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Increased plasma catalytic iron in patients may mediate acute kidney injury and death following cardiac surgery

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Catalytic iron, the chemical form of iron capable of participating in redox cycling, is a key mediator of acute kidney injury (AKI) in multiple animal models, but its role in human AKI has not been studied. Here we tested in a prospective cohort of 250 patients undergoing cardiac surgery whether plasma catalytic iron levels are elevated and associated with the composite outcome of AKI requiring renal replacement therapy or in-hospital mortality. Plasma catalytic iron, free hemoglobin, and other iron parameters were measured preoperatively, at the end of cardiopulmonary bypass, and on postoperative days 1 and 3. Plasma catalytic iron levels, but not other iron parameters, rose significantly at the end of cardiopulmonary bypass and were directly associated with bypass time and number of packed red blood cell transfusions. In multivariate analyses adjusting for age and preoperative eGFR, patients in the highest compared with the lowest quartile of catalytic iron on postoperative day 1 had a 6.71 greater odds of experiencing the primary outcome, and also had greater odds of AKI, hospital mortality, and postoperative myocardial injury. Thus, our data are consistent with and expand on findings from animal models demonstrating a pathologic role of catalytic iron in mediating adverse postoperative outcomes. Interventions aimed at reducing plasma catalytic iron levels as a strategy for preventing AKI in humans are warranted.

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Catalytic iron is the chemical form of iron that is neither transferrin nor protein bound and is therefore capable of catalyzing the Fenton and Haber–Weiss reactions, generating hydroxyl radicals, and causing cellular oxidative damage.[1,2] Under conditions of tissue injury[2] or hemolysis,[3] sudden release of intracellular stores of iron may result in elevated circulating levels of catalytic iron that may have a pathologic role in a variety of disease states including acute coronary syndrome[4,5] and acute kidney injury (AKI). In animal models of AKI, catalytic iron has been implicated in nephrotoxicity resulting from ischemia/reperfusion,[6] aminoglycosides,[7] cisplatin,[8] rhabdomyolysis,[9] hemoglobinuria,[10] and iodinated radiocontrast.[11] In many of these models, treatment with an iron chelator is protective.[7,8,12,13] The generalizability of these findings to humans is unknown.

In humans undergoing cardiac surgery, postoperative AKI is common and is associated with a several-fold increased risk of death.[14] Although the mechanisms of AKI following cardiac surgery are incompletely understood, release of free heme and iron during cardiopulmonary bypass (CPB) is likely to play a key role.[15] During CPB, extracorporeally circulated blood is exposed to nonphysiologic surfaces and shear forces that may injure red blood cells, leading to the release of free hemoglobin and catalytic iron.[16–19] We hypothesized that plasma catalytic iron levels increase after cardiac surgery and are associated with an increased risk of AKI requiring renal replacement therapy or in-hospital mortality (RRT/death).

RESULTS

Baseline characteristics

We enrolled and collected plasma and urine samples from 250 patients who underwent cardiac surgery (248 with CPB; 2 without CPB). Baseline and operative characteristics are shown in Table 1.

Iron markers before and after cardiac surgery

Figure 1 shows pre- and postoperative plasma catalytic iron levels in patients who did or did not reach the composite end

Table 1 | Baseline characteristics of the patients

Characteristic	$N = 250$
Demographics	
Age (years)	79 (72–83)
Female	107 (43)
White	242 (97)
Preoperative renal function	
Plasma creatinine (mg/dl)	1.2 (1.0–1.5)
eGFR (ml/min per 1.73 m^2)	49.9 (41.0–66.1)
CKD (eGFR <60 ml/min per 1.73 m^2)	170 (68)
Comorbidities	
Hypertension	205 (82)
Congestive heart failure	103 (41)
Diabetes mellitus	93 (40)
Chronic lung disease	56 (22)
Malignancy	52 (21)
Chronic liver disease	8 (3)
Preoperative iron marker levels	
Catalytic iron (μmol/l)	0.35 (0.29–0.44)
Free hemoglobin (mg/dl)	14.5 (8.9–24.1)
Total iron (μg/dl)	97 (81–106)
Transferrin (mg/dl)	211 (167–244)
Transferrin saturation (%)	33 (26–42)
Ferritin (ng/ml)	86 (46–156)
Urinary NGAL (ng)/creatinine (mg)	18.9 (7.5–68.8)
Operative characteristics	
Type of procedure	
CABG	31 (12)
Valve	111 (44)
CABG and valve	78 (31)
Status of procedure	
Elective	152 (61)
Urgent	98 (39)
First or reoperative	
First cardiovascular surgery	172 (69)
Reoperative cardiovascular surgery	78 (31)
Cardiopulmonary bypass time (min)	140 (100–196)
Cross-clamp time (min)	93 (66–126)

Abbreviations: CABG, coronary artery bypass graft; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; NGAL, neutrophil gelatinase-associated lipocalin.
Data are presented as *n* (%) or median (interquartile range IQR, 25–75th percentile). Estimated GFR was determined using the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) equation.

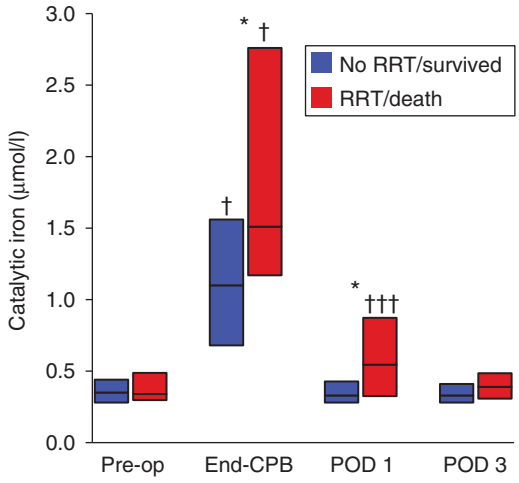

Figure 1 | Plasma catalytic iron levels—no RRT/survived versus RRT/death. $^{\dagger}P<0.001$, $^{\dagger\dagger\dagger}P<0.05$ for within-group comparisons to preoperative (Pre-op) levels; $^{*}P<0.01$ for between-group comparisons at individual time points. $N = 228$ (no RRT/survived); $N = 22$ (RRT/death). Bars represent median (25–75th interquartile range (IQR)). CPB, cardiopulmonary bypass; POD, postoperative day; RRT/death, renal replacement therapy or in-hospital mortality.

point of RRT/death. In both groups, plasma catalytic iron levels peaked at end-CPB, reaching levels ~2–3-fold higher than preoperative levels, and subsequently returned to baseline by postoperative day 3 (POD 3). At the first postoperative time point (end of CPB), plasma catalytic iron levels were significantly higher among patients who did versus who did not experience RRT/death, and remained significantly higher on POD 1 (Figure 1).

Figure 2 shows levels of pre- and postoperative plasma and urinary iron markers in patients who did or did not experience RRT/death. Similar to plasma catalytic iron, plasma free hemoglobin and urinary neutrophil gelatinase-associated lipocalin (NGAL) levels peaked at end-CPB in both groups. In addition, plasma ferritin and urinary NGAL levels were significantly higher on POD 1 among patients who did versus who did not experience RRT/death. In contrast to plasma catalytic iron, no other plasma or urinary iron markers were significantly different between groups at end-CPB (Figure 2).

Catalytic iron and adverse postoperative outcomes

Among the 250 patients, 64 developed AKI and 22 reached the primary composite end point of RRT/death (3 required RRT and survived, 13 died without RRT, and 6 required RRT and died). The causes of death were septic shock ($N = 9$), myocardial infarction ($N = 3$), respiratory failure ($N = 2$), mesenteric ischemia ($N = 1$), gastrointestinal bleed ($N = 1$), cerebrovascular accident ($N = 1$), cardiogenic shock ($N = 1$), and ventricular fibrillation ($N = 1$).

Univariate analyses between iron markers from each time point, baseline/operative characteristics, and RRT/death are shown in Table 2. Univariate analyses between change in iron markers over time and RRT/death are shown in Supplementary Table S1 online. Plasma catalytic iron and plasma ferritin levels measured at end-CPB and POD 1 and urinary NGAL levels measured on POD 1 were directly associated with RRT/death (Table 2). CPB time was directly associated, whereas age and estimated glomerular filtration rate (eGFR) were inversely associated, with RRT/death (Table 2). Additional univariate associations are shown in Table 2. As the strongest associations were observed on POD 1, subsequent analyses on adverse postoperative outcomes focused on iron markers at this time point.

Figure 3 shows the association between quartiles of catalytic iron on POD 1 and adverse outcomes. After adjusting for age and preoperative eGFR, patients with

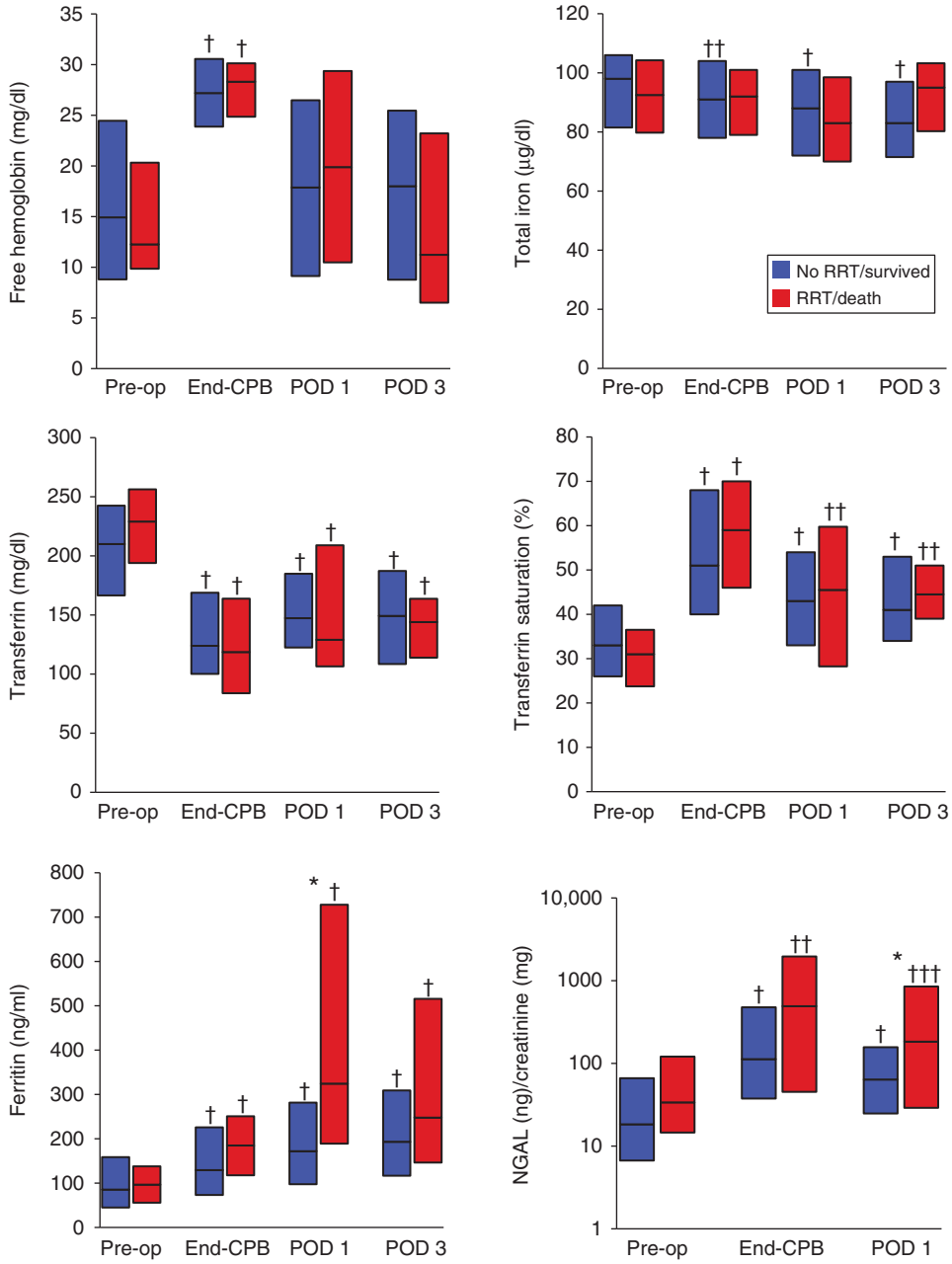

Figure 2 | Plasma and urinary iron markers—no RRT/survived versus RRT/death. All iron markers were measured in plasma except neutrophil gelatinase-associated lipocalin (NGAL), which was measured in urine. $^{\dagger}P<0.001$, $^{\dagger\dagger}P<0.01$, and $^{\dagger\dagger\dagger}P<0.05$ for within-group comparisons to preoperative (Pre-op) levels; $^{*}P<0.01$ for between-group comparisons at individual time points. $N=228$ (no RRT/survived); $N=22$ (RRT/death). Bars represent median (25–75th interquartile range (IQR)). CPB, cardiopulmonary bypass; POD, postoperative day; RRT/death, renal replacement therapy or in-hospital mortality.

catalytic iron levels in the highest versus lowest quartile had a significantly increased odds of RRT/death, AKI, and hospital mortality, but not 1-year mortality ($P=0.06$). Baseline characteristics were similar between patients with POD 1 catalytic iron levels in quartiles 1, 2, and 3 versus quartile 4, and are shown in Supplementary Table S2 online. Unadjusted and adjusted odds ratios for quartiles of catalytic iron on POD 1 and adverse outcomes are shown in Table 3 (additional odds ratios from different time points are shown in Supplementary Table S3 online). Patients with catalytic iron levels in the highest versus lowest quartile had a greater than sixfold increased odds of RRT/death. After adjustment for age and preoperative eGFR, this association remained unchanged. In exploratory analyses, further adjustment for CPB time >120 min, intraoperative packed red blood cell (pRBC) transfusions, and POD 1 plasma free hemoglobin levels resulted in an odds ratio of 6.23 (95% confidence interval 1.21–32.05). As other comorbidities

Table 2 | Univariate associations between plasma and urinary iron markers and baseline/operative characteristics with the primary end point of RRT/death

Time point	Odds ratio (95% confidence interval)	*P*-value
Catalytic iron		
Pre-op	1.06 (0.69–1.64)	0.79
End-CPB	1.61 (1.05–2.45)	0.03
POD 1	1.71 (1.18–2.47)	<0.01
POD 3	1.41 (0.96–2.07)	0.08
Free hemoglobin		
Pre-op	1.00 (0.63–1.59)	0.99
End-CPB	1.10 (0.67–1.80)	0.71
POD 1	1.23 (0.73–2.08)	0.44
POD 3	0.73 (0.47–1.13)	0.16
Total iron		
Pre-op	0.95 (0.61–1.47)	0.81
End-CPB	0.95 (0.62–1.46)	0.80
POD 1	0.89 (0.56–1.43)	0.64
POD 3	1.49 (0.84–2.66)	0.17
Transferrin		
Pre-op	1.33 (0.81–2.19)	0.26
End-CPB	0.82 (0.54–1.26)	0.37
POD 1	0.87 (0.54–1.41)	0.58
POD 3	0.93 (0.57–1.50)	0.75
TSAT		
Pre-op	0.76 (0.48–1.21)	0.25
End-CPB	1.17 (0.74–1.86)	0.50
POD 1	1.06 (0.65–1.72)	0.83
POD 3	1.38 (0.83–2.30)	0.21
Ferritin		
Pre-op	1.12 (0.70–1.78)	0.63
End-CPB	1.67 (1.08–2.60)	0.02
POD 1	2.09 (1.33–3.31)	<0.01
POD 3	1.45 (0.91–2.32)	0.12
Urinary NGAL/creatinine		
Pre-op	1.41 (0.90–2.23)	0.14
End-CPB	1.38 (0.87–2.18)	0.17
POD 1	2.08 (1.24–3.49)	<0.01
Baseline/operative characteristics		
Age (per 10 years)	0.63 (0.41-0.97)	0.04
eGFR (per 10 ml/min per 1.73 m^2)	0.69 (0.52-0.92)	0.01
CPB time >120 min	3.51 (0.99–12.51)	0.05

Abbreviations: CPB, cardiopulmonary bypass; eGFR, estimated glomerular filtration rate; NGAL, neutrophil gelatinase-associated lipocalin; POD, postoperative day; Pre-op, preoperative; RRT/death, renal replacement therapy or in-hospital mortality; TSAT, transferrin saturation.
All iron markers were measured in the plasma except for NGAL, which was measured in the urine. All markers were natural-log transformed, given their skewed distribution, and standardized to s.d. 1 to allow comparison across biomarkers.

and operative characteristics could also affect catalytic iron levels, additional models adjusting for these potential confounders are shown in Supplementary Table S4 online.

In addition to increased odds of RRT/death, patients with plasma catalytic iron levels in the highest versus lowest quartile also had increased odds of AKI, hospital mortality, postoperative myocardial injury, and postoperative vasopressor requirement (Table 3). These associations persisted after

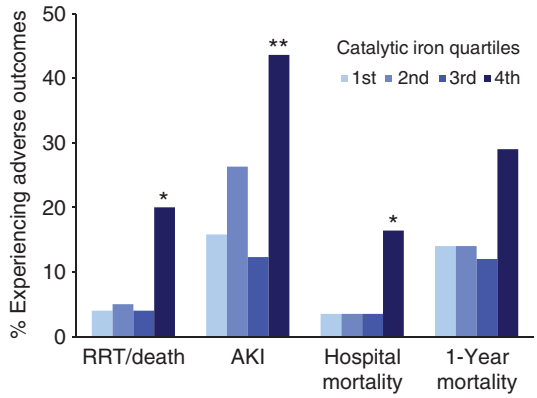

Figure 3 | Association between quartiles of catalytic iron on postoperative day 1 (POD 1) and adverse outcomes. *$P<0.05$ and **$P<0.01$, after adjustment for age and preoperative estimated glomerular filtration rate (eGFR). Catalytic iron quartiles (μmol/l): quartile 1 (0.19–0.28); quartile 2 (0.28–0.33); quartile 3 (0.33–0.46); and quartile 4 (0.46–4.96). AKI, acute kidney injury; RRT/death, renal replacement therapy or in-hospital mortality.

adjustment for age and preoperative eGFR. In addition, significant inverse correlations were observed between catalytic iron levels and hospital-free days ($r_s = -0.15$, $P = 0.03$) and ventilator-free days ($r_s = -0.17$, $P = 0.01$), implying longer duration of hospitalization and mechanical ventilation.

Factors associated with pre- and postoperative plasma catalytic iron

To explore potential sources of catalytic iron, we investigated the following biologically plausible factors for their association with plasma catalytic iron levels.

Baseline/operative characteristics. We found no association between preoperative plasma catalytic iron levels and any of the following: age, elective versus urgent surgery, congestive heart failure, diabetes mellitus, chronic lung disease, chronic liver disease, and malignancy. Furthermore, we found no correlation between preoperative catalytic iron levels and eGFR (Supplementary Figure S1 online), suggesting that decreased filtration does not significantly affect plasma levels.

Duration of CPB. Duration of CPB was associated with plasma catalytic iron levels at the end of CPB ($r_s = 0.39$, $P < 0.001$) and on POD 1 ($r_s = 0.15$, $P = 0.03$). Duration of CPB was associated with plasma free hemoglobin levels at the end of CPB ($r_s = 0.29$, $P < 0.001$) but not on POD 1 ($r_s = 0.11$, $P = 0.10$; Supplementary Figure S2 online).

pRBC transfusions. As pRBCs undergo hemolysis over time,[20] resulting in the release of free hemoglobin, we investigated the correlation between pRBC transfusions, catalytic iron levels, and free hemoglobin levels. We found a significant and positive correlation between number of pRBCs transfused through POD 3 and plasma catalytic iron levels on POD 3 ($r_s = 0.19$, $P < 0.01$, Figure 4a) but not plasma free hemoglobin levels on POD 3 (Figure 4b).

In an exploratory analysis, we also measured catalytic iron and free hemoglobin levels directly in 15 plasma aliquots

Table 3 | Association between quartiles of plasma catalytic iron on POD 1 and adverse outcomes

Outcome	Unadjusted		Adjusted	
	Odds ratio (95% CI)	*P*-value	Odds ratio (95% CI)	*P*-value
RRT/death (N = *22*)				
Quartile 1 (REF)	1.00	NA	1.00	NA
Quartile 2	1.52 (0.17–18.89)	1.00	1.64 (0.26–10.48)	0.60
Quartile 3	1.02 (0.07–14.52)	1.00	1.42 (0.19–10.77)	0.74
Quartile 4	6.62 (1.34–64.54)	0.01	6.71 (1.37–32.87)	0.02
AKI (N = *64*)				
Quartile 1 (REF)	1.00	NA	1.00	NA
Quartile 2	1.89 (0.69-5.46)	0.25	2.11 (0.82–5.45)	0.12
Quartile 3	0.76 (0.22–2.52)	0.82	0.94 (0.32–2.79)	0.91
Quartile 4	3.95 (1.53–10.00)	<0.01	3.99 (1.60–9.93)	<0.01
Hospital mortality (N = *19*)				
Quartile 1 (REF)	1.00	NA	1.00	NA
Quartile 2	1.00 (0.07–14.25)	1.00	1.05 (0.14–7.83)	0.96
Quartile 3	1.02 (0.07–14.52)	1.00	1.27 (0.17–9.56)	0.82
Quartile 4	5.20 (1.01–51.78)	0.05	5.11 (1.04–25.21)	0.05
One-year mortality (N = *44*)				
Quartile 1 (REF)	1.00	NA	1.00	NA
Quartile 2	1.00 (0.30–3.33)	1.00	1.07 (0.36–3.18)	0.91
Quartile 3	0.88 (0.25–3.01)	1.00	1.13 (0.37–3.50)	0.83
Quartile 4	2.43 (0.87–7.28)	0.10	2.29 (0.86–6.13)	0.10
Post-op myocardial injury (N = *27*)				
Quartile 1 (REF)	1.00	NA	1.00	NA
Quartile 2	5.00 (0.97–49.79)	0.06	5.53 (1.12–27.24)	0.04
Quartile 3	2.63 (0.41–28.73)	0.44	3.25 (0.59–17.87)	0.18
Quartile 4	5.10 (0.99–50.86)	0.05	4.92 (1.00–24.18)	0.05
Post-op pressors beyond 24 h (N = *71*)				
Quartile 1 (REF)	1.00	NA	1.00	NA
Quartile 2	1.59 (0.63–4.13)	0.39	1.75 (0.73–4.23)	0.21
Quartile 3	1.02 (0.38–2.79)	1.00	1.27 (0.50–3.23)	0.62
Quartile 4	2.59 (1.06–6.60)	0.04	2.67 (1.13–6.28)	0.03
Post-op sepsis (N = *50*)				
Quartile 1 (REF)	1.00	NA	1.00	NA
Quartile 2	0.89 (0.31–2.56)	1.00	0.92 (0.36–2.40)	0.87
Quartile 3	0.60 (0.18–1.87)	0.47	0.67 (0.24–1.91)	0.45
Quartile 4	1.67 (0.64–4.47)	0.35	1.64 (0.68–3.97)	0.28

Abbreviations: AKI, acute kidney injury; CI, confidence interval; NA, not applicable; POD, postoperative day; Post-op, postoperative; REF, reference; RRT/death, renal replacement therapy or in-hospital mortality.
Adjusted models include age and preoperative estimated glomerular filtration rate (eGFR).

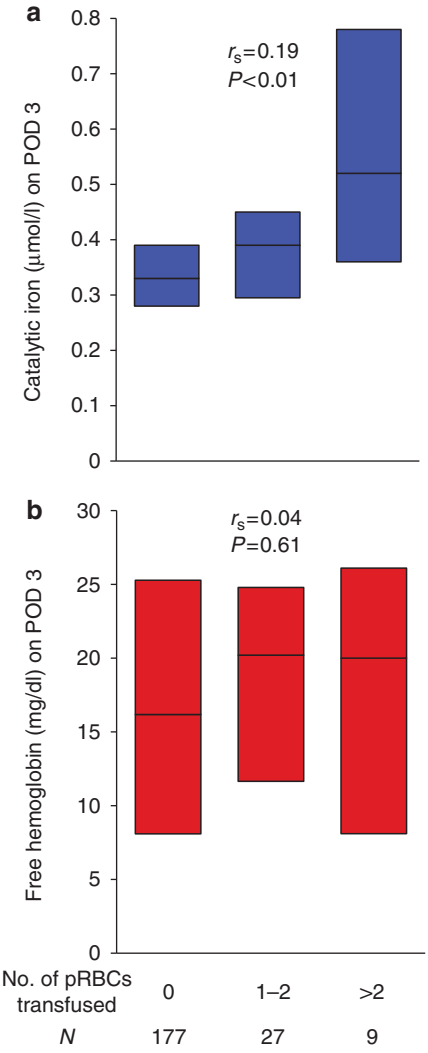

Figure 4 | Blood transfusions, catalytic iron, and free hemoglobin. Number of packed red blood cell (pRBC) transfusions and (**a**) plasma catalytic iron levels and (**b**) plasma free hemoglobin levels on postoperative day 3 (POD 3). Bars represent median (25–75th interquartile range).

prepared from blood bank-stored pRBCs of varying storage duration. Median (interquartile range) catalytic iron and free hemoglobin levels in these plasma aliquots were 47 (4–146) µmol/l and 1.2 (0.7–1.5) g/l, ~100-fold and 10-fold higher, respectively, than median levels observed in the plasma of study patients. In addition, catalytic iron and free hemoglobin levels appeared to be higher in aliquots from pRBCs of greater storage duration (Supplementary Figure S3 online), although these differences did not reach statistical significance ($P = 0.10$ and $P = 0.21$, respectively).

Free hemoglobin. As free hemoglobin resulting from CPB-induced hemolysis, pRBC transfusions, or other factors could be a source of catalytic iron, we investigated the correlation between plasma free hemoglobin and catalytic iron levels. Plasma free hemoglobin levels were significantly correlated with plasma catalytic iron levels at all four time points (Supplementary Figure S4 online).

Creatinine phosphokinase. As skeletal muscle injury occurs during CPB surgery,[21] and release of myoglobin into the circulation could be a source of plasma catalytic iron, we investigated the correlation between serum creatinine phosphokinase (CPK) values (as a surrogate for myoglobinemia)[22] and plasma catalytic iron levels. We found a significant correlation between plasma catalytic iron levels at the end of CPB and serum CPK levels obtained immediately postoperatively ($r_s = 0.28$, $P < 0.001$).

Acid-base status. As acidemia may lead to the release of iron from its transferrin-bound form to its free form,[23,24] we investigated the correlation between arterial pH and plasma

catalytic iron levels. We found no association at end-CPB ($r_s = -0.01$, $P = 0.89$), POD 1 ($r_s = -0.01$, $P = 0.85$), or POD 3 ($r_s = -0.02$, $P = 0.85$).

Urinary NGAL. As NGAL is an iron-sequestering protein and urinary levels are known to rise during CPB surgery, we investigated the correlation between urinary NGAL and plasma catalytic iron levels. We found no correlation preoperatively ($r_s = 0.01$, $P = 0.84$), a positive correlation at end-CPB ($r_s = 0.26$, $P < 0.001$), and a weak negative correlation on POD 1 ($r_s = -0.14$, $P = 0.05$; Supplementary Figure S5 online).

DISCUSSION

In this prospective cohort study we report the comprehensive analysis of multiple plasma and urinary iron markers before and after cardiac surgery. We found that levels of plasma catalytic iron, unlike other iron markers, rise early and considerably after cardiac surgery; are associated with increased CPB time and greater number of pRBC transfusions; and are associated with increased risk of RRT/death and other adverse postoperative outcomes including AKI and myocardial injury. These findings support the notion that plasma catalytic iron levels are an indicator of poor outcomes after cardiac surgery.

Iron and AKI in animal models

Iron has long been hypothesized to serve as a potential mediator of oxidative stress and cellular injury. By catalyzing the Haber–Weiss and Fenton reactions, catalytic iron combines with superoxide to ultimately form the very reactive and injurious hydroxyl radical.[1] In animal models of AKI, catalytic iron has been implicated in nephrotoxicity resulting from a wide range of insults including ischemia/reperfusion,[6] aminoglycosides,[7] rhabdomyolysis,[9] and hemoglobinuria.[10] In many of these models, treatment with an iron chelator attenuates renal function decline and histologic damage.[7,8,12,13]

Iron, free hemoglobin, and AKI in humans

Few studies have evaluated the associations between plasma catalytic iron, free hemoglobin, and AKI in humans, and all have been underpowered to evaluate hard clinical outcomes such as mortality. An observational study of 23 patients undergoing aortic valve replacement surgery reported elevated plasma catalytic iron levels postoperatively (no data on clinical outcomes were provided).[3] A recent study of 14 patients undergoing cardiac surgery reported elevated urinary catalytic iron levels 8 h postoperatively in patients who developed AKI.[19] The association between plasma free hemoglobin and postoperative AKI has been mixed, with one study ($N = 35$) reporting elevated levels in patients who developed AKI after major aortic surgery[25] and another study ($N = 30$) reporting no correlation between plasma free hemoglobin levels and AKI after CPB surgery.[26]

In contrast to the above studies, we simultaneously measured multiple iron markers at four time points in a large cohort of patients. We found that transferrin levels decrease and ferritin levels increase, likely as an acute-phase response,[27] and that plasma free hemoglobin levels increase after CPB. A key finding of this study is that although catalytic iron and free hemoglobin levels correlated with each other, an association with adverse postoperative outcomes was observed with the former but not the latter. This discordance suggests that catalytic iron may be more than a severity of illness marker and may be directly toxic. Importantly, the association between catalytic iron and RRT/death persisted after adjusting for free hemoglobin levels, suggesting that additional sources (other than RBC hemolysis) are likely to contribute to the generation of catalytic iron.

Iron and urinary NGAL

We found that plasma catalytic iron levels are associated with RRT/death at an earlier time point (end-CPB) than urinary NGAL (POD 1), an established AKI biomarker.[28] In addition, we found that urinary NGAL levels are directly associated with plasma catalytic iron levels at end-CPB. As NGAL is an iron-sequestering siderophore, these findings raise the interesting possibility that NGAL production is increased in response to elevated plasma catalytic iron levels.

Iron and acute coronary syndrome

In addition to its effects on AKI, iron also has a pathologic role in the process of lipid peroxidation,[29] the first step in the formation of an atherosclerotic lesion. Elevated plasma catalytic iron levels are independently associated with increased prevalence of coronary artery disease[30] and mortality in acute coronary syndrome.[4] In a pilot study of 45 patients undergoing coronary artery bypass grafting, intraoperative iron chelation versus placebo improved postoperative markers of oxidative stress and increased left ventricular ejection fraction at 12 months.[31] Adding to these findings, in this study we report a fivefold increased odds of postoperative myocardial injury among patients with elevated plasma catalytic iron levels on POD 1. Whether iron chelation might decrease myocardial injury in this setting has not been rigorously tested in a large randomized controlled trial.

Sources of catalytic iron

This study was not designed to determine the precise sources of catalytic iron. However, potential mechanisms by which cardiac surgery may induce elevation of plasma catalytic iron include: exposure of RBCs to nonphysiological surfaces resulting in hemolysis;[17] shear stress generated by pumps and suction systems; mechanical fragmentation of RBCs induced by valvular prostheses; skeletal muscle injury during CPB,[21] resulting in the release of iron-rich myoglobin into the circulation; pRBC transfusions;[20] and ischemia/reperfusion injury to the kidneys and other organs. Our findings of a positive correlation between both CPB duration and

number of pRBC transfusions with postoperative catalytic iron levels suggest that the rise is at least partially iatrogenic and hence potentially modifiable.

Preserved RBCs undergo progressive functional and structural changes during storage that ultimately result in accumulation of proinflammatory substances[32] and hemolysis.[20] Consistent with these observations, we found that catalytic iron levels are ~100-fold higher in plasma aliquots prepared from blood bank-stored pRBCs than levels observed in the plasma of study patients. We also observed a trend toward higher catalytic iron levels in pRBC aliquots of longer storage duration. Interestingly, in a large observational study of 'newer' versus 'older' pRBCs during cardiac surgery, patients who received pRBCs of longer storage duration had a significantly higher incidence of AKI.[33] However, our observations of catalytic iron content in pRBC plasma aliquots are preliminary in nature and in need of confirmation.

An additional potential source of catalytic iron is ferritin. Ferritin is the main iron storage protein, and may release iron in the presence of superoxide produced under conditions of inflammation.[34] Conversely, excess iron released from other sources such as free hemoglobin may stimulate increased levels of ferritin as a protective chelating mechanism. Consistent with this notion, a pilot study of 30 patients found that low preoperative ferritin levels were associated with a greater incidence of AKI following CPB surgery.[26] However, a larger subsequent study ($N = 120$) was unable to confirm this association.[35] Thus, whether ferritin acts as a source versus mitigator of elevated catalytic iron levels remains unclear.

Limitations

We acknowledge the limitations of this study including observational design. We did not measure all potentially relevant iron markers such as plasma haptoglobin, hemopexin, and hepcidin. We did not have data on storage time for transfused pRBCs in the overall cohort. To evaluate skeletal muscle injury as a source of plasma catalytic iron, we used CPK levels as a surrogate for myoglobin, as previous studies have demonstrated an excellent correlation between these two markers of rhabdomyolysis.[22] In addition, we did not have access to data on urine output, and therefore did not use oliguria as an AKI criterion. Finally, because of the large number of tests performed, we acknowledge the possibility of a type I error among the secondary end points, particularly the association between quartiles of plasma catalytic iron and hospital mortality, postoperative myocardial injury, and postoperative pressor requirement, all of which were significant at P-values between 0.01 and 0.05.

Threshold effect

Although we found a significant association between catalytic iron levels (evaluated as a continuous variable) and RRT/death, the association with RRT/death and other adverse outcomes was most striking for patients with levels in the highest quartile. This nonlinear association suggests a threshold effect, a phenomenon not uncommon in biological systems and which has been observed in previous studies of catalytic iron.[36]

Confounding

The association between catalytic iron levels and RRT/death could be confounded by a number of factors. In multivariable models we adjusted for several biologically plausible confounders including CPB time, intraoperative pRBC transfusions, plasma free hemoglobin levels, and demographic/comorbidity data. Adjustment for these factors did not affect the association between catalytic iron levels and RRT/death. Nonetheless, residual confounding from unmeasured variables cannot be excluded.

Plasma catalytic iron levels may increase due to decreased filtration rather than increased generation. However, iron is excreted mainly through sloughed mucosal cells in the gastrointestinal tract rather than in the urine. In addition, urinary catalytic iron levels are increased, not decreased, in patients with AKI.[19] Finally, we found no correlation between eGFR and preoperative plasma catalytic iron levels. Nonetheless, we cannot exclude the possibility that among patients with severely diminished urine output, decreased excretion of catalytic iron could have contributed to elevated plasma levels.

Conclusion

Plasma catalytic iron levels rise during cardiac surgery, increase with longer CPB time and number of pRBC transfusions, and are associated with multiple adverse postoperative outcomes. Given the known pathologic role of iron in a variety of disease states, plasma catalytic iron may be a director mediator of adverse outcomes after cardiac surgery and thus a potential therapeutic target. Interventional strategies to reduce catalytic iron levels—such as iron chelation, minimization of pRBC transfusions, and novel CPB devices—should be tested to minimize morbidity and mortality following cardiac surgery.

MATERIALS AND METHODS

Study design

We conducted a prospective cohort study among patients undergoing cardiac surgery at Brigham and Women's Hospital. Patients were recruited between August 2007 and March 2012. All patients provided written informed consent and all protocols were approved by the hospital's Institutional Review Board.

Study patients

The inclusion and exclusion criteria were chosen to capture patients at high risk of AKI and other adverse outcomes after cardiac surgery. Inclusion criteria were baseline eGFR ≤ 30 ml/min per 1.73 m^2 or any two of the following: baseline eGFR 31–60 ml/min per 1.73 m^2, diabetes mellitus, left ventricular ejection fraction $\leq 40\%$, previous cardiac surgery, combined coronary artery bypass/valve procedure, urgent procedure, and preoperative intraaortic balloon pump.

Exclusion criteria included preoperative AKI (defined as a 0.3 mg/dl rise in serum creatinine over 24 h or a 0.5 mg/dl rise over 48 h), serum creatinine >4.5 mg/dl, end-stage renal disease receiving dialysis, renal transplantation, pregnancy, and recent aminoglycoside use.

Study procedures

We collected plasma and urine samples preoperatively, at the end of CPB, and on PODs 1 and 3 (four total time points). We stored plasma aliquots at –80 °C within 2 h of collection.

Factors associated with plasma catalytic iron levels

We investigated the following biologically plausible factors for their association with plasma catalytic iron levels: baseline and operative characteristics, number of pRBC transfusions, acid-base status, urinary NGAL levels, and immediate postoperative serum CPK levels. CPK measurements were obtained in all patients undergoing cardiac surgery as part of routine clinical care.

End points

Investigator DEL adjudicated all outcomes by reviewing discharge summaries and progress notes, and was blinded to all study measurements at the time of adjudication. The prespecified primary end point was RRT/death. Secondary end points were AKI, defined as a 0.3 mg/dl rise in serum creatinine over 24 h or a 0.5 mg/dl rise over 48 h,[37] in-hospital mortality, 1-year mortality, postoperative myocardial injury, postoperative vasopressor requirement beyond 24 h, and postoperative sepsis. We defined postoperative myocardial injury using cut points for creatine kinase myocardial B fraction (CK-MB) specific for valvular or nonvalvular surgery, given intrinsic differences in degree of surgical trauma induced by the procedures.[38] For nonvalvular surgery, postoperative myocardial injury was defined as >10-fold rise in CK-MB above the upper reference limit of 5 ng/ml within 48 h of surgery.[39] For valvular surgery, >20-fold rise in CK-MB within 48 h defined myocardial injury.

Additional end points included duration of mechanical ventilation and hospital length of stay. To avoid the confounding effect of mortality, we calculated ventilator-free days and hospital-free days as 28 minus the number of ventilator-dependent days or hospitalization days, respectively, assuming survival to 28 days or discharge from the hospital. Patients who died before 28 days were assigned a score of zero.[40]

Laboratory analyses

We sent frozen plasma samples at −70 °C to the Muljibhai Patel Society for Research in Nephro-Urology, Nadiad, India, for blinded analysis of plasma catalytic iron, free hemoglobin, total iron, transferrin, total iron-binding capacity, and ferritin. Catalytic iron and other plasma iron markers were measured at all four time points. Catalytic iron levels were measured using the modified bleomycin assay.[4] In an exploratory analysis we also measured catalytic iron levels in a small number of aliquots of plasma prepared from blood bank-stored pRBCs of varying storage duration.

Serum creatinine was measured for clinical purposes using a modified kinetic Jaffe method. Urinary NGAL levels were measured at the first three time points, and were normalized to the urinary creatinine concentration. The interassay coefficient of variation for plasma catalytic iron, estimated using blinded split samples from study patients, was 5.4%. Interassay coefficients of variation for all other plasma and urinary assays were <10%. Additional details on the plasma and urinary assays are provided in the Supplementary Methods online.

Statistical analyses

Statistical analysis was performed with SAS Version 9.3 (Cary, NC). Data are reported as median and interquartile range (25–75 percentiles). Comparison of iron markers between patients who reached the composite end point (RRT/death) versus those who did not was assessed using the Wilcoxon rank-sum test. Comparison of iron markers over time (relative to preoperative levels) was assessed using the Wilcoxon signed-rank test. Correlations between catalytic iron levels and number of pRBC transfusions were analyzed using Spearman's rank correlation coefficient.

Univariate logistic regression was used to assess the relation between iron markers and baseline/operative characteristics with the primary end point. Univariate and multivariate logistic regression models (adjusted for age and preoperative eGFR) were used to compute odds ratios comparing quartiles of catalytic iron on POD 1 and the primary and secondary end points. Odds ratios and *P*-values from exact logistic regression models were qualitatively similar to those from logistic regression models and are provided for univariate analyses. Catalytic iron levels were analyzed by quartile because previous data suggested a nonlinear association with adverse outcomes, with the highest risk occurring in patients with the highest quartile of catalytic iron.[36] All comparisons are two tailed, with $P<0.05$ considered significant.

DISCLOSURE

MR and SSL report holding a US patent for the methods and kit for measurement of serum catalytic iron for early detection of acute coronary syndrome and prediction of adverse cardiac events. All the other authors declared no competing interests. SSW served as a consultant to Abbvie, CVS Caremark, Harvard Clinical Research Institute, and Takeda; provided expert testimony or consultation for litigation related to nephrogenic systemic fibrosis (GE Healthcare) and mercury exposure; and has received grants from the National Institute of Diabetes and Digestive Kidney Diseases, Genzyme, Merck, Otsuka, Pfizer, and Satellite Healthcare.

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SUPPLEMENTARY MATERIAL

Figure S1. Lack of association between preoperative eGFR and preoperative plasma catalytic iron levels.
Figure S2. Correlations between CPB time, plasma catalytic iron, and plasma free hemoglobin levels. CPB = Cardiopulmonary Bypass.
Figure S3. Catalytic iron and free hemoglobin levels in pRBC aliquots of varying storage duration. Bars represent median (25th-75th interquartile range).
Figure S4. Correlations between plasma catalytic iron and plasma free hemoglobin levels.
Figure S5. Correlations between plasma catalytic iron and urinary NGAL levels

Table S1. Univariate associations between change in plasma and urinary iron markers over time and RRT/death.
Table S2. Comparison of demographics between patients with POD#1 plasma catalytic iron levels in quartiles 1, 2, and 3 versus quartile 4.
Table S3. Association between quartiles of plasma catalytic iron and RRT/death.
Table S4. Association between quartiles of plasma catalytic iron on POD#1 and the composite endpoint of in-hospital mortality or AKI requiring RRT (death/RRT).
Supplementary material is linked to the online version of the paper at http://www.nature.com/ki

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