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Original Article

Hemolysis and Acute Kidney Injury Following Cardiac Surgery With Cardiopulmonary Bypass in Patients With Preexisting Renal Dysfunction

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Objectives: Acute kidney injury (AKI) is a common complication following cardiac surgery with cardiopulmonary bypass (CPB). This study investigated whether cell-free hemoglobin (CFHb) levels after CPB are associated with endothelial damage and postoperative AKI in patients with preexisting renal dysfunction.

Design: A substudy of a randomized controlled trial.

Setting: Secondary referral and tertiary hospital.

Participants: Adult patients undergoing cardiac surgery with CPB and an estimated glomerular filtration rate (eGFR) <50 mL/min/1.73 m^2 or diabetes mellitus with an eGFR of <60 mL/min/1.73 m^2.

Interventions: Clinical data and plasma samples were collected after induction of anesthesia, within 1 hour at the intensive care unit, and 24 and 48 hours postoperatively.

Measurements and Main Results: Of the 89 patients included, 21% developed AKI. CFHb peaked at 1 hour postoperatively (22.5 *v* 5.4 mg/dL, $p < 0.001$), and lactate dehydrogenase rose until 48 hours postoperatively (119 *v* 339 U/L, $p < 0.001$). Tumor necrosis factor α and intercellular adhesion molecule 1 increased following surgery (7.06 *v* 9.21 ng/mL, $p = 0.020$; 247 *v* 388 ng/mL, $p < 0.001$). Angiopoietin-2 rose until 48 hours postoperatively and was higher in patients with AKI at 24 hours (4,162 *v* 3,374 pg/mL, $p = 0.027$). Similarly, neutrophil gelatinase-associated lipocalin increased in patients with AKI (43.1 *v* 88.0 ng/mL, $p < 0.001$). CFHb at 1 hour postoperatively was not associated with angiopoietin-2 at 24 or 48 hours. Adding CFHb to a prediction model of AKI did not improve model fit ($p = 0.28$) or discrimination ($p = 0.32$).

Conclusions: This study demonstrates that CPB induces hemolysis and endothelial activation and damage in patients with preexisting renal dysfunction. Although CFHb concentrations were higher in those developing AKI, CFHb did not predict AKI or correlate with markers of endothelial damage.

Key Words: acute kidney injury; cardiopulmonary bypass; cell-free hemoglobin; chronic kidney disease; endothelial injury; hemolysis

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ACUTE KIDNEY INJURY (AKI) remains one of the most frequent postoperative complications following cardiac surgery with cardiopulmonary bypass (CPB).[1] Postoperative AKI leads to a longer hospital stay and is associated with worse outcomes and mortality.[2] Furthermore, patients who develop postoperative AKI have an elevated long-term risk of progressing to end-stage renal disease.[3] However, the pathophysiology of postoperative AKI remains incompletely understood, and although CPB is a relatively safe technique, it is associated with a wide range of pathophysiological disturbances.

During CPB, blood is exposed to nonphysiological conditions that can trigger a cascade of unfavorable processes. The extracorporeal circuit subjects erythrocytes to increased abnormal mechanical forces, including high shear stress and turbulence. These conditions can cause hemolysis, characterized by the rupture of erythrocyte membranes and the release of toxic cell-free hemoglobin (CFHb) into the circulation. Under physiological conditions, haptoglobin binds to CFHb, after which the complex is metabolized in the liver. During extensive hemolysis, the binding capacity of haptoglobin is exceeded, allowing CFHb to circulate in an unbound state. Unbound CFHb is redox-active and contributes to oxidative stress, nitric oxide scavenging, and endothelial injury. These processes promote vasoconstriction, impaired microcirculatory perfusion, and increased vascular permeability, which together compromise tissue oxygenation.[4-6] Several studies have linked elevated CFHb levels during CPB with the development of postoperative AKI,[6,7] supporting a pathogenic role of hemolysis in renal injury. However, these studies largely focused on patients with preserved renal function, while patients with preexisting renal dysfunction may be more susceptible to hemolysis-induced renal and endothelial injury.

In parallel, the foreign surfaces of the CPB circuit trigger a systemic inflammatory response through activation of immune and coagulation pathways.[8-10] The released inflammatory mediators can activate the endothelium, thereby increasing endothelial permeability and damage, as well as impairing microcirculatory perfusion.[10-12] We have previously shown that levels of CFHb are associated with microcirculatory perfusion disturbances and kidney injury in a rat model of extracorporeal circulation.[13] In this model, CFHb levels correlated with circulating angiopoietin-2, a marker of endothelial damage, suggesting that hemolysis-induced endothelial damage is an important mechanism contributing to CPB-induced AKI.

The kidneys are the primary route for CFHb clearance from the blood, leaving them particularly vulnerable. Once filtered from the blood, CFHb can be taken up by proximal tubular cells, where it can induce oxidative stress and tubular cytotoxicity. These mechanisms may be especially harmful in patients with preexisting chronic kidney disease, who have diminished renal reserve and impaired antioxidant defense, rendering them more susceptible to additional insults during CPB.[14] In addition, patients with renal dysfunction often exhibit chronic endothelial damage, making their endothelium even more susceptible to CFHb-induced injury during CPB.[15] To date, little is known about the relationship between CPB-induced hemolysis and postoperative AKI in patients with preexisting renal impairment. Therefore, we have investigated whether hemolysis after CPB is associated with the development of postoperative AKI in these vulnerable patients. In addition, we explored the relationship between hemolysis and endothelial damage in this high-risk population. We hypothesized that higher CFHb levels following CPB are associated with both increased endothelial damage and a higher risk of postoperative AKI in patients with preexisting renal dysfunction.

Material and Methods

Study Design

This study is a substudy of a multicenter, randomized controlled trial comparing pulsatile and continuous nonpulsatile flow during CPB. The study was approved by the Institutional Review Board of the VU University Medical Center (2014.367, NL-OMON21086, Amsterdam, the Netherlands). Adult patients scheduled for nonaortic elective cardiac surgery with CPB with an estimated glomerular filtration rate (eGFR) <50 mL/min/1.73 m^2 or a combination of diabetes mellitus and an eGFR of <60 mL/min/1.73 m^2 were included. Informed consent was obtained from all patients prior to inclusion. Exclusion criteria were emergency operations, a history of cardiac surgery, renal failure requiring preoperative renal replacement therapy, and a body surface area >2.3 m^2.

Patients underwent anesthesia and CPB according to local protocols.[12] Anesthesia was induced using intravenous sufentanil and midazolam, combined with rocuronium. This was followed by continuous propofol infusion as maintenance. Furthermore, patients received dexamethasone and cefazolin after induction. A radial artery catheter was placed for continuous hemodynamic monitoring and blood gas analysis during surgery. CPB consisted of a centrifugal pump (Stockert Instrumente GMBH Munich), a hollow-fiber oxygenator (Affinity; Medtronic), and a phosphorylcholine-coated tubing system (P. h.i.s.i.o.; The Sorin Group). Heparin (300 IU/kg) was administered to reach a target activated clotting time >480 seconds, after which CPB was initiated. CPB flow was maintained at 2.2 to 2.5 L/min/m^2. After surgery, anticoagulation was counteracted using protamine in a 1:1 ratio to achieve normal activated clotting time. Autologous red blood cell transfusion was performed using a cell saver (Autolog; Medtronic).

Collection of Blood Samples

Arterial blood samples were taken for blood gas analyses and plasma analyses. Samples for plasma analyses were collected in citrate tubes for plasma storage at set time points: after induction of anesthesia, within 1 hour after surgery in the intensive care unit (ICU), and 24 and 48 hours postoperatively. Additional blood gas analyses were performed at the start and end of aortic cross-clamping. Blood samples were centrifuged twice to obtain platelet-free plasma, which was stored at −80°C.

Study Endpoints

All patients were admitted to the ICU after surgery, where their urine output was continuously monitored and recorded. Serum creatinine and urinary creatinine were measured daily according to routine laboratory procedures. Acute kidney injury, our primary endpoint, was defined as an increase in serum creatinine by ≥26.5 μmol/L within 48 hours, an increase in serum creatinine to ≥1.5 times baseline within the previous 7 days, or a urine output ≤0.5 mL/kg/h for 6 hours, according to the Kidney Disease: Improving Global Outcomes guidelines.[16] The primary exposure of interest was plasma CFHb measured within 1 hour in the ICU. Secondary endpoints included circulating markers of hemolysis, inflammation, endothelial activation and damage, and kidney injury.

Plasma Protein Analyses

Plasma levels of tumor necrosis factor α (TNFα), intercellular adhesion molecule 1 (ICAM-1), angiopoietin-1, soluble Tie2, neutrophil gelatinase-associated lipocalin (NGAL), and cystatin C were measured using a Luminex platform (Biotechne). Circulating levels of angiopoietin-2 (DY623; Biotechne) and haptoglobin (DHAPG0; Biotechne) were measured using an enzyme-linked immunosorbent assay in accordance with the manufacturer's instructions. Haptoglobin concentrations were determined only at the time of anesthesia induction and at 1 and 24 hours in the ICU due to limited plasma volume.

CFHb in the plasma samples was determined using Kahn's algorithm.[17] The absorbance of the samples was measured using the Spectramax M2e microplate reader (Molecular Devices). Absorbances were measured at 562, 578, and 598 nm, and the extinction values were used to calculate the concentration of CFHb. To account for changes in the plasma compartment, all plasma marker concentrations were adjusted for hematocrit-level fluctuations relative to the values at induction.

Lactate Dehydrogenase Activity

During hemolysis, lactate dehydrogenase (LDH) is released from erythrocytes into the circulation. LDH activity was analyzed by spectrophotometry (Spectramax M2e; Molecular Devices) at 340 nm and 25°C. Potassium phosphate buffer (0.5M) and Triton (10%) were added to the sample. Sodium pyruvate was added as the substrate, after which the rate of nicotinamide adenine dinucleotide hydrogen oxidation was measured.[18] The decrease in nicotinamide adenine dinucleotide hydrogen concentration reflects the total LDH activity.

Extracellular Vesicles

As a pilot study to explore the consequences of erythrocyte membrane shedding, extracellular vesicle formation was measured using flow cytometry. Ten patients were randomly selected from our study cohort, consisting of 5 patients who developed postoperative AKI and 5 who did not. Prior to staining, plasma was diluted in filtered Dulbecco's phosphate-buffered saline to prevent swarm detection. Erythrocyte-derived extracellular vesicles (EVs) were identified using an anti-human CD235a-phycoerythrin (M0819; Dako Agilent) antibody, with appropriate isotype and buffer controls included. Samples were analyzed on a calibrated flow cytometer (A60-Micro; Apogee Flow Systems), operated at a flow rate of 3.01 μL/min for 120 seconds, with a side-scatter trigger threshold equivalent to an optical diameter of 121 nm. $CD235a^+$ particles <1,000 nm were considered erythrocyte-derived EVs. Custom-built analysis software (MATLAB R2020b; MathWorks) was used for data calibration and analysis (Supplementary File A).

Statistical Analysis

Variables are expressed as mean ± standard deviation or median with interquartile range. Patients were divided into 2 groups based on the development of postoperative AKI. The normality of the data was checked using quantile-quantile plots and Shapiro-Wilk tests. Differences in patient characteristics and perioperative data were analyzed using a Mann-Whitney U test for continuous variables or a chi-square test for categorical variables. Linear mixed-effects models with Tukey post hoc analyses were used to test time-dependent differences between patients who developed AKI and those who did not. Logistic regression was used to test the predictive value of CFHb at 1 hour after ICU admission. Correlations between different circulating markers were tested using Spearman correlation or repeated-measures correlation. To explore whether plasma CFHb levels improved the prediction of postoperative AKI, we constructed logistic regression models. A baseline model (model 1) included clinical risk factors that differed between patients with and without AKI in our cohort (diabetes, red blood cell transfusion, and preoperative serum creatinine). In model 2, CFHb measured 1 hour after ICU admission was added to this baseline model, and in model 3, peak CFHb was added instead. Model performance was compared using likelihood ratio tests, Akaike's information criterion (AIC), and receiver operating characteristic (ROC) curve analysis with calculation of the area under the curve (AUC). Differences in AUC between models were assessed using DeLong's test for correlated ROC curves. A p value <0.05 was considered statistically significant. Data were analyzed using RStudio (version 4.3.2; Posit, PBC), and graphs were created using GraphPad Prism 10 (GraphPad Software).

Results

Patient Characteristics

Patient characteristics and intraoperative parameters are shown in Table 1. A total of 89 patients were included in the study. Most patients were male (67%), with a median age of 75 [71-79] years. In our cohort, 19 patients (21%) developed postoperative AKI. Those patients showed a higher prevalence of diabetes mellitus compared with patients who did not develop postoperative AKI (11 [58%] *v* 21 [30%], p = 0.048).

Table 1
Patient Characteristics and Perioperative Data

Characteristic	Total (N = 89)	No AKI (n = 70)	AKI (n = 19)	p Value
Male sex	60 (67)	49 (70)	11 (58)	0.470
Age, y	75 [71-79]	76 [70-79]	73 [71-77]	0.295
BMI, kg/m^2	28.6 [25.7-30.6]	27.0 [25.5-30.3]	29.8 [27.3-31.5]	0.053
Smoking	9 (10)	9 (13)	0 (0)	0.223
Comorbidities				
Diabetes mellitus	32 (36)	21 (30)	11 (58)	0.048
Hypertension	72 (81)	55 (79)	17 (90)	0.457
Hypercholesterolemia	35 (39)	26 (37)	9 (47)	0.586
Type of surgery				0.865
CABG	23 (26)	19 (27)	4 (21)	
Valve replacement	31 (35)	27 (39)	8 (42)	
CABG + valve replacement	35 (39)	24 (34)	7 (37)	
Preoperative serum creatinine, μmol/L	119 [106-139]	115 [103-136]	137 [128-159]	0.005
Preoperative eGFR, mL/min/1.73 m^2	44 [39-50]	46 [41-52]	39 [28-45]	0.001
ASA score	3 [3-3]	3 [3-3]	3 [3-3]	0.087
Time of ECC, min	117 [95-148]	114 [95-144]	121 [101-152]	0.394
Time of aortic cross-clamping, min	85 [65-104]	81 [64-104]	89 [67-106]	0.531
Red blood cell transfusion, mL	0 [0-290]	0 [0-275]	278 [0-558]	0.042
ICU stay, d	1 [1-2]	1 [1-2]	1 [1-2]	0.460
Total hospital stay, d	7 [5-11]	7 [5-10]	8 [6-11]	0.263
Mortality	5 (6)	3 (4)	2 (11)	0.627

NOTE. Data represent frequencies (percentages) or median [interquartile range].
Abbreviations: AKI, acute kidney injury; ASA, American Society of Anesthesiologists; AVR, aortic valve replacement; BMI, body mass index; CABG, coronary artery bypass grafting; ECC, extracorporeal circulation; eGFR, estimated glomerular filtration rate; ICU, intensive care unit; MVR, mitral valve replacement.

Preoperative serum creatinine levels were higher in patients who developed AKI compared with those who did not (137 [128 -159] *v* 115 [103 -136] μmol/L, p = 0.005). Consequently, preoperative eGFR was lower in patients with postoperative AKI (39 [28-45] *v* 46 [41 - 52] mL/min/1.73 m^2, p = 0.001). Time of extracorporeal circulation and aortic cross-clamping was comparable in both patient groups. However, patients with postoperative AKI received more red blood cell transfusion products than patients without AKI (278 [0-558] *v* 0 [0-275] mL, p = 0.042). In the entire study cohort, the ICU stay was 1 [1-2] day, and the total hospital stay was 7 [5-11] days.

Cardiopulmonary Bypass-Induced Hemolysis

Plasma levels of CFHb significantly increased after surgery with CPB, peaking at 1 hour in the ICU compared with induction of anesthesia (22.5 *v* 5.4 mg/dL, p < 0.001; Fig 1A). CFHb levels returned to baseline at 24 and 48 hours postsurgery. To exclude a possible effect of flow mode during CPB, we verified that CFHb concentrations did not differ between patients receiving pulsatile or nonpulsatile flow. Analyses of the effect of CPB mode on all circulating markers are summarized in Table S1 (Supplementary File B). LDH levels also showed an increase directly after surgery but kept rising until 48 hours after surgery (119 *v* 339 U/L, p < 0.001; Fig 1B) in the entire cohort. Plasma haptoglobin concentrations decreased significantly from induction to 1 hour in the ICU (1.70 *v* 0.80 g/L, p < 0.001; Fig 1C) and remained lower at 24 hours (1.70 *v* 1.14 g/L, p = 0.0011; Fig 1C).

When stratified by AKI status, patients who developed postoperative AKI showed higher levels of CFHb and LDH postsurgery compared with those who did not (respectively, 21.1 *v* 29.6 mg/dL, p = 0.019; Fig 1A and 301 *v* 216 U/L, p = 0.0051; Fig 1B). Nonetheless, CFHb and LDH levels 1 hour in the ICU could not predict the development of AKI using logistic regression. Additionally, pilot data on erythrocyte-derived extracellular vesicles, formed during hemolysis, significantly increased from induction to 1 hour in the ICU (11.4×10^6 *v* 51.4×10^6, p = 0.033), but levels were not different between patients who developed AKI and those who did not (Fig 1D). Carboxyhemoglobin (COHb) and methemoglobin (MetHb), proposed markers of hemolysis,[19-22] are easily available from blood gas analyses. In our cohort, both COHb and MetHb levels were not different between patients with or without postoperative AKI (Fig 1, E and F).

Inflammation and Endothelial Damage

Both TNFα and ICAM-1, markers of systemic inflammation and endothelial activation, gradually increased from 1 to 48 hours postoperatively (respectively, 7.06 *v* 9.21 ng/mL, p = 0.020; Fig 2A and 247 *v* 388 ng/mL, p < 0.001; Fig 2B). However, there was no significant difference over time between patients who developed AKI and those who did not. Angiopoietin-2, a marker of endothelial damage, progressively increased until 48 hours postoperatively in the entire cohort. Circulating levels were significantly higher in patients with AKI compared to patients without AKI at 24 hours in the ICU (4,162 *v* 3,374 pg/mL, p = 0.027; Fig 2D). Nonetheless, higher angiopoietin-2 levels were not significantly associated with AKI development, after accounting for repeated measures (p = 0.25). Moreover, CFHb at 1 hour postoperatively was not significantly associated with higher

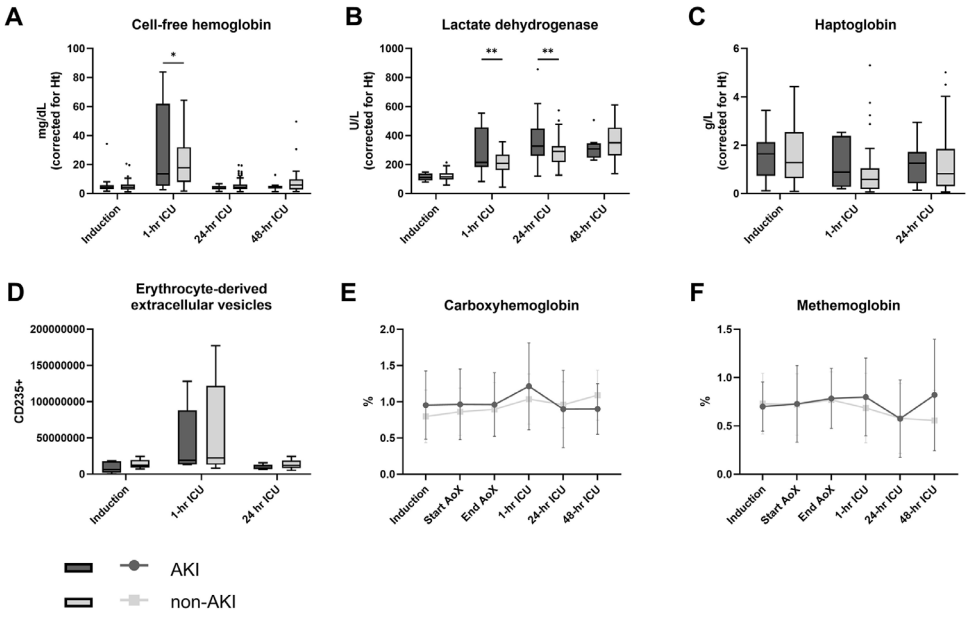

Fig 1. Circulating markers of hemolysis in plasma. Circulating levels of cell-free hemoglobin (A), lactate dehydrogenase (B), and haptoglobin (C), erythrocyte-derived extracellular vesicles (n = 10; D), carboxyhemoglobin (E), and methemoglobin (F) were measured in plasma or whole blood. Patients who developed acute kidney injury (AKI) are represented in dark gray, and patients without AKI are represented in light gray. Concentrations in plasma were corrected according to hematocrit levels. Data are presented as median with interquartile range and mean with standard deviation. *$p < 0.05$, **$p < 0.01$ between groups.

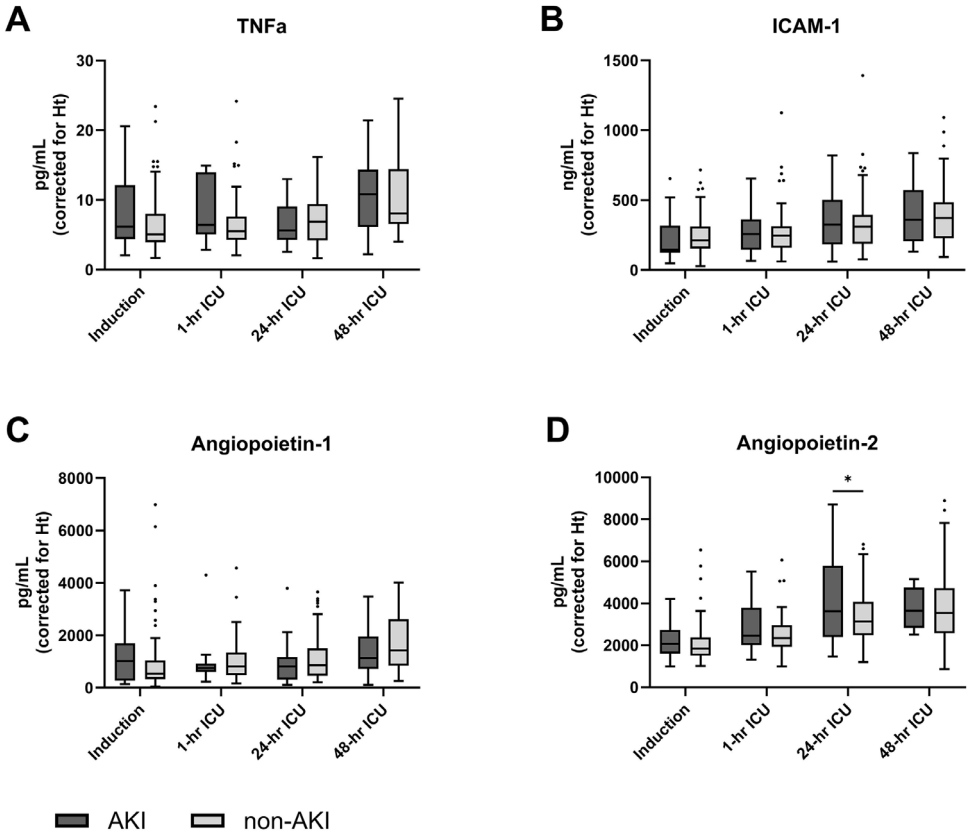

Fig 2. Circulating markers of inflammation and endothelial damage. Circulating levels of tumor necrosis factor α (TNFα; A), intercellular adhesion molecule 1 (ICAM-1; B), angiopoietin-1 (C), and angiopoietin-2 (D) in plasma. Patients who developed acute kidney injury (AKI) are represented in dark gray, and patients without AKI are represented in light gray. Concentrations in plasma were corrected according to hematocrit levels. Data are presented as medians with interquartile range. *$p < 0.05$ between groups.

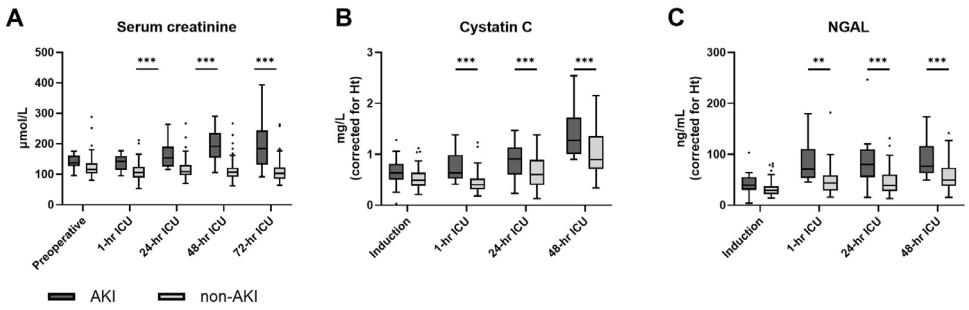

Fig 3. Circulating markers of kidney function and injury. Circulating levels of serum creatinine (A), cystatin C (B), and neutrophil gelatinase-associated lipocalin (NGAL; C) in plasma. Patients who developed acute kidney injury (AKI) are represented in dark gray, and patients without AKI are represented in light gray. Concentrations of cystatin C and NGAL in plasma were corrected according to hematocrit levels. Data are presented as medians with interquartile range. **p < 0.01, ***p < 0.001 between groups.

angiopoietin-2 levels at 24 hours (β = 3.30, p = 0.716) or 48 hours (β = 4.81, p = 0.779) after surgery. Angiopoietin-1 competes with angiopoietin-2 to maintain vascular stability. In our cohort, circulating levels of angiopoietin-1 elevated over time in the group of patients who did not develop AKI (998 *v* 1,660 ng/mL, p = 0.017), whereas levels remained stable in patients with AKI (Fig 2C).

Kidney Function and Injury

Markers of renal function and injury changed significantly over time, with distinct patterns between patients who developed AKI and those who did not (Fig 3). Serum creatinine remained stable in patients without AKI, whereas it progressively increased in patients with AKI (140 *v* 192 µmol/L, p < 0.001; Fig 3A) from induction to 48 hours postoperatively. Cystatin C rose gradually, peaking at 48 hours after surgery, particularly in patients with AKI (0.948 *v* 1.317 mg/L, p < 0.001; Fig 3B). NGAL levels increased in the entire cohort early after surgery, but the rise from induction to 1 hour postoperatively was markedly greater in patients who subsequently developed AKI (43.1 *v* 88.0 ng/mL, p < 0.001 and 33 *v* 48.2 ng/mL, p = 0.001; Fig 3C). Additionally, serum creatinine (odds ratio [OR], 1.03; 95% CI, 1.02-1.06; p = 0.0011), cystatin C (OR, 62.9; 95% CI, 5.34-1304.57; p = 0.0025), and NGAL (OR, 1.03; 95% CI, 1.01-1.06; p = 0.0072) at 1 hour in the ICU were all early predictors of AKI development.

CFHb Does Not Improve the Prediction of Postoperative AKI

To evaluate whether circulating CFHb levels provided additional predictive value for postoperative AKI, we constructed logistic regression models. The base model included only the established predictors of diabetes mellitus, perioperative packed red blood cells transfusion, and baseline serum creatinine. This model demonstrated good discrimination for postoperative AKI (AUC = 0.80; Fig 4, model 1). This model was compared with a model that additionally included CFHb levels measured 1 hour after surgery. Inclusion of CFHb did not significantly improve model fit (AIC 50.6 *v* 51.4, p = 0.28) or discrimination (AUC = 0.83, p = 0.32; Fig 4, model 2). Similarly, when peak CFHb values were added to the base model instead of CFHb at 1 hour in the ICU, no significant improvement was observed (AIC 50.6 *v* 51.5, p = 0.31; AUC = 0.83, p = 0.29; Fig 4, model 3). Lastly, adding angiopoietin-2 levels at 1 hour in the ICU also did not improve the prediction of AKI compared with the initial model (AIC 50.6 *v* 50.7, p = 0.17; AUC = 0.82, p = 0.56).

Discussion

Acute kidney injury is one of the most frequent complications of cardiac surgery with CPB, although the pathophysiology is still incompletely understood. CPB-induced hemolysis leads to increased levels of CFHb, to which the kidneys are particularly vulnerable. Nonetheless, little is known about the relation between CFHb and postoperative AKI in patients with preexisting renal dysfunction, a population already predisposed to renal and endothelial vulnerability. In this study, we

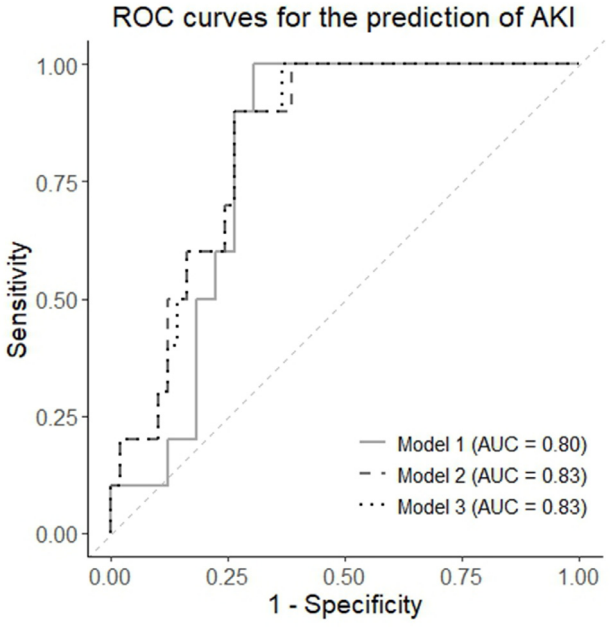

Fig 4. ROC curves for the prediction of postoperative acute kidney injury (AKI). ROC curves predicting postoperative AKI using diabetes mellitus, perioperative red blood cell transfusion, and baseline serum creatinine (model 1; continuous line), adding cell-free hemoglobin levels at 1 hour in the intensive care unit (model 2; dashed line), or adding peak cell-free hemoglobin levels (model 3; dotted line). AUC, area under the curve; ROC, receiver operating characteristic.

show that plasma CFHb concentrations increase after CPB, peaking at 1 hour after ICU admission compared with baseline in cardiac surgery patients with preexisting renal dysfunction. Furthermore, patients who developed AKI (21%) had higher postoperative CFHb and LDH levels. Nonetheless, CFHb did not improve the prediction of AKI when added to established clinical risk factors. Markers of inflammation and endothelial activation increased postoperatively but were not distinctive between patients developing AKI and those who did not. However, patients developing AKI had elevated levels of angiopoietin-2, a marker of endothelial damage, compared to those without AKI. Similarly, NGAL, a marker of kidney injury, increased significantly in the early postoperative period in patients who developed AKI. Interestingly, CFHb levels at 1 hour postoperatively were not associated with increased levels of angiopoietin-2. Together, these findings indicate that while CPB induces more pronounced hemolysis and endothelial damage in patients with AKI, CFHb does not provide additional predictive value for AKI in this high-risk population.

In patients with preexisting renal dysfunction, cardiac surgery with CPB induces hemolysis, as shown by increased plasma CFHb concentrations, peaking within 1 hour after ICU admission. This was accompanied by a reduction in haptoglobin levels that remained suppressed for 24 hours. These findings are in line with previous studies in patients undergoing cardiac surgery with CPB, in whom CFHb levels rose rapidly and peaked shortly after CPB alongside a reduction in haptoglobin.[6,23-25] However, those studies were conducted in patients with preserved kidney function prior to surgery, whereas our cohort included patients with preexisting renal dysfunction. The absolute CFHb concentrations observed in our study were lower than those reported in earlier studies.[6,23] This may be explained by shorter CPB duration or by differences in the type of surgery, as isolated CABG procedures may induce less hemolysis than aortic or combined valvular surgeries.[7] However, we observed no significant differences in CFHb concentrations between the different types of surgery in our cohort. Despite the occurrence of hemolysis, CFHb did not improve the prediction of postoperative AKI beyond established risk factors. This aligns with Davis et al.[25] and Wetz et al.,[24] but these findings contrast with the results of Hu et al.[6] and Hokka et al.,[23] who identified CFHb as an independent predictor of AKI. All previous studies included patients with preserved kidney function prior to surgery, whereas our study is the first to specifically look at patients with preexisting renal dysfunction. In this context, preoperative serum creatinine remained the strongest predictor of postoperative AKI, suggesting that in patients with reduced renal reserve, additional hemolytic burden may contribute less to overall AKI risk than in patients with normal preoperative renal function.

When erythrocytes are damaged due to exposure to increased shear stress, as well as abnormal flow and pressure conditions, they release extracellular vesicles into the circulation.[26,27] In line with this, our pilot data showed a postoperative increase in erythrocyte-derived EVs, supporting the thought that CPB triggers vesiculation as part of the hemolytic process. Despite the limited number of patients in whom EVs were analyzed, these pilot findings are encouraging and highlight the potential value of exploring this further in future studies.

Additionally, we explored whether COHb and MetHb are accessible markers of hemolysis, as previously reported.[19-22] Interestingly, COHb and MetHb did not differ between patients with and without AKI in our cohort. However, no values exceeded 2% in our cohort, suggesting that COHb and MetHb primarily reflect more severe hemolysis than typically occurs during routine CPB. Together, these findings indicate that CPB induces measurable mechanical erythrocyte injury and vesicle formation, although these secondary markers may lack sensitivity to detect the more moderate degree of hemolysis observed in our cohort.

Endothelial damage is a recognized feature of CPB-related organ dysfunction. CPB triggers systemic inflammation, initiates coagulation, and activates the endothelium, subsequently leading to endothelial hyperpermeability, tissue edema, and organ dysfunction. Angiopoietin-2 is a key regulator of endothelial permeability and promotes vascular leakage by competitively inhibiting angiopoietin-1–mediated Tie2 activation.[28,29] In this study, TNFα and ICAM-1 increased postoperatively, confirming systemic inflammation and endothelial activation in our cohort. In line with previous findings from our group[11] and others,[30,31] we found that circulating angiopoietin-2 levels increased up to 48 hours after surgery, indicating sustained postoperative endothelial injury. Angiopoietin-1 levels rose in patients without AKI but remained unchanged in those who developed AKI, suggesting a loss of endothelial barrier function in the latter group. Although angiopoietin-2 levels were higher in patients who developed AKI compared with those who did not, no independent association with AKI was observed after accounting for repeated measures. Similar results have been reported previously in patients with preserved renal function prior to surgery.[31] The absence of an association suggests that other factors may influence this relationship. Our cohort primarily only consisted of patients with preexisting renal dysfunction, which is often accompanied by chronic endothelial dysfunction.[15] Additionally, the lack of statistical significance may partly be explained by the limited number of AKI cases, which may have reduced power to detect modest effects. Similarly, postoperative CFHb concentrations were not associated with angiopoietin-2 levels at 24 or 48 hours after surgery. This contrasts with findings in other settings of hemolysis, such as severe malaria, where CFHb is correlated with elevated angiopoietin-2 levels.[32] In the present study, CFHb peaked shortly after CPB, whereas angiopoietin-2 levels continued to rise during the postoperative period. We hypothesized that CFHb could act as a trigger of endothelial damage, with postoperative angiopoietin-2 levels reflecting sustained endothelial damage. However, the absence of an association in our cohort suggests that the increase in angiopoietin-2 may be caused by distinct processes rather than CFHb-induced endothelial damage. Thus, while both CFHb and angiopoietin-2 increase after CPB, their trajectories may occur in parallel rather than be causally linked in these patients with preexisting renal and endothelial vulnerability.

Strengths and Limitations

This study provides novel insights into the relationship between hemolysis, endothelial damage, and kidney function in cardiac surgery patients with preexisting renal dysfunction, a group often excluded from previous studies. The inclusion of all available patients with plasma samples from an academic as well as secondary referral hospital strengthens the generalizability of the findings. However, the relatively small number of patients with AKI limited statistical power and the number of variables in multivariable analyses. In addition, the multiple comparisons in our analyses introduced a risk of type I error, but post hoc analyses were adjusted using Tukey correction to minimize the risk of false-positive findings. Furthermore, the absence of plasma samples directly after CPB prevented assessment of the immediate hemolytic response. Nevertheless, the trend in postoperative CFHb levels is comparable to those reported previously, and these studies also showed minimal change between the end of CPB and ICU admission.[24,25] Thus, CFHb concentrations measured within 1 hour after ICU admission can be considered a reliable reflection of CPB-induced hemolysis.

Conclusions

In conclusion, this is the first study to specifically investigate the relationship between CPB-induced hemolysis, endothelial damage, and postoperative AKI in patients with preexisting renal dysfunction. Our findings demonstrate that CPB induces hemolysis and endothelial damage in these high-risk patients, with higher CFHb and angiopoietin-2 levels observed in patients who developed AKI. However, CFHb did not independently predict AKI or correlate with markers of endothelial damage. These findings suggest that in patients with preexisting reduced renal and endothelial reserve, the additional contribution of CPB-induced hemolysis to AKI development appears limited. Future studies in larger cohorts are needed to confirm these observations and to explore interventions aimed at reducing hemolysis and endothelial injury during cardiac surgery in this vulnerable population.

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Declaration of competing interest

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CRediT authorship contribution statement

Carolien Volleman: Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation. **Philippa G. Phelp:** Writing – review & editing, Methodology. **Dionne P.C. Dubelaar:** Writing – review & editing. **Anita M. Tuip-de Boer:** Writing – review & editing, Investigation. **Rebecca V.G. Hollander:** Writing – review & editing, Investigation. **Rienk Nieuwland:** Writing – review & editing. **Alexander P.J. Vlaar:** Writing – review & editing, Supervision, Resources. **Charissa E. van den Brom:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition, Conceptualization.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1053/j.jvca.2026.02.039.

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