Low preoperative hepcidin concentration as a risk factor for mortality after cardiac surgery: A pilot study

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Objective: Hepcidin regulates iron absorption and recycling and is central to host defense, protection from reactive iron species, and a biomarker of iron-related pathophysiology. We assessed the value of hepcidin measured preoperatively for the prediction of in-hospital mortality and renal outcomes.

Methods: We studied 100 adult patients undergoing cardiac surgery in the control arm of a randomized, controlled trial. Plasma and urine were sampled before induction of anesthesia, and hepcidin-25 was quantified by competitive enzyme-linked immunoassay. Renal outcomes were acute kidney injury defined by risk, injury, failure, loss of function, end-stage renal disease (RIFLE) classification and need for renal replacement therapy. Variables with the potential to influence hepcidin expression were investigated.

Results: Low preoperative hepcidin concentration in urine (median, 15.3 ng/mL; 25-75 percentiles, 0-129.1) and plasma (median, 49.2 ng/mL; 25th-75th percentile, 0-52.2) predicted mortality (area under the curve–receiver operating characteristic [AUC-ROC] for urine hepcidin, 0.89; 95% confidence interval, 0.73-0.99; cutoff, 130 ng/mL; sensitivity, 73%; specificity, 100%; and AUC-ROC for plasma hepcidin, 0.90; 95% confidence interval, 0.80-0.99; cutoff, 55 ng/mL; sensitivity, 83%; specificity, 100%). Survivors had median preoperative hepcidin concentrations of 325.3 ng/mL (25th-75th percentile, 120-770.1 ng/mL) in urine and 113.1 ng/mL (25th-75th percentile, 77.7-203.1 ng/mL) in plasma. Preoperative serum creatinine did not predict mortality (AUC-ROC, 0.50; 95% confidence interval, 0.10-0.94). Furthermore, preoperative urine, plasma hepcidin, and serum creatinine did not distinguish patients requiring postoperative renal replacement therapy from those without (urine: AUC-ROC, 0.62; 95% confidence interval, 0.38-0.86; plasma: AUC-ROC, 0.63; 95% confidence interval, 0.34-0.91; serum creatinine: AUC-ROC, 0.61; 95% confidence interval, 0.22-0.99). Preoperative renal function and hemoglobin did not correlate with hepcidin indices whereas plasma markers of inflammation did.

Conclusions: Low preoperative hepcidin concentration might be a risk factor for in-hospital mortality. Findings should be validated in larger patient cohorts with a greater number of events. (J Thorac Cardiovasc Surg 2013;145:1380-6)

Cardiac surgery with cardiopulmonary bypass (CPB) is one of the most common major surgical procedures worldwide and improves quality of life and long-term survival.[1] However, CPB facilitates labile iron toxicity through hemolysis and generation of oxidoinflammation.[2,3] Iron is an essential trace element for molecules sensing, transporting, and storing oxygen, and for enzymes involved in the oxidation and reduction of substrates and the generation of reactive oxygen for host defense.[4]

Novel biomarkers involved in central iron homeostasis may prove useful in the early identification of patients at increased risk for postoperative adverse events. The peptide hormone hepcidin is the principal regulator of iron absorption and tissue distribution, with gene regulation being

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Abbreviations and Acronyms
AKI = acute kidney injury
CI = confidence interval
CKD = chronic kidney disease
CPB = cardiopulmonary bypass
CRP = C-reactive protein
eGFR = estimated glomerular filtration rate
IL = interleukin
RRT = renal replacement therapy

influenced by iron loading, hypoxia, or inflammation.[5-7] Hepcidin is a low-molecular weight peptide (2.78 kD); it passes through the glomerular membrane and is reabsorbed and degraded in the proximal tubules with only a small fraction (3-5%) of the filtered hepcidin passing intact into urine.[8] Therefore, hepcidin concentrations in biofluid may indicate disorders of iron metabolism and may be associated with prognosis. Recently, several work groups found early postoperative hepcidin to be useful in predicting protection from acute kidney injury (AKI) after CPB.[9-12] These studies provide further support for the role of labile iron-associated reactive oxygen species in the pathophysiology of AKI after the use of CPB in light of the demonstrated utility of other iron-associated protein biomarkers (eg, neutrophil gelatinase–associated lipocalin, liver-type fatty acid–binding protein, cystatin C) for the prediction of tubular damage.[2] The need for improved preoperative risk prediction, the central biologic role of hepcidin, and the importance of CPB in augmenting labile iron-induced organ toxicity suggest hepcidin may have value as a biomarker for risk stratification.

In this study, we aimed (1) to assess the predictive value of preoperative urine and plasma hepcidin concentration for in-hospital mortality, need for renal replacement therapy (RRT) initiation, and the development of AKI; and (2) to investigate modifiers of hepcidin, including preoperative chronic kidney disease (CKD), concentrations of hemoglobin, and markers of inflammation.

METHODS

Patient Population

For the purpose of the study, we took advantage of the control arm of a multicenter, randomized, controlled trial (N = 100) at its German study center (www.clinicaltrials.gov, NCT00672334). This study compared perioperative sodium bicarbonate versus placebo (sodium chloride 0.9%) for the prevention of AKI and the exploration of renal biomarkers in patients at increased renal risk undergoing cardiac surgery necessitating the use of CPB.

CKD was defined as a preoperative estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m^2.[13] eGFR was estimated using the CKD Epidemiology Collaboration equation[14] using serum creatinine values standardized to isotope dilution mass spectroscopy. RRT was initiated if the patient fulfilled at least 1 of the following clinical criteria: oliguria (urine output, <100 mL/>6 hours) unresponsive to fluid resuscitation measures, hyperkalemia (K > 6.5 mmol/L), severe acidosis (pH < 7.2), or clinically significant organ edema (eg, lung) in the setting of AKI.

Samples of ethylenediamine tetraacetic acid–plasma and urine were obtained simultaneously directly after insertion of an arterial line before induction of anesthesia. Aliquots of plasma and urine were stored frozen at –80°C immediately after collection and centrifugation, and were kept frozen on dry ice during transport. Patients were recruited between January 2009 and June 2010. The local institutional review board approved this study and written informed consent was obtained from each patient including the investigation of novel renal biomarkers. The study was carried out in compliance with the Helsinki Declaration.

Data Collection and Outcome Definition

Demographic and clinical data were collected at baseline and during the patient's stay in the intensive care unit. Serum creatinine was measured at baseline and daily within the first postoperative week and, if required, until hospital discharge. AKI was defined based on the baseline-to-peak serum creatinine increase (>50%) or urine output decrease (<0.5 mL/kg/hour for at least 6 hours) during the first 7 postoperative days using the risk, injury, failure, loss of function, end-stage renal disease (RIFLE) consensus definition.[15] The value of this AKI definition has been reported previously.[16,17] As mentioned, RRT was initiated if the patient fulfilled at least 1 of the following clinical criteria: oliguria (urine output, <100 mL/>6 hours) unresponsive to fluid resuscitation measures, hyperkalemia (K > 6.5 mmol/L), severe acidosis (pH < 7.2), or clinically significant organ edema (eg, lung) in the setting of renal failure. Mortality was defined as in-hospital death during the primary postoperative hospital stay. The Strengthening the Reporting of Observational Studies in Epidemiology recommendations[18] for reporting observational studies were used.

Biochemical Analysis

Co-investigators performing hepcidin assays (M.W., V.O.) were blinded to patient details and outcomes. Human urine and plasma hepcidin-25 isoforms were measured by competitive enzyme-linked immunoassay in duplicate, as described previously.[8] The lower limit of hepcidin detection was 5.5 ng/mL. The median coefficient of variation was <10% for intraassay precision and 6% for interassay reproducibility. Urine hepcidin adjusted to urine creatinine was calculated and expressed as nanograms hepcidin per milligrams creatinine. Serum creatinine assays were carried out using the modified Jaffé method. Fractional excretion of hepcidin (ie, the proportion of filtered hepcidin that appears in urine) was calculated by the following formula:

$$FE_{hepcidin} = \left(\left[U_{hepcidin}\right] \times \left[Pl_{creat}\right] / \left[Pl_{hepcidin}\right] \times \left[U_{creat}\right]\right) \times 100\%$$

where FE is the fractional excretion, $U_{hepcidin}$ is the urine hepcidin concentration, $Pl_{hepcidin}$ is the plasma hepcidin concentration, Pl_{creat} is the plasma creatinine concentration, and U_{creat} is the urine creatinine concentration.

In preoperative samples, we measured interleukin (IL) 6 in urine and plasma by means of enzyme-linked immunosorbent assay (IL-6; ECLIA, Roche Diagnostics, Mannheim, Germany) or immunoturbidimetry for C-reactive protein (CRP; ECLIA, Roche Diagnostics) in plasma. The coefficient of variation for each assay was <10%.

Statistics

Statistical analysis was performed using SPSS 16.0 (SPSS Inc, Chicago, Ill) and MedCalc 11.5 (Mariakerke, Belgium). Categorical data were reported as percentages with the 95% confidence interval (CI) of the mean percentage, and compared using the Fisher exact test. After testing for normal distribution, continuous data were reported as median with 25th to 75th percentiles, and nonparametric data were compared using the Mann-Whitney *U* test. We used nonparametric bivariate correlation and report Spearman correlation coefficients. The ability of hepcidin to predict in-hospital mortality, RRT, or AKI was assessed by plotting receiver operating characteristic

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