represented here. Our results suggest that in future studies of AKI using SCr, dedicated efforts may be required to investigate the relationship of PHb and AKI across the pediatric age spectrum.

Male gender was a risk factor for AKI. Females have a greater microvascular vasodilatory response to nitroglycerin [39] and males have a genetic predisposition to worse outcomes after trauma [40]. In adults with sickle cell, males have a greater risk for relative hypertension [41] and higher systemic and pulmonary vascular resistance [42]. In addition, both NO bioavailability and responses to exogenous NO were reduced in males [43]. Given that PHb is known to bind NO, this suggests that there may be a greater deleterious impact from similar levels of PHb in males. Only 16/60 (27%) of the patients in our cohort were >10yrs (the age of onset of secondary sexual characteristics in the U.S. [44, 45]) with no differences in proportion between genders. This suggests a non-hormonal mechanism that needs further exploration.

Consistent with other studies [4–7], patients with AKI had longer ICU and HospLOS. Thus, even in these low-risk patients, PHb-related AKI can have an impact on health care resources and cost. AKI can lead to renal replacement therapy, uremia, bleeding issues, and disturbances in pharmacokinetics, all of which can lead to longer LOS. AKI may also be an indicator of overall critical illness and probably does not solely contribute to LOS.

Recent evidence suggests that fluid overload after cardiac surgery leads to prolonged mechanical ventilation [46, 47]. In this study, subjects without AKI had LOS that were correlated with factors that suggested an increased need for cardiac support in the immediate postoperative period such as longer CPB duration, higher surgical complexity, higher VIS scores, and need for mechanical ventilation. However, subjects with AKI had LOS that did not correlate with this same profile and seemed to be more convincingly correlated with the duration of mechanical ventilation.

Our study has several limitations. Our results are not generalizable to all pediatric CPB. In addition, since CPB represents a complex therapy, there are likely many additional predictors of AKI. Nevertheless, our observations in comparatively healthy patients likely underestimates the potential effect of PHb which would suggest a much more robust impact on sicker or more complex patients undergoing CPB. In this study overall, PHb was associated with a higher incidence of AKI. The medium effect size in our whole population improved in the subset of subjects >2yrs. We found a similar result with a AUC for the full logistic regression model that improved in those >2yrs. A larger study involving all populations will be necessary to further elucidate these differences.

CONCLUSIONS

In conclusion, in low risk pediatric patients undergoing CPB, PHb levels increased during CPB, was associated with renal dysfunction in patients especially >2yrs of age, and may be more common in males. Studies of the impact of PHb on long term renal function and vulnerability are warranted. Future studies are also needed that target the evaluation of novel therapies to mitigate the effects of PHb on AKI after CPB.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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	Start CPB	END CPB	2h REP	24h REP
PHb (mg/dL)	8.0 (3.8-12.9)	52.8 (35.0-91.8)	35.9 (14.9-62.7)	8.5 (4.2-12.7)
Haptoglobin (mg/dL)	43.0 (24.5-62.5)	38.0 (26.0-55.0)	35.0 (24.0-48.5)	23.5 (1.0-53.5)
LDH (IU/L)	157 (127-197)	263 (203-319)	328 (273-405)	360 (297-470)

Figure 1.

(A) Cell-free plasma hemoglobin (PHb) levels increased during cardiopulmonary bypass (CPB) and returned to baseline by 24h reperfusion (REP). There was a difference (* $p < .01$) in PHb levels at EndCPB and 2hREP from StartCPB. (B) Haptoglobin levels (Hp) decreased during CPB and continued to fall for 24h after reperfusion. There was a significant difference (* $p < .01$) in Hp levels at 2hREP and 24hREP from StartCPB. (C) Lactate dehydrogenase (LDH) levels increased during CPB and continued to rise for 24h after reperfusion. There was a difference (* $p < .01$) in LDH levels at EndCPB, 2hREP, and 24hREP from StartCPB. Data is presented as median (IQR).

Figure 2.

(A) There was an overall association between fold Cr with PHb ($R^2=0.12$, $p<.01$). This association persisted in patients aged >2 yrs ($R^2=0.22$, $p<.01$) (B) but was not significant in patients <2 yrs ($R^2=0.03$, $p=0.42$) (C).

Table 1

Baseline characteristics by AKI

Characteristic	All patients (n=60)	Non-AKI (34/60, 57%)	AKI (26/60, 43%)	p
Age (in Years)	2.51 (0.48–10.52)	5.12 (0.52–12.63)	1.66 (0.38–4.74)	0.10
Weight (kg)	12.3 (6.3–30.9)	14.2 (6.3–40.4)	9.9 (6.0–15.9)	0.11
Gender - Male	30 (50%)	13 (38%)	17 (65%)	0.04
Change in PHb (mg/dL)	43.8 (31.3–83.4)	38.25 (18.5–60.6)	53.9 (39.7–86.7)	0.03
CPB duration (min)	78 (59–109)	72.5 (48–91)	97.5 (71–111)	0.03
Cross-clamp duration (min)	38 (22.5–61.5)	32 (23–52)	52.5 (21–72)	0.33
Blood prime	45/60 (75%)	24/34 (71%)	21/26 (81%)	0.37
RACHS-1				0.06
Risk Category 1	6 (10%)	6 (18%)	0 (0%)	
Risk Category 2	23 (38%)	14 (41%)	9 (35%)	
Risk Category 3	22 (37%)	9 (26%)	13 (50%)	
Risk Category 4	9 (15%)	5 (15%)	4 (15%)	
VIS score (highest score)				
In first 24h after CPB	10 (6–13)	10 (5–12.5)	12 (10–14)	0.07
24–48h after CPB	2 (0–5.5)	0 (0–5)	5 (1–7.5)	0.01
Lactate (highest)				
In first 24h after CPB	3.1 (2.4–4.1)	3.1 (2.3–4.3)	3.1 (2.5–3.8)	0.99
SvO2 (lowest)				
In first 24h after CPB	60.5 (50.5–72)	67 (54–73)	57.5 (44–64)	0.02
Mechanical ventilation (days)	0 (0–0)	0 (0–0)	0 (0–1)	0.09
ICU LOS (days)	2 (1–6)	1 (1–3)	4 (2–10)	<0.01
Hospital LOS (days)	5 (3–9)	4 (3–6)	9 (5–16)	<0.01

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

Table 2

Factors independently associated with AKI by KDIGO

Risk Factor	Unadjusted analysis		Adjusted analysis*	
	OR (95% CI)	p	OR (95% CI)	p
Age (yrs)	0.93 (.85–1.02)	0.10		
Male gender	3.05 (1.05–8.84)	0.04	3.78 (1.17–12.25)	0.03
Change in PHb (mg/dL)	1.01 (1.00–1.02)	0.07	1.02 (1.00–1.03)	0.03
CPB duration (min)	1.02 (1.00–1.03)	0.03		
RACHS-1 score	1.82 (0.96–3.43)	0.07		
VIS score (highest) in first 24h after CPB	1.13 (1.00–1.29)	0.05		
VIS score (highest) 24–48h after CPB	1.12 (0.99–1.26)	0.06		
Lactate (highest) in first 24h after CPB	0.98 (0.74–1.30)	0.89		
SvO2 (lowest) in first 24h after CPB	0.95 (0.92–0.99)	0.02		

* Adjusted for (age, male gender, change in PHb, CPB duration, RACHS-1 score, highest VIS score in first 24h after CPB, highest VIS score 24–48h after CPB, lowest SvO2 in first 24h after CPB)

Table 3

Factors independently associated with AKI by KDIGO in subjects >2yrs

Risk Factor	Unadjusted analysis		Adjusted analysis*	
	OR (95% CI)	p	OR (95% CI)	p
Age (yrs)	0.99 (.87–1.13)	0.85		
Male gender	6.22 (1.07–36.21)	0.04	26.01 (1.13–599.36)	0.04
Change in PHb (mg/dL)	1.02 (1.00–1.04)	0.06	1.04 (1.00–1.07)	0.03
CPB duration (min)	1.02 (1.00–1.04)	0.04		
RACHS-1 score	2.79 (1.06–7.37)	0.04		
VIS score (highest) in first 24h after CPB	1.25 (1.01–1.54)	0.04		
VIS score (highest) 24–48h after CPB	1.22 (0.95–1.55)	0.12		
Lactate (highest) in first 24h after CPB	1.15 (0.82–1.60)	0.42		
SvO2 (lowest) in first 24h after CPB	0.93 (0.86–1.01)	0.07		

* Adjusted for (male gender, change in PHb, CPB duration, RACHS-1 score, highest VIS score in first 24h after CPB, lowest SvO2 in first 24h after CPB)