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**Title Page**

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Perioperative Serum Free Hemoglobin and Haptoglobin Levels in Valvular and Aortic Surgery with Cardiopulmonary Bypass: Their Associations with Postoperative Kidney Injury

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**Declarations of interest:** none

**Abstract**

**Objective:** To observe the perioperative free hemoglobin and haptoglobin levels and to assess their associations with the risk of postoperative acute kidney injury (pAKI) in adult patients undergoing valvular and aortic surgery requiring cardiopulmonary bypass (CPB)

**Design:** A single-center prospective observational study

**Setting and participants:** Adult patients without chronic renal failure who underwent cardiovascular surgery requiring CPB from 2014 to 2020 in a public teaching hospital

**Measurements and main results:** We obtained perioperative free hemoglobin and haptoglobin levels during the study period. The primary outcome was pAKI defined by the KDIGO criteria. We finally included 74 patients in this study, and pAKI occurred in 25 patients (33.8%). Free hemoglobin level began to increase after the initiation of CPB and reached a peak level at 30 minutes after weaning from CPB. It returned to the baseline level on postoperative day 1. Haptoglobin levels were highest after anesthesia induction and decreased continuously until postoperative day 1. In our multivariate analysis, max free hemoglobin and minimum haptoglobin were independently associated with increased risk of pAKI (adjusted odds ratio=1.33 (95% CI:1.12, 1.58),

p=0.001 and 0.95 (95% CI:0.91, 1.00), p=0.03, respectively). We found that free hemoglobin level had begun to have an independent association with pAKI at 1 hour after commencement of CPB and that the independent association had disappeared at postoperative day 1.

**Conclusions:** We found that the perioperative increase of free hemoglobin level and decrease of haptoglobin level had independent associations with the risk of pAKI.

(243words)

**Key Words**

Free hemoglobin, Haptoglobin, Hemolysis, Cardiopulmonary bypass, Postoperative acute kidney injury

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## Introduction

Acute kidney injury (AKI) is frequently observed after cardiovascular surgery (CVS) with cardiopulmonary bypass (CPB) (1,2). Such postoperative AKI (pAKI) is known to be associated with a high rate of postoperative mortality and long stays in the intensive care unit (ICU) and hospital (2-4). There are many factors that contribute to pAKI after CVS, including age, anemia, diabetes, chronic lung disease, chronic heart failure, chronic renal dysfunction, administration of nephrotoxic agents, hypoperfusion, hypotension, embolization, and increased aortic clamp time. However, there is still no active treatment for pAKI after CVS (5,6). Thus, the focus of clinicians is on prevention and management of risk factors. Although various factors have been reported to be associated with pAKI after CVS, countermeasures for some of risk factors such as advanced age, co-morbidities, and use of CPB (6) are difficult.

Hemolysis is caused by CPB, blood transfusion and the use of a cell salvage system (7) and thus hemolysis frequently occurs in patients undergoing CVS with CPB. Hemolysis leads to an increase in serum free hemoglobin (fHb), which might lead to reduction of NO bioavailability and cause microcirculation dysfunction, possibly contributing to organ dysfunction including pAKI (8-10).

Considering the characteristics of hemolysis, administration of an fHb scavenger such

as external haptoglobin might be an effective strategy for preventing AKI in patients undergoing CVS with CPB (11,12). To explore such a possible strategy for preventing pAKI, it is necessary to consider 1) the time trends of serum fHb and haptoglobin levels during the perioperative period and 2) their associations with the risk of pAKI, 3) the value of fHb as an indicator of treatment timing.

However, there have been few studies in which perioperative changes in serum fHb and haptoglobin levels were investigated and their associations with pAKI in adult patients undergoing CVS with CPB were assessed.

Accordingly, we conducted a prospective observational study to obtain the time trends of perioperative fHb and haptoglobin levels and to assess the independent associations of fHb and haptoglobin levels with incidence risk of pAKI in adult patients undergoing CVS with CPB. In current study, our null-hypothesis was that perioperative serum free hemoglobin and haptoglobin concentration was not differed between patients with and without postoperative AKI.

## Methods

### Design

This study was a single-center prospective observational study that was carried out to determine perioperative fHb and haptoglobin concentrations and to assess their associations with the risk of postoperative AKI in patients undergoing CVS that required CPB. The study was conducted in a public teaching hospital with 880 beds and 32 ICU beds.

This study was approved by the Kobe University Hospital Ethics Committee (Approval No. 1614, principle investigator: Moritoki Egi, date of registration: August 11, 2014; UMIN000014991). A trained researcher collected data and entered the information into a database. Data monitoring and source-data verification were conducted according to a predefined plan. Written informed consent was obtained from all participants preoperatively according to the legal requirements of the ethics committee.

### Setting and participants

We screened patients over 20 years of age who were scheduled to undergo cardiovascular surgery requiring CPB during the period from 2014 to 2020 in our hospital. We excluded patients with preoperative serum creatinine (sCr) level over 2 mg/dl and

patients who had already required renal replacement therapy (RRT) preoperatively. In study site, CABG were most likely performed without CPB, therefore, we did not include isolated CABG. Among the patients from whom we obtained informed consent, we excluded patients who were administered external haptoglobin in the perioperative period and who needs multiple runs on bypass during one operation or reoperation was performed. We define that multiple runs of bypass as re-commencement of CPB after occlusion of venous return line in first CPB.

Patients' characteristics

We collected data for patients' characteristics including age, sex, weight, height, American Society of Anesthesiology physical status classification (ASA-PS), European System for Cardiac Operative Risk Evaluation II (EuroSCORE2), presence of hypertension and diabetes mellitus, preoperative estimated glomerular filtration rate (eGFR) and left ventricular ejection fraction (LVEF). We also collected operative information including operation time, cross-clamp time, duration of CPB, amount of transfusion during surgery and type of operation. As there was potential that low mean arterial pressure (MAP) might be associated with the incidence of AKI, we extracted MAP during CPB every minute and calculated average MAP in each patient.

Haptoglobin administration

In current study, haptoglobin administration were decided by the attending certificated anesthesiologist, who blinded with serum fHb and haptoglobin concentration during study periods. During study period, the anesthesiologist considered the administration of haptoglobin by observing the phenomenon of the urinary color becoming red. Medical insurance covers up to 4000 units of haptoglobin in this cohort.

The information on the CPB circuit, pump, and cell salvage system

After heparinization, cardiopulmonary bypass was established using a CPB circuit （S5/Liva Nova） and centrifugal pump(Revolution/ Liva Nova, Mixflow /JMS, Gyro Pump /Medtronic) with membrane oxygenation. During aortic cross clamping, perfusion pressure was closely monitored and kept at 40–80 mm Hg, and Prefusion Index(PI) was kept 2.2-2.5ml/$m^2$/min, respectively. A cell-salvage device (XTRA/Liva Nova, Cell Savar 5/HAEMONETICS, Cell Saver Elite/ HAEMONETICS) was used in every patient.

Measurements of serum free hemoglobin and haptoglobin concentrations

We measured serum fHb and haptoglobin concentrations at the following 6 time points: 1) after anesthesia induction (Pre), 2) 1 hour after initiation of CPB (CPB1), 3) 2 hours after initiation of CPB (CPB2), 4) 30 minutes after weaning from CPB (pCPB0.5), 5) 2 hours after weaning from CPB (pCPB2), and 6) the morning of postoperative day (POD) 1. Measurement of fHb was performed with Plasma/Low Hemoglobin (Hemocue R, Radiometer, Denmark), and determined by the azide-methemoglobin method. Serum haptoglobin was measured using polyacrylamide gel electrophoresis.

Study outcomes

The primary outcome was postoperative AKI defined by Kidney Disease: Improving Global Outcomes（KDIGO）(13). Postoperative AKI was determined by an increase in creatinine level of 0.3 mg/dl or more above the preoperative value or an increase in creatinine level of 50% or more within 48 hours after the operation. Serum creatinine levels were measured every morning after the operation. Although AKI was defined by the increase of serum creatinine level and decrease of urine output, we decide not to adopt the criteria of urine output for the definition of pAKI in this study to avoid the influence of use of diuretic drug in this cohort.

Statistical analysis

All variables are expressed as medians (interquartile range, IQR) or proportions as appropriate. We divided the patients into patients with and those without AKI. Comparisons between the two groups were performed using the Mann-Whitney U test for continuous variables and the chi square test for categorical variables. To assess the predictability and estimated cutoff values of serum fHb level at each timing, we performed receiver operator characteristics curve (ROC) analysis.

To assess the independent associations of perioperative increase of fHb and decrease of Hp with AKI, we first calculated preoperative maximum fHb (maxfHb) and minimum haptoglobin (minHp) concentrations and then performed multivariate logistic analysis to explore their independent associations with pAKI. If maxfHb and/or minHp had independent associations with AKI, we further performed multivariate analysis to assess the associations of fHb and Hp at each timing with AKI in order to determine their time trends. To perform multivariate analysis for the risk of pAKI, we used indices of fHb and Hp and variables that significantly differed between patients with and those without pAKI as independent factors.

To calculate the sample size for the current study, we considered a difference of 70% of standard deviation for free hemoglobin or haptoglobin concentration to be meaningful. Assuming an incidence of AKI of 33%, a power of 0.80, and an α level of 0.05, approximately 75 participants were required. Considering the proportion of patients who would be administrated external haptoglobin as 33.3% and dropout rate as 5%, we planned to study 120 patients. All statistical analyses were performed using JMP 14.0 (SAS Institute Inc., North Carolina, USA). A P value<0.05 was defined as a statistically significant difference. This manuscript adheres to the applicable STROBE guidelines.

## Results

Study flow

We evaluated 120 patients without chronic renal failure who underwent cardiac surgery that required CPB (Supplemental figure). Among them, we excluded patients who were administered external haptoglobin in the perioperative period and patients in whom more than one CPB or reoperation was performed. We finally included 74 patients in this study. Postoperative AKI occurred in 25 (33.8%) of those patients. There were no missing data.

Patient's demographic characteristics

Table 1 shows a comparison of demographics for patients with and those without AKI. Patients with AKI were significantly older than patients without AKI (p=0.01) and had a lower preoperative eGFR (p=0.04) than that in patients without AKI. The median value of MAP during CPB in patients with AKI was 51.3 (43.9-63.4) mmHg, which was not significantly different from the median value of 54.5 (45.3-64.8) mmHg (p=0.49). There was no significant difference in other demographics.

Changes in the levels of free hemoglobin and haptoglobin

Figures 1-a and 1-b show the perioperative changes in serum fHb and haptoglobin levels, respectively. The median fHb level after induction of anesthesia was 0.03 (0.01-0.05) g/dL. It began to increase after the initiation of CPB and reached a peak value of 0.08 (0.06-0.12) g/dL at 30 minutes after weaning from CPB. Then it returned to the baseline level on POD 1 (Figure 1-a). The median haptoglobin concentration after induction of anesthesia was 92 (67.3-130.3) mg/dL. After the initiation of CPB, it began to decrease and continued to decrease throughout the study period. The median concentration on POD 1 was 27 (12.8-40.3) mg/dL (Figure 1-b).

Comparison of serum free hemoglobin levels in patients with and those without AKI

Figure 2 shows a comparison of perioperative serum fHb levels in patients with AKI and those without AKI. After induction of anesthesia, the median fHb level in patients with AKI was 0.04 (0.02-0.04) g/dL, which was not significantly different from the median level of 0.03 (0-0.05) g/dL in patients without AKI ($p=0.65$). During and after CPB, fHb levels in patients with AKI were significantly higher than those in patients without AKI (CPB1: $p<0.001$, CPB2: $p=0.01$, pCPB0.5: $p<0.001$, pCPB2: $p<0.001$). The significant difference disappeared on POD 1 ($p=0.62$). The median maxfHb level in patients with AKI was 0.13 (0.12-0.15) g/dL, which was significantly higher than that of 0.08 (0.06-

0.11) g/dL in patients without AKI ($p<0.001$).

ROC analysis of perioperative serum free hemoglobin level for pAKI

We performed ROC analysis and showed the area under ROC curve and we estimated the optimal cutoff value for fHb at each timing (Table 2). Area under ROC curve >0.7 was observed at CPB1, pCPB0.5, pCPB2 and maxfHb. These estimated cutoff values were 0.06g/dL, 0.12g/dL, 0.09g/dL and 0.12g/dL, respectively.

Comparison of serum haptoglobin levels in patients with and those without AKI

Figure 3 shows a comparison of perioperative haptoglobin levels in patients with AKI and those without AKI. After induction of anesthesia, the median haptoglobin level in patients with AKI was 86 (70–104) mg/dL, which was not significantly different from the median level of 98 (64–136) mg/dL in patients without AKI ($p=0.63$). The haptoglobin levels in patients with AKI were significantly lower than those in patients without AKI from pCPB0.5 to POD 1 (pCPB0.5: $p=0.04$, pCPB2: $p=0.01$ and POD 1: $p=0.01$). The median minHp level in patients with AKI was 15 (8–22) mg/dL, which was significantly lower than the median level of 29 (12-41) mg/dL in patients without AKI ($p=0.01$).

Multivariate analysis for the risk of pAKI

We performed a multivariate analysis for the risk of pAKI using maxfHb, minHp, age, and preoprerative eGFR as independent variables (Table 3). In this analysis, maxfHb and minHp were independently associated with increased risk of pAKI (maxfHb: adjusted odds ratio=1.33 (95% CI: 1.12, 1.58), p=0.001, minHp: adjusted odds ratio= 0.95 (95% CI: 0.91, 1.00), p=0.03).

We further performed a multivariate analysis of perioperative serum fHb (Figure 4-a) and haptoglobin (Figure 4-b) concentrations for the risk of pAKI. We found that fHb level had begun to have an independent association with pAKI at CPB1 and that the independent association had disappeared at POD 1.

## Discussion

In this prospective study conducted in valvular and aortic surgery with cardiopulmonary bypass, perioperative increase of fHb levels and decreased of Hp levels were independently associated with the risk of AKI. In additional analysis, we found that the increase of fHb had begun to have an independent association with the risk of AKI at 1 hour after initiation of CPB.

There have been three studies on perioperative fHb and haptoglobin concentrations and their associations with the risk of AKI in adult patients undergoing CVS with CPB. In two of those three studies, the subjects were mainly patients who underwent coronary artery bypass grafting (CABG). Wetz et al. conducted an observational study on fHb and haptoglobin levels in 154 patients who underwent CABG with CPB (14). Connie Davis et al. studied fHb levels in 30 patients who underwent cardiac surgery, mainly CABG (60%), requiring CPB (15). In both studies, there was no significant difference in the values of fHb and haptoglobin between patients with AKI and those without AKI. Our study was performed mainly in patients undergoing valve or aortic arch surgery. Windsant et al. showed that free hemoglobin concentration was significantly lower in isolated CABG that in other types of cardiac surgery (16). Therefore, the difference between our

results and the results of the two previous studies might come from the case mix, related to the severity of hemolysis.

Another study by Windsant et al. was conducted in patients who underwent thoracoabdominal aortic aneurysm surgery (17). They obtained perioperative fHb levels at the following time points: preoperative, before the start of CPB, end of CPB, 15 min, 2 hours, and 6 hours after cessation of CPB, and PODs 1 and 2. They found that fHb had a peak level at 2 hours after cessation of CPB and that the peak level was significantly higher in patients with AKI than in patients without AKI. They also found a positive correlation between total fHb release and NAG excretion during the study period.

Although our results are in line with those obtained by Windsant et al, we additionally obtained data for perioperative haptoglobin concentrations and reported the time trends of adjusted odds ratios of perioperative serum fHb and haptoglobin concentrations.

We found a significant association between an increase of fHb and risk of postoperative AKI. There are potential explanations for this association. First, hemolysis would be accelerated due to the blood pumping, suction, cell salvage system and turbulent flow in CPB circuits (7). Therefore, more complex surgery might have increased fHb (16). Then, complex surgery might be associated with the risk of pAKI, in compared with simple

surgery. Such a bias might have skewed our results and could not be completely adjusted. However, it should be noted that the type of surgery and the time of the operation were not significantly different between patients with and those without AKI in our study. Nonetheless, we believe that our findings should be refuted or confirmed by a future interventional study to assess the effect of an administration of scavenger of fHb, such as haptoglobin, on the risk of AKI.

Second, a regurgitated flow or jet related to a paravalvular leak or turbulence of sub-valvular stenosis after valve surgery may increase both the severity of hemolysis (18) and the risk of AKI, which might have biased our findings. However, such hemolysis should occur after surgery (19). Our results showed that fHb level decreased after CPB and returned to the preoperative level in patients with AKI. Therefore, we believe that our findings had little chance of being biased by postoperative valve malfunction.

Third, an increase of fHb might be potentially harmful for kidney function in patients undergoing cardiovascular surgery with CPB (20). Free hemoglobin induced by hemolysis binds to haptoglobin and forms a hemoglobin-haptoglobin complex (21), which is mediated and degraded by endocytosis (22). However, if release of fHb occurs rapidly, serum haptoglobin might be depleted. In this setting, excess fHb would bind to the vasodilator nitric oxide (NO), resulting in microvascular vasoconstriction and

impairment of micro-perfusion (8-10). Furthermore, methemoglobin produced by fHb combining with NO forms a cast and occludes the renal tubule (23). Windsant et al. found a correlation between hemolysis and NO consumption in patients undergoing CVS (16). These findings may support the hypothesis that hemolysis is one of causes of AKI and intestinal tissue damage through limiting NO bioavailability. In addition, fHb released by hemolysis is reabsorbed in the renal tubles, and decomposed into heme and elemental iron, both of which are intensely oxidative and damage the renal tubular epithelium. These mechanisms may contribute to renal dysfunction that accompanies hemolysis.

Considering the above-described possible mechanisms, hemolysis might be associated with various types of postoperative organ damage such as renal dysfunction and cerebrovascular disease. Finally, there might be another unknown mechanism or a combination of the above-described mechanisms.

To prevent AKI caused by hemolysis, there are various methods to reduce fHb and iron have been proposed. Gleason TG, et al reported that Filters to remove fHb (CytoSorb) use was safe and capable of reducing concentrations of fHb and activated complement, especially in patients undergoing valve replacements surgery(24). Hepcidine which binds to free iron may be a novel approach to treat AKI by decreasing the excessive iron.

Administration of exogenous hepcidin might be provide therapeutic benefit in postoperative AKI after CVS(25). And, administration of haptoglobin is one of the possible treatments for postoperative AKI(11,12). The results of our study might be useful to decide the timing of administration of these therapies. It is possible to generate the hypothesis that to use them if serum fHb≥0.06 g/dL at 1 hour after commencement of CPB or ≥0.12 g/dL in anytime may prevent pAKI. Although this study was an observational study and our findings thus showed an association but not a causality relationship, this hypothesis should be confirmed or refuted by a future interventional trial.

We should note that there are limitations in this study. First, this study was a small, single-center study in Japan. So, these findings cannot be generalized to all races. Thus, our findings should be validated outside of the study sites. Also, other potential mechanisms underlying the relation between intravascular hemolysis and kidney damage were not investigated and should thus be investigated in a future study. Second, as administration of external haptoglobin for patients with hemolytic urine were local common practice in japan, our study excluded the patients who were administrated external haptoglobin, instead of avoiding to use it by protocol. Accordingly,

approximately one third of patients (43/120) were excluded due to this reason. This fact may skew our findings, therefore, our finding should be confirmed in the different medical circumstance. Third, we excluded patients with preoperative chronic renal failure. It might be one of explanation why preoperative GFR did not associate with the risk of AKI. Accordingly, our findings can not be generalized to patients with chronic renal failure. Forth, this study performed over 6years. Therefore, the change in practice over the years might skew our results. Therefore, we assessed interaction of year of operation on the association of maxfHb and minHp with the risk of pAKI, and found that there was no significant interaction. Fifth, as we studied in 74 patients, the number of variables for multivariate analysis was limited. Therefore, there are another relevant confounders as like type of fluids, transfusion of RCC, vital signs and so on. As the association of transfusion of RCC with the incidence of pAKI were nearly significant (p=0.06), we performed sensitive multivariate analysis to add transfusion of RCC as additional independent variables. In this analysis, there was no independent association (adjusted odds ratio=1.07(95% CI: 0.92, 1.24), p=0.39) and MaxfHb and minHp keep their independent association. Finally, urinary fHb level might be relevant information to explore the association between hemolysis and the risk of pAKI, In our preliminary study, fHb concentration in urine in the cohort was too low to detect our deice, If more sensitive

method to measure fHb level could be available, future study should obtain urinary fHb as well.

## Conclusions

In our patients, serum fHb level began to increase after the initiation of CPB, reached a peak value at 30 minutes after weaning from CPB, and then returned to the baseline level on POD1. Serum haptoglobin level began to decrease after the initiation of CPB and continued to decrease until POD 1. The increase of fHb level and decrease of haptoglobin level had independent associations with pAKI. These results call for increased awareness of the adverse consequences of even modest increases in serum fHb in patients with CPB. Future interventional studies are needed to determine whether inactivation of fHb significantly attenuates the development of pAKI.

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## Figure Legends

Figure 1-a and 1-b. Perioperative serum free hemoglobin and haptoglobin concentrations.

These two figures show the time courses of perioperative serum free hemoglobin (figure 1-a) and haptoglobin (figure 1-b) concentrations at the following 6 time points: 1) after anesthesia induction (Pre), 2) 1 hour after initiation of CPB (CPB1hr), 3) 2 hours after initiation of CPB (CPB2hr), 4) 30 minutes after weaning from CPB (pCPB0.5hr), 5) 2 hours after weaning from CPB (pCPB2hr), and 6) the morning of postoperative day 1 (POD 1).

Figure 2. Comparisons of perioperative serum free hemoglobin concentrations in patients with and those without AKI.

This figure shows a comparison of perioperative serum free hemoglobin concentrations in patients with and those without AKI at the following 6 time points: 1) after anesthesia induction (Pre), 2) 1 hour after initiation of CPB (CPB1hr), 3) 2 hours after initiation of CPB (CPB2hr), 4) 30 minutes after weaning from CPB (pCPB0.5hr), 5) 2 hours after weaning from CPB (pCPB2hr), and 6) the morning of postoperative day 1 (POD 1). White box plots indicate the values for patients without AKI and black box plots indicate the values for patients with AKI. * means $p<0.05$.

AKI: acute kidney injury, CPB: cardiopulmonary bypass.

Figure 3. Comparison of perioperative serum haptoglobin concentrations in patients with and those without AKI.

This figure shows a comparison of perioperative serum haptoglobin concentrations in patients with and those without AKI at the following 6 time points: 1) after anesthesia induction (Pre), 2) 1 hour after initiation of CPB (CPB1hr), 3) 2 hours after initiation of CPB (CPB2hr), 4) 30 minutes after weaning from CPB (pCPB0.5hr), 5) 2 hours after weaning from CPB (pCPB2hr), and 6) the morning of postoperative day 1 (POD 1). White box plots indicate the values for patients without AKI and black box plots indicate the values for patients with AKI. * means $p<0.05$.

AKI: acute kidney injury, CPB: cardiopulmonary bypass.

Figure 4-a and 4-b. Adjusted odds ratios of perioperative serum free hemoglobin and haptoglobin concentrations for the risk of AKI.

These two figures show the time courses of adjusted odds ratios for perioperative serum free hemoglobin (figure 4-a) and haptoglobin (figure 4-b) levels at the following 6 time points: 1) after anesthesia induction (Pre), 2) 1 hour after initiation of CPB (CPB1hr), 3) 2 hours after initiation of CPB (CPB2hr), 4) 30 minutes after weaning from CPB (pCPB0.5hr), 5) 2 hours after weaning from CPB (pCPB2hr), and 6) the morning of postoperative day 1 (POD1).

Supplemental figure. Study flow.

CPB: cardiopulmonary bypass, sCr: serum creatinine, RRT: renal replacement therapy, PCPS: percutaneous cardiopulmonary support, ICU: intensive care unit, AKI: acute kidney injury

Figure 1-a.

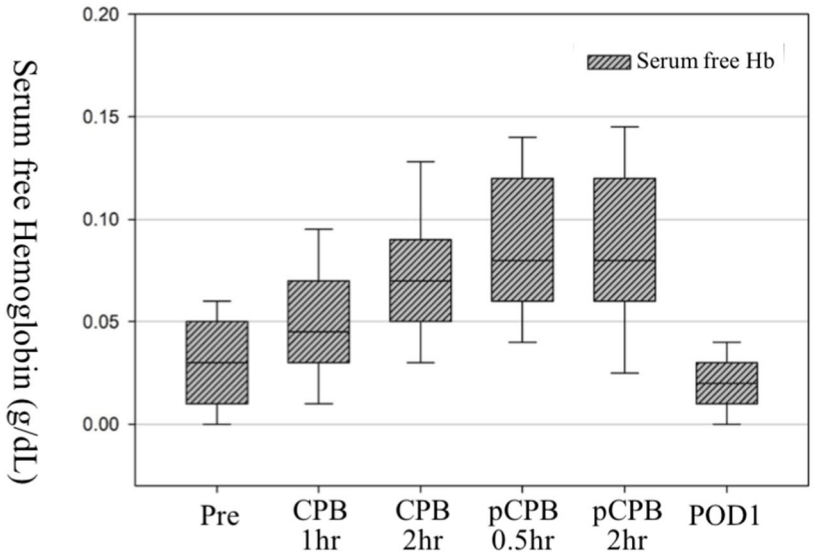



Figure 1-b.

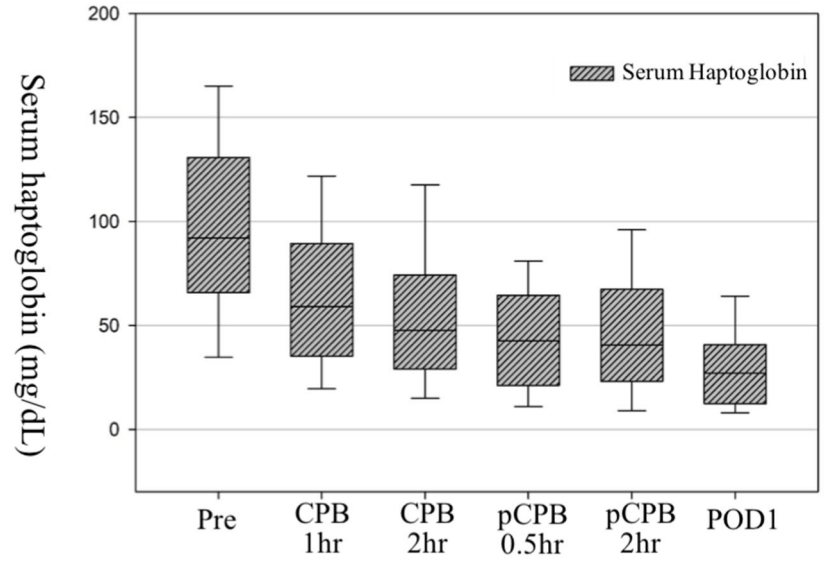


Figure 2.

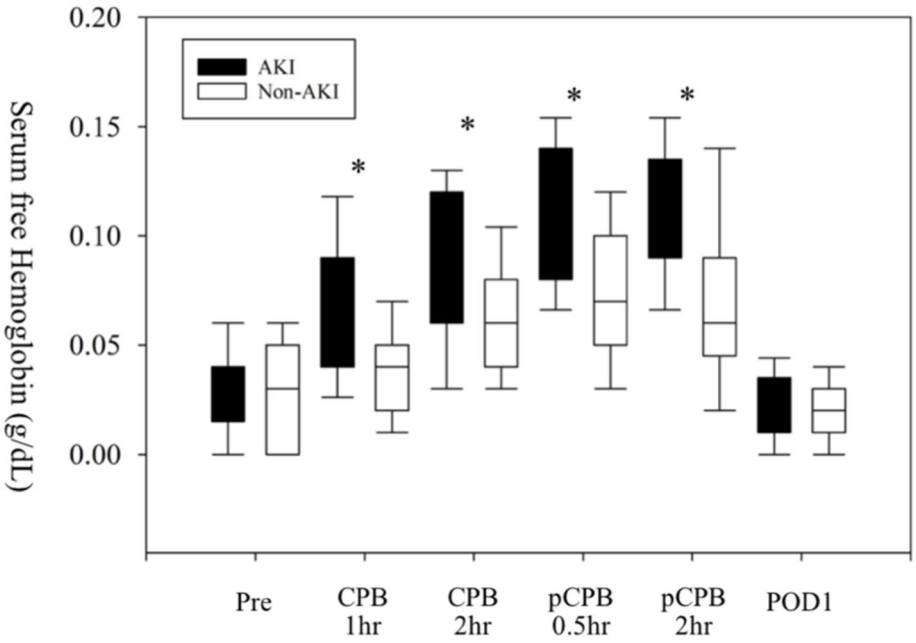



Figure 3.

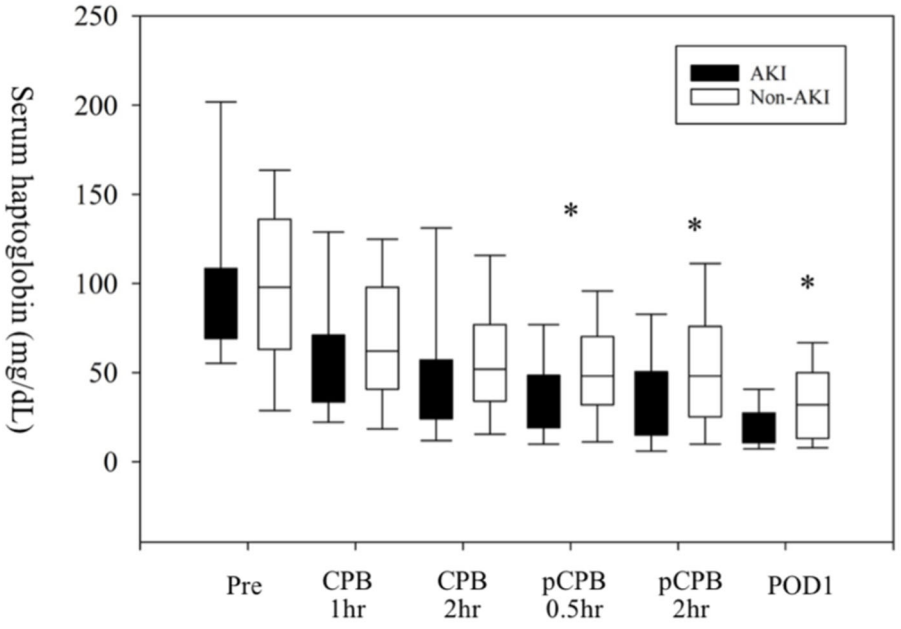


Figure 2.

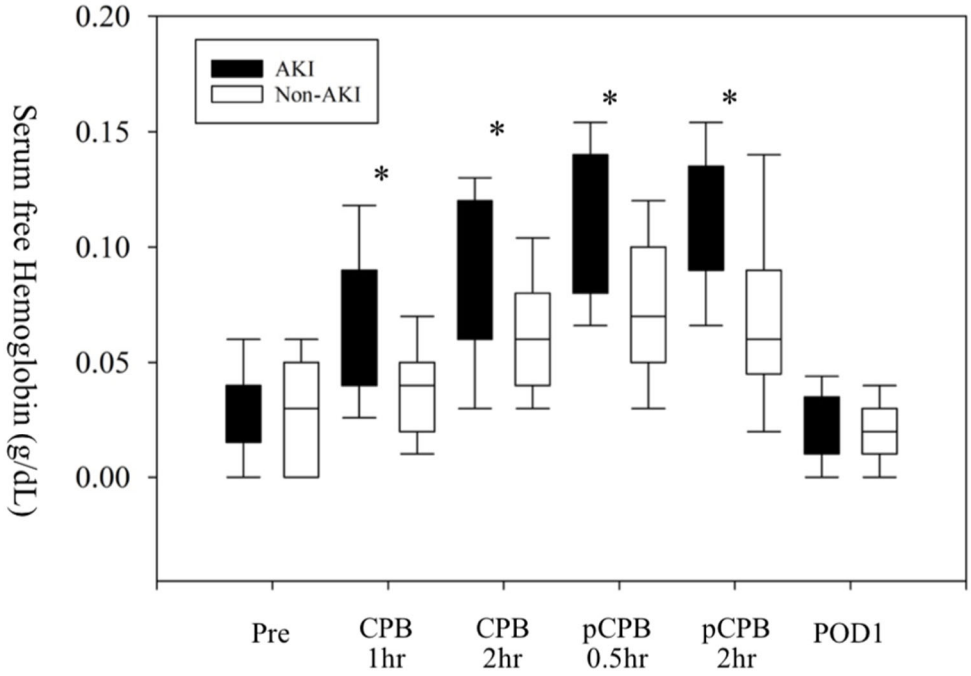



Figure 3.

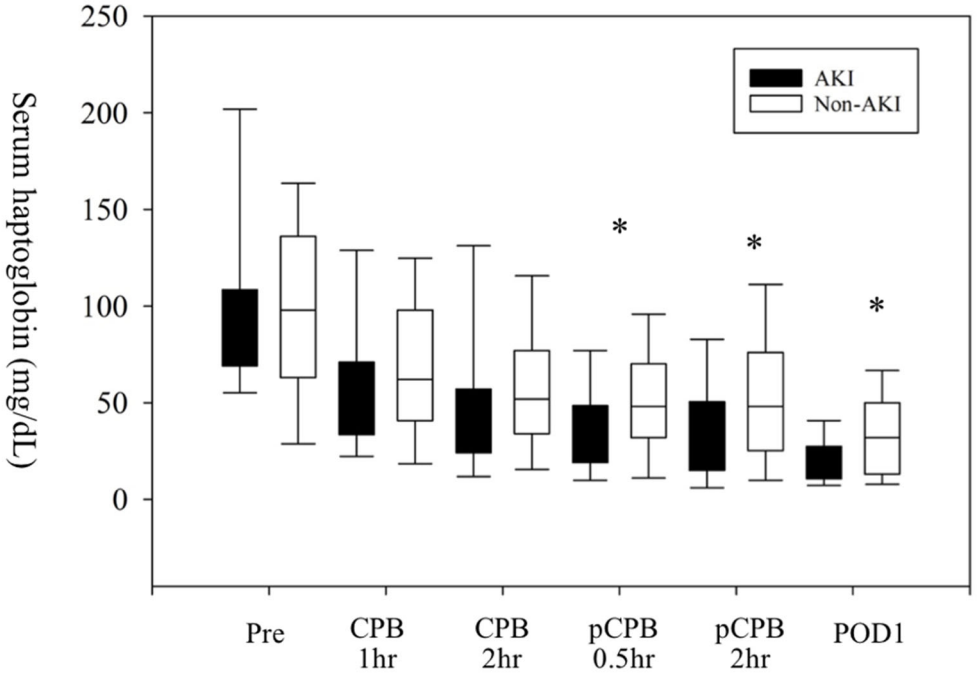


Figure 4-a.

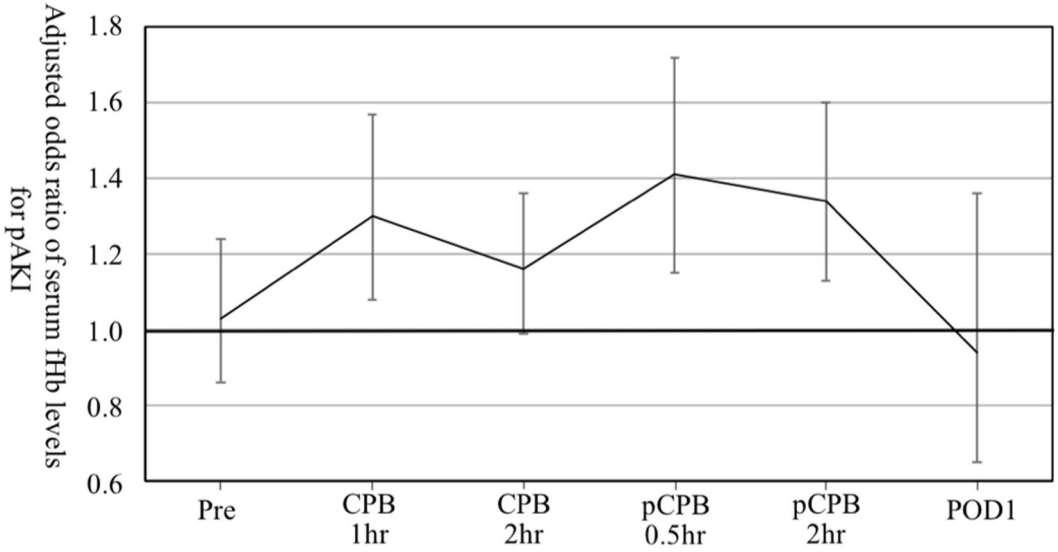
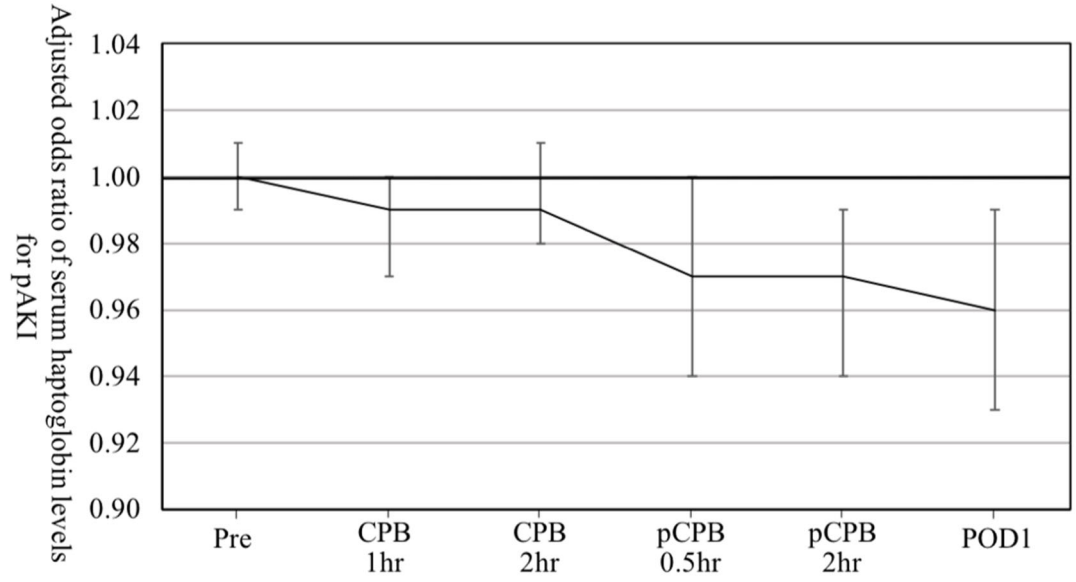



Figure 4-b.



Supplemental Figure: Study flow.

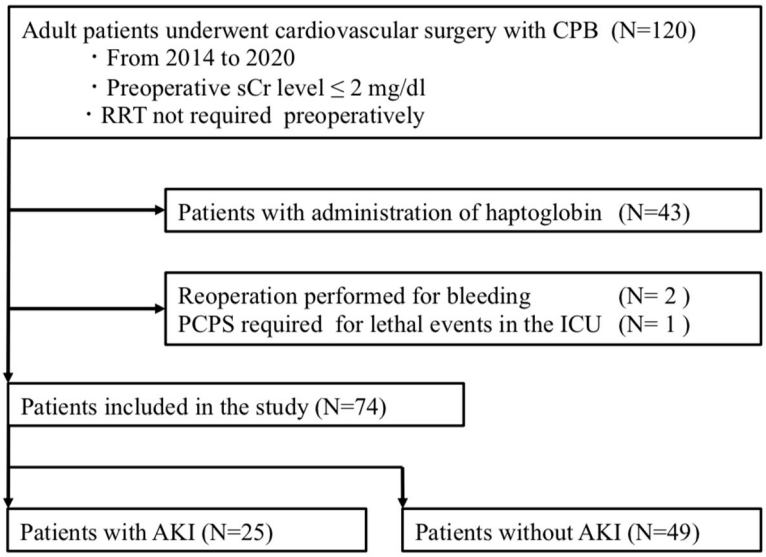


Table 1. Characteristics of patients with and those without postoperative AKI

| | With AKI (n=25) | Without AKI (n=49) | P value |
|---|---|---|---|
| Age (years) | 75 (67-83) | 67 (62-76) | 0.01 |
| Female, n. (%) | 6(24%) | 18(36.7%) | 0.27 |
| Height (cm) | 162 (150-168) | 163 (156-170) | 0.41 |
| Weight (kg) | 59.5 (52.3-66) | 56.2(48-65) | 0.76 |
| ASA-PS | 3(2-3) | 3(2-3) | 0.34 |
| EuroSCORE II | 2.81 (1.98-5) | 2.62(1.6-4.8) | 0.22 |
| Hypertension, n. (%) | 15(60%) | 32(65.3%) | 0.65 |
| Diabetes mellitus, n. (%) | 4(16%) | 5(10.2%) | 0.47 |
| Preoperative eGFR ($ml/min/1.73m^2$) | 54.8(45.3-68.1) | 65.7 (53.4-78.2) | 0.04 |
| LVEF(%) | 64 (59-71) | 61 (55.4-66.5) | 0.23 |
| Operation time (mins) | 322 (276-389) | 315(261-368) | 0.34 |
| Cross-clamp time (mins) | 108 (80-148) | 104 (79-131) | 0.54 |
| Duration of CPB (mins) | 176 (141-213) | 169 (132-202) | 0.46 |
| Mean perfusion pressure during CPB (mmHg) | 51.3(43.9-63.4) | 54.5(45.3-64.8) | 0.49 |
| Transfusion (RCC) (unit) | 6(6-10) | 6(2-8) | 0.06 |
| Transfusion (FFP) (unit) | 6(4-10) | 6(0-10) | 0.63 |
| Type of procedure, n. (%) | | | 0.74 |
| Total arch replacement | 6(24%) | 14(28.6%) | |
| Valve surgery | 17(68%) | 29(59.2%) | |
| Total arch replacement +Valve surgery | 2 (8%) | 6(12.2%) | |

AKI: acute kidney injury, ASA-PS: American Society of Anesthesiology physical status, EuroSCOREII: European System for Cardiac Operative Risk Evaluation II, eGFR: estimated glomerular filtration rate, LVEF: left ventricular ejection fraction, TAR: total arch replacement.

Table 2. Area under receiver operator characteristic curve of perioperative serum free hemoglobin levels for the risk of postoperative acute kidney injury.

| | Area under ROC curve (95%CI) | Estimated optimal cut off value | sensitivity | specificity |
|---|---|---|---|---|
| Pre | 0.53 (0.40, 0.67) | 0.02 | 0.76 | 0.37 |
| CPB1hr | 0.74 (0.62, 0.87) | 0.06 | 0.64 | 0.74 |
| CPB2hr | 0.68 (0.54, 0.83) | 0.09 | 0.58 | 0.79 |
| pCPB0.5hr | 0.80(0.70, 0.91) | 0.12 | 0.60 | 0.88 |
| pCPB2hr | 0.80 (0.70, 0.90) | 0.09 | 0.60 | 0.88 |
| POD1 | 0.54(0.40, 0.68) | 0.04 | 0.24 | 0.86 |
| maxfHb | 0.81(0.71, 0.91) | 0.12 | 0.76 | 0.88 |

ROC: receiver operator characteristic, CPB: cardiopulmonary bypass, pCPB: post cardiopulmonary bypass, POD: postoperative day, maxfHb: maximum plasma free hemoglobin level during the study period

Table 3 Multivariate analysis for the risk of pAKI

| Variables | Adjusted odds ratio (95%CI) | P value |
|---|---|---|
| maxfHb (10mg/dL) | 1.33 (1.12, 1.58) | 0.001 |
| minHp (mg/dL) | 0.95 (0.91, 1.00) | 0.03 |
| Age (years old) | 1.06 (0.99, 1.12) | 0.08 |
| Preoperative eGFR (ml/min$^{1}$/1.73m$^{2}$) | 1.00 (0.97, 1.04) | 0.99 |

pAKI: postoperative acute kidney injury, maxfHb: maximum serum free hemoglobin level during the study period, minHp: minimum serum haptoglobin level during the study period, eGFR: estimated glomerular filtration rate