ORIGINAL RESEARCH

Haptoglobin 2-2 Phenotype Is Associated With Increased Acute Kidney Injury After Elective Cardiac Surgery in Patients With Diabetes Mellitus

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***Background*—**Recent studies reported an association between the 2-2 phenotype of haptoglobin (Hp 2-2) and increased cardiorenal morbidity in nonsurgical diabetic patients. Our goal was to determine whether the Hp 2-2 phenotype was associated with acute kidney injury (AKI) after elective cardiac surgery in patients with diabetes mellitus.

***Methods and Results*—**We prospectively enrolled 99 diabetic patients requiring elective cardiac surgery with cardiopulmonary bypass. Haptoglobin phenotypes were determined by gel electrophoresis. Cell-free hemoglobin, haptoglobin, and total serum bilirubin were quantified as hemolysis markers. The primary outcome was postoperative AKI, as defined by the Acute Kidney Injury Network classification. The incidence of AKI was significantly higher in Hp 2-2 patients compared with patients without this phenotype (non–Hp–2-2; 55.6% versus 27%, $P<0.01$). The need for renal replacement therapy was also significantly higher in the Hp 2-2 group (5 patients versus 1 patient, $P=0.02$). Thirty-day mortality (3 versus 0 patients, $P=0.04$) and 1-year mortality (5 versus 0 patients, $P<0.01$) were also significantly higher in patients with the Hp 2-2 phenotype. In multivariable analysis, Hp 2-2 was an independent predictor of postoperative AKI ($P=0.01$; odds ratio: 4.17; 95% confidence interval, 1.35–12.48).

***Conclusions*—**Hp 2-2 phenotype is an independent predictor of postoperative AKI and is associated with decreased short and long-term survival after cardiac surgery in patients with diabetes mellitus. **(*J Am Heart Assoc*. 2017;6:e006565. DOI: 10.1161/JAHA.117.006565.)**

Key Words: acute kidney injury • diabetes mellitus • haptoglobin • surgery

Postoperative acute kidney injury (AKI) is associated with poor outcome after cardiac surgery.[1] Although progression to severe postoperative kidney failure requiring renal replacement therapy (RRT) is relatively uncommon (occurring in 2–4% of patients), the incidence of postoperative AKI can be as high as 50% in high-risk patients such as those with diabetes mellitus (DM).[2,3] Studies have shown that DM is a major risk factor for complications after cardiac surgery that could result in increased morbidity, longer hospital stay, and higher short and long-term mortality.[4–9]

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Over the past several years, polymorphism in the hemoglobin binding protein haptoglobin has been reported as an important risk factor, predicting early development of cardiovascular[10,11] and renal[12,13] complications in patients with DM. Located on chromosome 16q22 in humans, the *HP* (haptoglobin) gene contains 2 classes of alleles denoted as 1 and 2, giving rise to 3 structurally and functionally distinct haptoglobin protein phenotypes: Hp 1-1, Hp 2-1, and Hp 2-2. The protein product of the Hp 2 allele has an inferior ability to bind to hemoglobin. This may lead to iron overload during periods of hemolysis and has been linked with renal injury.[14,15] In addition, the Hp 2 allele product is also an inferior antioxidant compared with the Hp 1 allele product,[16] which can further exacerbate tissue injury. Several observational studies in nonsurgical patient populations have demonstrated that diabetic patients with the Hp 2-2 phenotype have higher incidence of cardiorenal morbidity compared with those who carry the other 2 phenotypes (Hp 1-1 or Hp 2-1). This has been attributed to decreased binding of cell-free hemoglobin by Hp 2-2, resulting in iron accumulation, which, in turn, may lead to increased oxidative stress and inflammation, resulting in cardiovascular and renal injury.[17,18]

Clinical Perspective

What Is New?

- Patients with diabetes mellitus who possess the 2-2 phenotype of haptoglobin have increased risk for acute kidney injury and mortality after cardiac surgery.
- This may be caused by inability to handle increased hemolysis and iron overload in the context of cardiopulmonary bypass.

What Are the Clinical Implications?

- Cardiac surgery patients with diabetes mellitus who have the Hp 2-2 phenotype may require a modified clinical approach.
- This may include performing more off-bypass coronary revascularizations and catheter-based valvular procedures in an attempt to minimize cardiopulmonary bypass–associated hemolysis.

Interestingly, similar increased risk of cardiorenal morbidity has not been reported in nondiabetic patients who have the Hp 2-2 phenotype,[19] presumably because they lack of the increased baseline oxidative and inflammatory burden that is associated with chronic hyperglycemia and other metabolic disturbance in patients with DM. Given the high incidence of AKI in diabetic patients undergoing cardiac surgery[20] and the poor postoperative outcomes associated with it, as well as the known relationship between the Hp 2-2 phenotype and kidney disease in diabetic patients,[12,13] our aim was to identify whether there was any correlation between haptoglobin phenotypes and post–cardiac surgery AKI in patients with DM. We hypothesized that the Hp 2-2 phenotype in patients with DM is associated with an increased risk for AKI after elective cardiac surgery with cardiopulmonary bypass (CPB).

Methods

The study was approved by the University of Virginia institutional review board (IRS-HSR 14691). Written informed consent was obtained from all study participants before enrollment. Between July 2012 and January 2014, we prospectively enrolled consecutive adult diabetic patients (aged >18 years) who self-reported European ancestry and who required elective cardiac surgery (coronary artery bypass grafting [CABG], valve repair/replacement, or combined CABG and valve repair/replacement) with CPB. All patients had been diagnosed with DM (type 1 or 2) for ≥10 years. Patients with chronic hemolytic disorders, hematological malignancies, other hemoglobinopathies or recent (<30 days) allogeneic blood transfusion were excluded from the study. In addition, patients with a recent exposure to intravenous contrast dye (≤7 days before surgery) and patients with kidney dysfunction (acute and/or chronic) requiring renal replacement therapy were also excluded from the study.

Anesthesia Induction and Maintenance

Induction and maintenance of general anesthesia was based on our standard institutional practice. Monitoring included standard American Society of Anesthesiologists monitors as well as a radial arterial line catheter, a pulmonary artery catheter, transesophageal echocardiography, and cerebral near-infrared spectroscopy. All patients had a standard surgical approach. Heparin was used for anticoagulation during CPB per standard of care protocols. High-potassium cold crystalloid cardioplegia was used to arrest the heart during the CPB period. Pharmacological support with vasopressors and/or inotropes for assistance in separation from CPB was used based on the attending anesthesiologist's discretion and the patient's hemodynamic status. Protamine was administered after separation from CPB for heparin reversal. Intraoperative and postoperative glucose management was achieved with insulin and was based on serum glucose measurements, using a computer-based algorithm (Glucommander 2.0 Enterprise Edition; Glytec Systems) to maintain serum glucose between 6.7 and 8.9 mmol/L (120–160 mg/dL) intraoperatively and <10 mmol/L (180 mg/dL) postoperatively.[21]

Patient Data Collection

Perioperative data, including demographic data, comorbidities, relevant preoperative laboratory results, medication use, intraoperative variables, intraoperative and postoperative laboratory values, and postoperative outcomes were prospectively collected from the electronic anesthetic record and the patient's medical chart and recorded on an electronic case report form. For each patient, the Society of Thoracic Surgeons (STS) predictive risk of operative mortality was calculated preoperatively. The STS national database was used for further verification of perioperative data and outcomes. Data collection was performed by 2 independent investigators (S.T. and D.C.S.) who were not aware of the patients' haptoglobin phenotype.

Haptoglobin Phenotyping

All materials and reagents needed for haptoglobin phenotyping were purchased from Sigma Aldrich. Haptoglobin phenotyping was performed using gel electrophoresis, as previously described by Hochberg et al.[22] This phenotypic determination fully correlates with haptoglobin genotyping using polymerase chain reaction, as reported by Koch and colleagues.[23] Briefly,

arterial blood samples (5 mL) were collected after anesthesia induction, placed on ice, and allowed to clot. The serum was collected and stored at 4°C. A 10% human hemoglobin solution was prepared by using heparinized blood and washing the red blood cells 5 times in phosphate buffered saline and then lysing it with sterile water (ratio of red cells to sterile water: 1:9). The lysate was then centrifuged at 10 000*g* for 40 minutes, and the supernatant (that contained hemoglobin) was collected and frozen in −70°C until analyzed. For the analysis, 10 μL of the patient's serum were mixed with 2 μL of the 10% hemoglobin solution. To allow haptoglobin/hemoglobin (Hp/Hb) complexes to form, samples were rested for 10 minutes at room temperature. An equal volume of sample buffer containing 125 mmol/L TrisBase (pH 6.8), 20% (wt/vol) glycerol, and 0.001% (wt/vol) bromophenol blue was then added to each sample. The Hp/Hb complexes were resolved by polyacrylamide gel electrophoresis. To visualize Hp/Hb complexes, the gels were then soaked in a staining solution containing 5 mL of 0.2% (wt/vol) 3,3′,5,5′-tetramethylbenzidine in methanol 0.5 mL dimethylsulfoxide, 10 mL of 5% (vol/vol) glacial acetic acid, 1 mL of 1% (wt/vol) potassium ferricyanide, and 150 μL of 30% (wt/wt) hydrogen peroxide. This procedure produces easily distinguishable bands for each haptoglobin phenotype and has been accurately correlated with haptoglobin genotyping performed by polymerase chain reaction.[23] All gels were photographed and read by an independent investigator who was not aware of the patient's postoperative outcomes.

Blood Samples and Laboratory Tests Collection

All laboratory tests were collected per institutional protocol and performed at the University of Virginia Medical Center core biochemistry laboratory. Baseline creatinine was defined as the most recent recorded serum creatinine concentration before surgery (but not on the day of surgery). Postoperatively, creatinine was measured on admission to the intensive care unit (ICU) and then daily for the first 7 days postoperatively or until hospital discharge, whichever was earlier. Because AKI was diagnosed in the first 72 hours after surgery, only the values on ICU admission and on postoperative days 1 to 3 for postoperative serum creatinine are reported.

Intravascular hemolysis was quantified by measuring haptoglobin and cell-free hemoglobin concentrations at the following time points: after anesthesia induction (baseline), 60 minutes after initiation of CPB, on admission to the ICU, and on the first 2 days postoperatively. Serum total bilirubin was also measured as an adjunct marker of hemolysis at the following time points: preoperatively (baseline), postoperatively at admission to the ICU, and on postoperative days 1 to 3. Haptoglobin and bilirubin levels were measured using a validated Beckman LX20 clinical chemistry analyzer (Beckman Coulter) in the core biochemistry laboratory of our institution. Cell-free hemoglobin was quantified using the 2−wavelength method, as previously described in detail by Billings et al.[24]

Study Outcomes and End Points

The study's primary end point was postoperative AKI as defined by the Acute Kidney Injury Network classification using the creatinine criteria.[25] Urine output criteria were not used because of the potential confounding effects of hypovolemia and the use of diuretic medications, both of which are common postoperatively after cardiac surgery.[26,27] AKI risk was calculated for all patients according to the Acute Kidney Injury in Cardiac Surgery score.[28] Secondary end points included 30-day and 1-year all-cause mortality. Additional evaluated outcomes included postoperative myocardial infarction, stroke, postoperative mechanical ventilation >48 hours, new-onset postoperative atrial fibrillation, and the length of postoperative ICU and hospital stay. Postoperative myocardial infarction was defined according to the third universal definition of myocardial infarction.[29] Other outcomes were defined according to standard definitions as used by the STS (Table 1). Survival data were obtained by directly contacting patients by telephone and were further verified using data from our institutional central data repository and the State of Virginia Department of Health Death Registry.

Sample Size Estimate

The incidence of postoperative AKI in patients with DM has been reported to range from 30% to 50%.[20,30] Assuming an overall postoperative AKI incidence of 40% in our study cohort, we hypothesized that an overall 20% increase in the incidence of AKI in Hp 2-2 patients would represent a clinically significant effect size. Given that the frequency of the Hp-2-2 phenotype in white persons is ≈35%,[31] this requirement would assume that 60% of the patients with Hp 2-2 and 30% of the patients with a non–Hp 2-2 phenotype developed postoperative AKI. Based on these assumptions, 96 patients were needed for the study to reach a power of 80% and an α level at 0.05 by population analysis.

Statistical Analyses

Descriptive statistics were used in delineating the characteristics of the cohort by haptoglobin phenotype. Differences in continuous variables between 2 groups were tested using the 2-sample Student *t* test or the Wilcoxon rank test, depending on whether the data were normally distributed. Categorical variables were compared with the χ^2 test and the Fisher exact test, as appropriate. A linear mixed-effects model for repeated

Table 1. Definitions of the Primary and Secondary Outcomes

Outcome	Definition
AKI	
Stage I	Increase in serum creatinine >0.3 mg/dL of baseline or increase of 1.5 to 2-fold from baseline
Stage II	Increase in serum creatinine >2 to 3-fold from baseline
Stage III*	Increase in serum creatinine >3 from baseline or serum creatinine >4 mg/dL with an acute rise of at least 0.5 mg/dL
Postoperative MI	Elevation of cardiac biomarker values >10× the 99th percentile (in patients with normal preoperative cTN levels) in addition to (1) new pathological Q waves or new LBBB, (2) angiographically documented new graft or new native coronary artery occlusion, or (3) imaging evidence of new loss of viable myocardium or new regional motion abnormality
Stroke	Postoperative central neurologic deficit persisting >72 h
New-onset atrial fibrillation	New onset of atrial fibrillation/flutter requiring treatment; does not include recurrence of atrial fibrillation/flutter that was present preoperatively
Ventilation >48 h	Pulmonary insufficiency requiring ventilatory support that includes (but is not limited to) causes such as acute respiratory distress syndrome and pulmonary edema and/or any patient receiving ventilation >48 h postoperatively

AKI indicates acute kidney injury; cTN, cardiac troponin; LBBB, left bundle-branch block; MI, myocardial infarction.
*Patients who require renal replacement therapy are considered stage III AKI.

measures was used to evaluate intra- and intergroup differences for creatinine, total bilirubin, haptoglobin, and cell-free hemoglobin levels. A univariate logistic regression followed by a multiple logistic regression model was developed to assess whether an association between haptoglobin phenotype and the primary study outcome (AKI) existed. The odds ratios for the likelihood of AKI with 95% confidence intervals and the corresponding *P* values were calculated and reported. Additional variables that were evaluated in the multiple logistic regression for the risk of AKI included age (in 10-year increments), sex, preoperative creatinine level, preoperative glycosylated hemoglobin (HbA1c), preoperative ejection fraction <40%, CPB time, aortic cross-clamp time, intraoperative blood transfusion, and the log-transformed STS mortality risk score.

Model fitting was evaluated by the Hosmer–Lemeshow goodness-of-fit test and deviance and Pearson χ^2 tests, and the predictive power was assessed by the C statistic (the area under the receiver operating characteristic curve). In addition, to assess whether the effect of the Hp 2-2 phenotype was independent, its interactions with other covariates were tested in multiple logistic regression, and significant interactions were retained.

The Kaplan–Meier method was used to estimate the postoperative survival of the 2 groups and to describe the prognostic importance of haptoglobin phenotype with respect to postoperative mortality. The log-rank test was used to evaluate differences between survival curves.

Data are presented as mean±SD for normally distributed continuous variables or median (with interquartile range) when parameters were not normally distributed. Group frequencies and percentages were used for categorical variables. $P<0.05$ was considered significant with a 2-sided test. SAS version 9.4 software (SAS Institute Inc) was used for data analysis.

Results

The study was conducted and reported according to the CONSORT (Consolidated Standards of Reporting Trials). The enrollment CONSORT diagram is presented in Figure 1. Overall, 168 diabetic patients who self-reported European ancestry and were admitted for elective cardiac surgery were screened, and 99 patients completed the study. Of the 69 patients who were excluded, 56 did not meet inclusion criteria, 10 refused to participate in the study, and 3 withdrew from the study after enrollment (Figure 1). Eligible patients were recruited between July 2012 and January 2014.

Patients were divided into 2 groups according to their haptoglobin phenotype: (1) patients with a Hp 2-2 phenotype (Hp 2-2 group) and (2) patients with a non–Hp 2-2 phenotype (Hp 1-1 or Hp 2-1 phenotype; non–Hp 2-2 group). Thirty-six patients (36.3%) had the Hp 2-2 phenotype, whereas 63 patients had a non–Hp 2-2 phenotype (18 patients [18.2%] demonstrated a Hp 1-1 phenotype, and 45 patients [45.4%] demonstrated a Hp 2-1 phenotype). These phenotypic frequencies are consistent with the findings published by others.[32,33]

Patients in the Hp 2-2 group were younger than those in the non–Hp 2-2 group (in years: 64.2±8.3 [range: 38–86] versus 69.6±9.2 [range: 44–89]; $P=0.03$). Preoperative glycosylated hemoglobin (HbA1c) levels were comparable between groups (7.1% in the Hp 2-2 group versus 7.3% in the non–Hp 2-2 group, $P=0.52$). Mean baseline creatinine concentration was also similar between the study groups (80.4±31.8 μmol/L in the Hp 2-2 group versus 83.1±35.4 μmol/L in the non–Hp 2-2 group, $P=0.89$). Other patient characteristics, comorbidities, and relevant preoperative clinical and laboratory data are presented in Table 2.

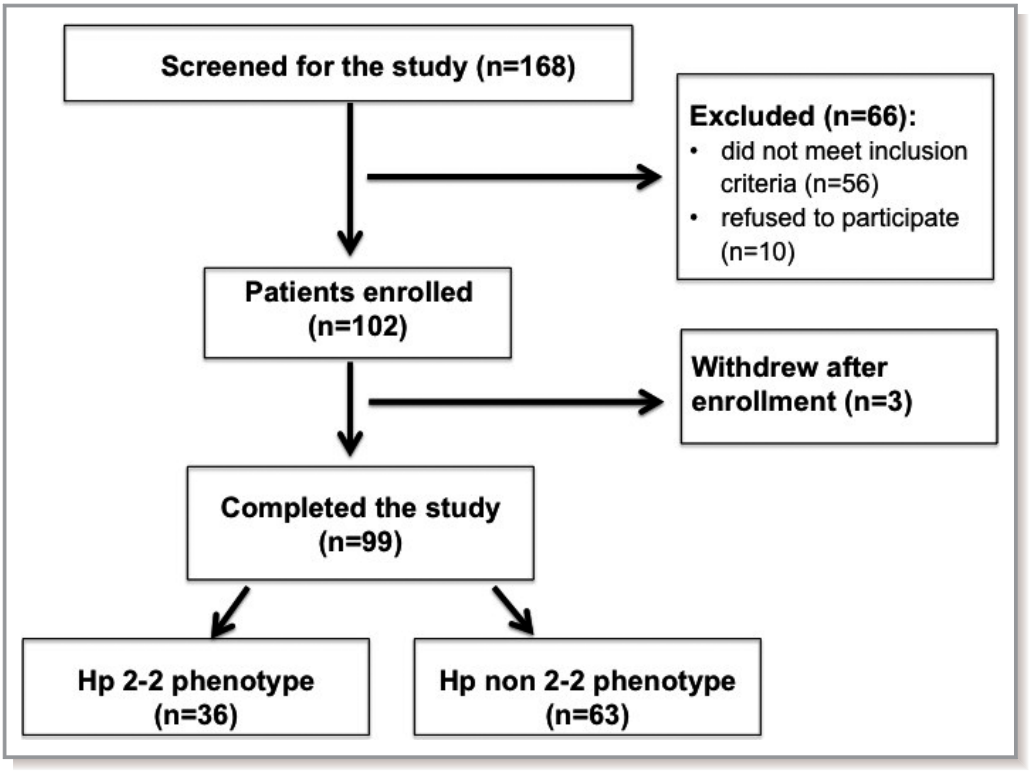

Figure 1. CONSORT (Consolidated Standards of Reporting Trials) trial flow diagram. Hp indicates haptoglobin.

Intraoperative variables are summarized in Table 3. Seventeen patients in the Hp 2-2 group (47.2%) and 27 patients in the non–Hp 2-2 group (42.9%) had isolated CABG (P=0.68). There were no significant differences in CPB time (103±31 minutes in the Hp 2-2 group versus 107±28 minutes in the non–Hp 2-2 group, P=0.79), aortic cross clamp time (82±22 in the Hp 2-2 group versus 84±25 minutes in the non–Hp 2-2 group, P=0.64), nor the number of coronary grafts (3±1 in the Hp 2-2 group versus 3±1 in the non–Hp 2-2 group, P=1.00) between the study groups. Postoperative variables and outcomes are summarized in Table 4.

Cell-Free Hemoglobin, Haptoglobin, and Serum Bilirubin Concentrations

Intergroup differences at predefined time points and within-group differences between baseline and individual time points were evaluated by a linear mixed-effects model.

CPB was associated with a very prominent rise in cell-free hemoglobin in both patient groups (Figure 2A). With the exception of similar baseline concentrations, free hemoglobin concentrations in the Hp 2-2 group were consistently and significantly higher than in the non–Hp 2-2 group at all times (P<0.001, from linear mixed-effects model). In both groups, cell-free hemoglobin levels decreased over the first 24 hours after surgery until returning to normal values on postoperative day (POD) 2.

Baseline haptoglobin levels were similar between the 2 patient groups (Figure 2B). Haptoglobin level significantly decreased over the course of surgery and the immediate postoperative period in both groups, with the lowest level recorded on POD 1 and then returned to normal by POD 2. Although both groups had a significant decrease from baseline in haptoglobin levels intraoperatively and on POD 1, a significantly greater decrease was evident in the non–Hp 2-2 group compared with the Hp 2-2 patients (P<0.001, from linear mixed-effects model).

Baseline serum bilirubin levels were similar between the 2 patient groups (Figure 2C). In both groups there was a significant rise in bilirubin concentration with a peak on POD 1, which then decreased over time until returning to normal values on POD 2. There were no significant differences, however, in bilirubin concentrations between the 2 patient groups at all 5 time points (P=0.81, from linear mixed-effects model).

Acute Kidney Injury

Serum creatinine levels at baseline, on admission to the ICU, and for the first 72 hours after surgery are presented in Figure 3. Mean serum creatinine on admission to the ICU was similar in the 2 patient groups (92.8±32.7 μmol/L in the Hp

Table 2. Preoperative Patient Characteristics in Hp2-2 Versus Non–Hp 2-2 Patients

Parameter	Hp-2-2 (n=36)	Non–Hp 2-2 (n=63)	*P* Value
Age, y, mean (SD)	64.2 (8.3)	69.6 (9.2)	0.03
Sex, male, n (%)	21 (58.3)	35 (55.5)	0.88
BMI, kg/m^2 (SD)	30.7 (5.7)	31.4 (6.9)	0.64
Medical history, n (%)			
Hypertension	28 (77.8)	52 (82.5)	0.79
COPD	11 (30.6)	19 (30.2)	0.99
Previous MI	13 (36.1)	15 (23.8)	0.25
EF <40%	9 (25)	13 (20.6)	0.62
Cerebrovascular disease	5 (13.9)	4 (6.3)	0.28
Hyperlipidemia	22 (61.1)	37 (58.7)	0.83
PVD	8 (22.2)	19 (30.2)	0.49
Previous coronary bypass surgery	3 (8.3)	4 (6.3)	0.70
Preoperative medications, n (%)			
β-blockers	25 (69.4)	40 (63.5)	0.52
ACEI	12 (33.3)	22 (34.9)	1.00
ARB	6 (16.7)	13 (20.6)	0.79
Statins	28 (77.8)	46 (73)	0.64
Insulin	7 (19.4)	14 (22.2)	0.78
Preoperative laboratory values			
HbA1c, % (SD)	7.1 (1.29)	7.3 (1.43)	0.52
Creatinine, μmol/L (SD)	80.4 (31.8)	83.1 (35.4)	0.89
Hemoglobin, g/dL (SD)	13.8 (1.7)	13.2 (1.8)	0.36
Serum glucose, μmol/L (SD)	6.9 (1.8)	6.3 (1.7)	0.45
Haptoglobin, g/L (SD)	1.34 (0.17)	1.27 (0.16)	0.67
Free hemoglobin, μmol/L (SD)	2.8 (1.1)	2.7 (1.35)	0.85
Cardiac index, L/min/m^2 (SD)	2.1 (0.2)	2.2 (0.3)	0.82
STS predicted mortality, mean (SD)	4.3 (2.6)	4.9 (3.3)	0.64
AKICS score, mean (SD)	4.6 (0.7)	5.1 (0.6)	0.57

ACEI indicates angiotensin-converting enzyme inhibitor; AKICS, Acute Kidney Injury in Cardiac Surgery; ARB, angiotensin receptor blocker; BMI, body mass index; COPD, chronic obstructive pulmonary disease; EF, ejection fraction; HbA1C, glycosylated hemoglobin; Hp, haptoglobin; MI, myocardial infarction; PVD, peripheral vascular disease; STS, Society of Thoracic Surgeons.

2-2 group versus 94.6±28.2 μmol/L in the non–Hp 2-2 group, *P*=0.56). However, serum creatinine levels over PODs 1 to 3 were consistently and significantly higher in the Hp 2-2 group compared with the non–Hp 2-2 group (*P*=0.02, from linear mixed-effects model).

Overall, the incidence of postoperative AKI was 37.3% (37 of 99 patients). Twenty patients (55.6%) in the Hp 2-2 group had AKI versus 17 patients (27%) in the non–Hp-2-2 group (*P*<0.01).

Table 3. Intraoperative Variables in Hp 2-2 Versus Non–Hp 2-2 Patients

Parameter	Hp-2-2 (n=36)	Non–Hp 2-2 (n=63)	*P* Value
CABG only, n (%)	17 (47.2)	27 (42.9)	0.68
CPB time, min (SD)	103 (31)	107 (28)	0.79
Aortic cross clamp time, min (SD)	82 (22)	84 (25)	0.65
Lowest hemoglobin during CPB, g/dL (SD)	9.2 (1.7)	8.7 (1.5)	0.53
Intraoperative PRBC transfusion, n (%)	9 (25)	19 (30.2)	0.65
Number of coronary grafts, n (SD)	3 (1)	3 (1)	1.00

CABG indicates coronary artery bypass grafting; CPB, cardiopulmonary bypass; Hp, haptoglobin; PRBC, packed red blood cells.

In addition, 7 patients (19.4%) in the Hp 2-2 group were diagnosed with stage III AKI versus only 1 patient (1.6%) in the non–Hp 2-2 group (*P*<0.01). Moreover, 5 patients in the Hp 2-2 group (13.9%) compared with only 1 patient in the non–Hp-2-2 group (1.6%) required renal replacement therapy (*P*=0.02).

The multivariable analysis revealed that significant risk factors associated with AKI (Table 5) were preoperative creatinine (odds ratio: 7.44; 95% confidence interval, 1.55–35.75, *P*=0.01), aortic cross clamp time (odds ratio: 2.52; 95% confidence interval, 1.11–4.68, *P*=0.03) and the haptoglobin 2-2 phenotype (odds ratio: 4.17; 95% confidence interval, 1.35–12.48, *P*=0.01).

The *P* value for the Hosmer–Lemeshow goodness-of-fit test was 0.379, and the *P* values for deviance and Pearson tests were 0.123 and 0.378, respectively, indicating that the logistic regression model fit well with an adequate predictive power. The C statistic (area under the curve; Figure 4) was 0.73 when the model included only traditional risk factors for AKI without the Hp 2-2 phenotype. When the Hp 2-2 phenotype was also included in the model, the C statistic increased to 0.79, suggesting that the predictive power of the model is improved by the addition of Hp 2-2. We have also evaluated the interaction between the Hp 2-2 phenotype and the other covariates that were included in the final model. A significant interaction (*P*=0.04) was discovered only between Hp 2-2 and the log-transformed STS risk score. For the model also including this interaction, the C statistic was increased to 0.83, further improving the predictive power of the model.

Survival Analysis

Patients were followed for a median of 2.4 years (interquartile range: 1.3–3.2 years). For descriptive purposes, we report 30-day and 1-year survival rates for the 2 patient groups. The

Table 4. Postoperative Variables and Outcomes in Patients With Hp 2-2 Versus Non–Hp 2-2 Phenotype

Parameter	Hp 2-2 (n=36)	Non–Hp 2-2 (n=63)	*P* Value
Myocardial infarction, n (%)	3 (8.3)	1 (1.6)	0.13
New-onset atrial fibrillation, n (%)	4 (11.1)	9 (14.13)	0.77
AKI, n (%)	20 (55.6)	17 (27)	<0.01
Postoperative RRT, n (%)	5 (13.9)	1 (1.6)	0.02
Stroke, n (%)	1 (2.8)	1 (1.6)	1.00
Ventilation >48 h, n (%)	6 (16.7)	7 (11.1)	0.54
PRBC transfusion, n (%)	9 (25)	18 (28.6)	0.82
ICU length of stay, h (IQR)	47.6 (24.2–76.8)	35.3 (22.8–66.4)	0.09
Postoperative hospital LOS, d (IQR)	9.3 (6.2–13.6)	7.2 (5.4–9.7)	0.17
30-d mortality, n (%)	3 (8.3)	0 (0)	0.04
1-y mortality, n (%)	5 (13.9)	0 (0)	<0.01

AKI indicates acute kidney injury; Hp, haptoglobin; ICU, intensive care unit; IQR, interquartile range; LOS, length of stay; PRBC, packed red blood cells; RRT, renal replacement therapy.

30-day all-cause mortality in the Hp 2-2 group was 8.3% (3 of 36 patients) versus 0% in the non–Hp 2-2 group (P=0.04). One-year all-cause mortality in the Hp 2-2 group was 13.9% (5 of 36 patients) versus 0% in the non–Hp 2-2 group (P<0.01). Kaplan–Meier survival estimates are presented in Figure 5. The curves for the 2 patient groups are significantly different (log rank P<0.01), suggesting a significant survival benefit for patients with non–Hp 2-2 phenotype compared with those with the Hp 2-2 phenotype.

Discussion

The results of the current investigation indicate that diabetic patients with the Hp 2-2 phenotype have increased risk for postoperative AKI after elective cardiac surgery, compared with patients with DM who do not have this phenotype. In addition, patients presenting the Hp 2-2 phenotype also have a higher risk of developing the severe form of AKI (stage III) and thus are more likely to require renal replacement therapy. The association between Hp 2-2 phenotype and AKI or renal replacement therapy is independent of other traditional risk factors, such as age, baseline kidney function, or aortic cross-clamp time. Furthermore, the addition of the haptoglobin phenotype into the model increased its predictive power for the primary outcome, as reflected by the receiver operator characteristic curves in Figure 4. Finally, we also demonstrated that 30-day and 1-year all-cause mortality was significantly higher in the Hp 2-2 patients, suggesting a significant survival benefit in diabetic patients with a non–Hp 2-2 phenotype.

Haptoglobin is a hepatically synthesized protein with expression that is elevated in response to oxidative and inflammatory stress. Several longitudinal observational studies demonstrated that diabetic patients with the Hp 2-2 phenotype have an increased risk for diabetes mellitus-associated cardiovascular and renal complications, including a 5-fold increase in the risk of coronary heart disease and a 2- to 3-fold higher risk of nephropathy.[10,11,34,35] Delanghe and coworkers showed that among CABG patients, those with the Hp 2-2 phenotype were younger and had more extensive coronary atherosclerosis, thus requiring more coronary grafts for revascularization.[36] Our results also demonstrate that Hp 2-2 patients were significantly younger than non–Hp 2-2 patients. Although this may suggest that patients with Hp 2-2 phenotype have more advanced atherosclerotic disease at presentation, presumably caused by less efficient oxidative stress and inflammation handling, and thus are more susceptible to postoperative complications, it seems that our patient groups were well matched, as reflected by similar baseline creatinine levels and similar STS predictive mortality scores.

Luo and coauthors reported in an abstract that advanced age, microalbuminuria, and Hp 2-2 phenotype were predictors of renal failure after CABG surgery.[37] Our findings confirm and further extend this observation. First, we present data regarding CPB-associated hemolysis, which may provide a mechanistic explanation for the association of Hp 2-2 with postoperative AKI. In addition, we report 30-day and 1-year mortality, whereas Luo et al only reported early postoperative mortality. Furthermore, the frequencies of the 3 haptoglobin phenotypes in Luo and colleagues' study population are not clear. Although the frequency of the Hp 2-2 phenotype in Europe and North America is 35% to 40%, in Southeast Asia, >90% of all individuals carry the Hp 2-2 genotype.[38] This may reflect a major difference between our findings and Luo's group, since 100% of their study participants were of southeast Asian origin.

The most important role of haptoglobin is to bind cell-free hemoglobin. The binding of haptoglobin to hemoglobin attenuates the oxidative damage of the heme-derived iron to surrounding proteins and lipids.[32] The formation of the Hp/Hb complex is especially critical during hemolysis, when cell-free hemoglobin concentration increases and catalytic iron, which is a major source of oxidative damage to cellular membranes, proteins, and DNA,[39] is being released from the

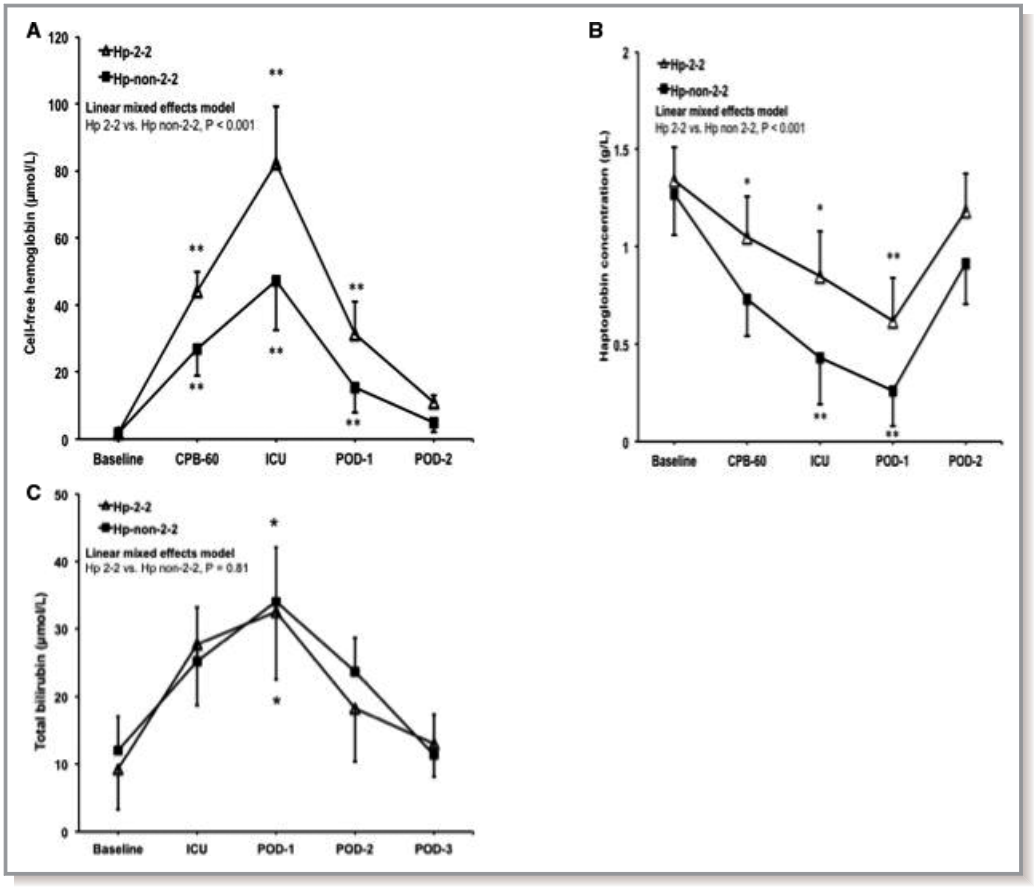

Figure 2. Plasma cell-free hemoglobin concentrations over time (A), haptoglobin concentrations over time (B), and total serum bilirubin over time (C) in patients with Hp 2-2 phenotype vs patients with non–Hp 2-2 phenotype. **P*<0.05 compared with baseline level. ***P*<0.01 compared with baseline level. Data are presented as mean±SD. CPB indicates cardiopulmonary bypass; ICU, intensive care unit; Hp, haptoglobin; POD, postoperative day.

heme molecule.[40,41] Less efficient Hp/Hb complex formation has been described with the Hp 2-2 phentype,[42,43] thus increased hemolysis in patients with Hp 2-2 phenotype may result in accumulation of catalytic iron in the kidneys, which has been associated with AKI and death after cardiac surgery.[44] Hemolysis has been reported in the context of CPB,[45] and hemoglobinemia is associated with increased oxidative stress and AKI after cardiac surgery.[24]

The Hp/Hb complex is cleared via the CD163 receptor, which is found on monocytes and macrophages.[46] Binding between Hp 1-1/Hb complexes and the receptor prompts the secretion of anti-inflammatory cytokines such as IL-10, thus promoting an anti-inflammatory state, whereas the binding of Hp 2-2/Hb complexes to the CD163 receptor induces a proinflammatory state.[47] This may further exacerbate organ damage, contributing to postoperative complications. It has been demonstrated that Hp 2-2 has a decreased affinity to hemoglobin and thus a reduced ability to prevent lipid peroxidation that is mediated by heme-derived catalytic iron via the production of hydroxyl radicals.[42,43] This phenomenon is increased in patients with DM due to the glycosylation of hemoglobin.[48] Furthermore, this effect is further exacerbated because Hp 2-2/Hb complexes are removed less effectively from the circulation compared with Hp 1-1/Hb or Hp 2-1/Hb complexes, resulting in excessive accumulation of free hemoglobin and catalytic iron, particularly in the proximal tubules of the kidney.[16,49] Moreover, because of its higher concentration, free hemoglobin can bind nitric oxide, resulting in decreased bioavailability of nitric oxide, which has been linked with endothelial dysfunction[50] and postoperative intestinal and kidney injury.[51]

Our results demonstrate an increase in plasma cell-free hemoglobin and a decrease in plasma haptoglobin both intraoperatively and in the immediate postoperative period (compared with baseline levels) that are most probably the results of CPB-induced hemolysis. Nevertheless, significant differences existed between the 2 patient groups: Cell-free hemoglobin levels were significantly higher in the Hp 2-2 group compared with the non–Hp 2-2 patients. This may be explained, at least in part, by the decreased binding ability of Hp 2-2 to

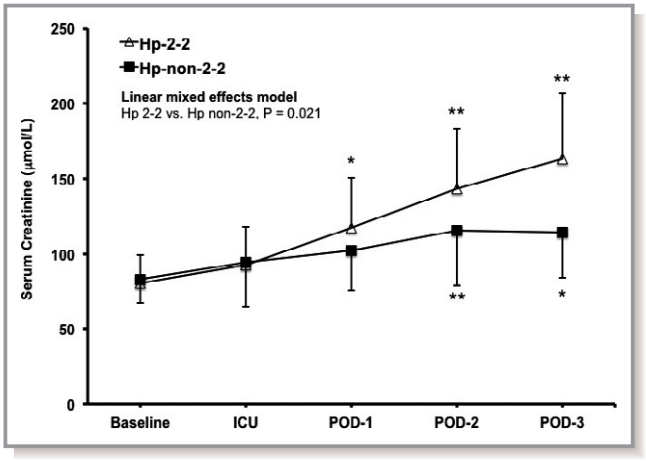

Figure 3. Serum creatinine concentrations over time in patients with Hp 2-2 phenotype vs patients with non–Hp 2-2 phenotype. **P*<0.05 compared with baseline level. ***P*<0.01 compared with baseline level. Data are presented as mean±SD. ICU indicates intensive care unit; Hp, haptoglobin; POD, postoperative day.

hemoglobin and decreased clearance of the Hp/Hb complex,[42,52] resulting in higher levels of plasma haptoglobin compared with the non–Hp 2-2 group. These effects may result in excessively elevated cell-free hemoglobin levels and thus release of large amounts of catalytic iron, which may have contributed to the increased incidence of AKI found in the Hp 2-2 group. We have also quantified total serum bilirubin levels as an adjunct marker of hemolysis. Hyperbilirubinemia is reported in 25% to 30% of patients after cardiac surgery with CPB[53,54]; therefore, as expected, there was a significant increase in postoperative serum bilirubin levels in both groups. However, there were no significant differences in bilirubin concentration between the 2 study groups, a result that is different from our predication that patients with the Hp 2-2 phenotype may have increased serum bilirubin due to of the increased levels of its

Table 5. Predictors of Postoperative AKI by Multivariable Analysis

Variable	Odds Ratio	95% CI	*P* Value
Age (10-y increments)	0.97	(0.91–1.02)	0.17
Sex	1.39	(0.45–4.36)	0.57
Hp 2-2 phenotype	4.17	(1.35–12.84)	0.01
HbA1c	1.18	(0.76–1.83)	0.46
EF <40%	2.84	(0.87–9.27)	0.08
Preoperative creatinine	7.44	(1.55–35.75)	0.01
CPB time	1.02	(0.98–1.03)	0.63
Aortic cross clamp time	2.52	(1.11–4.68)	0.03
PRBC transfusion	1.13	(0.44–1.41)	0.28

AKI indicates acute kidney injury; CI, confidence interval; CPB, cardiopulmonary bypass; EF, ejection fraction; HbA1c, glycosylated hemoglobin; Hp, haptoglobin; PRBC, packed red blood cells.

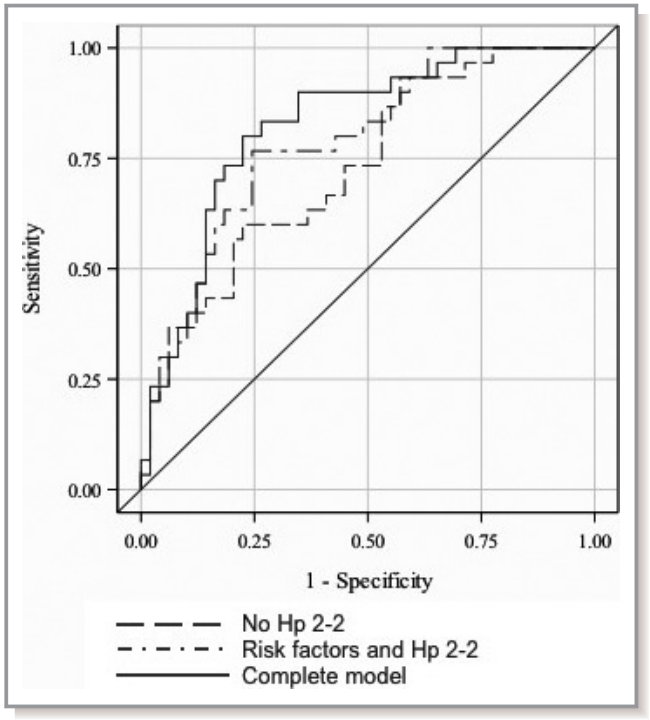

Figure 4. Receiver operator characteristic curves presenting the predictive power for postoperative AKI of the 3 statistical models: (1) clinical risk factors only (non–Hp 2-2); (2) clinical risk factors and Hp 2-2 phenotype (risk factors and Hp 2-2); and (3) clinical risk factors, Hp 2-2 phenotype, and the interaction with the Society of Thoracic Surgeons risk score (complete model). AKI indicates acute kidney injury; Hp, haptoglobin.

substrate: cell-free hemoglobin. The reasons for this unexpected finding are not known and require further investigation. Many biochemical steps including transporting cell-free

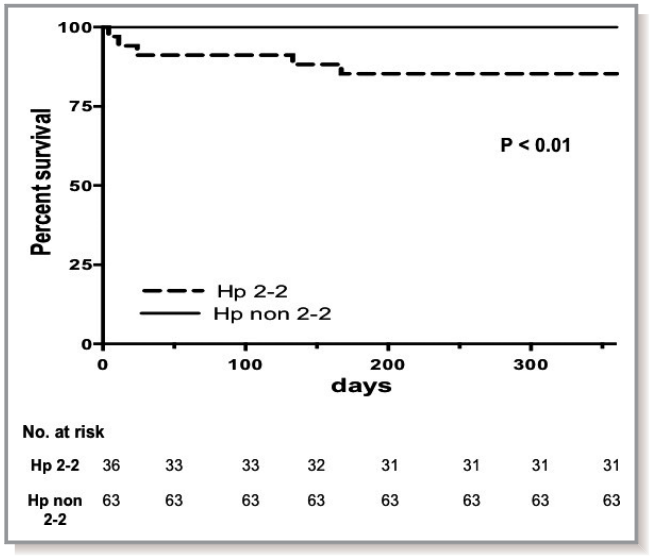

Figure 5. Kaplan–Meier estimates of mortality over time in patients with Hp 2-2 phenotype vs patients with non–Hp 2-2 phenotype. Hp indicates haptoglobin.

hemoglobin into cells and several enzymatic reactions are needed to produce bilirubin from hemoglobin. It may be that some of the steps are not upregulated in patients with the Hp 2-2 phenotype despite the increase of cell-free hemoglobin.

Taken together, our results indicate that Hp 2-2 is a novel independent predictor of AKI and mortality in patients with DM after cardiac surgery with CPB. This, at least in part, could be caused by increased oxidative stress and inflammation caused by excessive cell-free hemoglobin and its byproduct, catalytic iron, which may have accumulated in the kidneys.

Our findings may be clinically relevant in several respects. First, the association of Hp 2-2 with increased CPB-induced hemolysis and AKI may improve our knowledge about the pathogenesis of post–cardiac surgery AKI. Second, it is possible that diabetic patients with the Hp 2-2 phenotype, being at increased risk for postoperative complications, may require a modified clinical approach to fluid management and blood product utilization, as well as an attempt to minimize CPB-associated hemolysis. This may include performing off-bypass coronary revascularizations and catheter-based valvular procedures and the use of high-dose vitamin E, which is emerging as a novel effective therapy alleviating inflammatory and oxidative stress in diabetic patients with the Hp 2-2 phenotype.[19,55] Nevertheless, any modification in patient care will require additional extensive clinical research including appropriately powered multicenter clinical trials.

Our results must be interpreted within the constraints of several potential limitations. We have suggested that excessive cell-free hemoglobin and catalytic iron levels, in the context of CPB-induced hemolysis, may promote increased oxidative stress and thus kidney injury. We have not quantified lipid peroxidation or other markers of oxidation in our study patients, nor have we measured catalytic iron levels. Nonetheless, others have already reported that diabetic patients with the Hp 2-2 phenotype have increased oxidative stress[56] and increased levels of catalytic iron,[57,58] and an association between excessive oxidation and AKI after cardiac surgery has been reported.[24]

Second, we assumed that the elevated cell-free hemoglobin and the blunted decrease in haptoglobin in the Hp 2-2 group are the results of decreased Hp/Hb complex formation and decreased binding of the Hp 2-2/Hb complex to the CD163 receptor in the context of CPB-induced hemolysis; however, these findings may also occur as a result of decreased expression of the CD163 receptor. We have not measured the level of the CD163 receptor in the current study. Nevertheless, several studies have demonstrated upregulation of the CD163 receptor following CABG surgery (regardless of the use of CPB).[47,59] Consequently, it is more likely that the observed differences in cell-free hemoglobin and haptoglobin between our patient groups result from decreased Hp 2-2/Hb complex formation and/or reduced binding of the Hp 2-2/Hb complex to the CD163 receptor rather than downregulation of the receptor itself. Finally, we had a total of 37 events of AKI and performed multivariable analysis to identify possible risk factors for AKI. All statistical analyses indicated a very good fit for our model; however, caution is needed in interpretation of the results, given our relatively small patient cohort.

Conclusions

This study found that the Hp 2-2 phenotype is independently associated with increased risk of AKI in diabetic patients after elective cardiac surgery and further improves the predictive power of traditional clinical relevant risk factors for postoperative AKI. In addition, short- and long-term all-cause mortality was also significantly higher in these patients. These findings are important and unique and may assist clinicians in identifying patients who are at increased risk for AKI after cardiac surgery, thus improving patient care and risk stratification. Our findings also suggest a possible role for cell-free hemoglobin in the pathogenesis of AKI after cardiac surgery. This could lead to potential therapies that would reduce the risk of cardiac surgery–associated AKI in diabetic patients with the Hp 2-2 phenotype.

Authors' Contributions

Study conception and design: Zuo, Raphael. Study conduct: Feng, Scalzo, Thammishetti, Xin, Ma, Raphael. Data analysis: Naik, Xin, Ma, Thiele, Zuo, Raphael. Writing article: all authors. Revising article: all authors.

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Disclosures

None.

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