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Elevated free hemoglobin and decreased haptoglobin levels are associated with adverse clinical outcomes, unfavorable physiologic measures and altered inflammatory markers in pediatric cardiac surgery patients

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Abstract

Background—There are clinical, mechanistic and animal model data suggesting that free hemoglobin, heme and iron may contribute to infection, thrombosis, multi-organ failure and death in critically ill patients. These outcomes may be mitigated by hemoglobin binding proteins such as haptoglobin.

Methods—164 consecutively treated children undergoing surgery for congenital heart disease were evaluated for associations between free hemoglobin/haptoglobin and clinical outcomes, physiologic metrics and biomarkers of inflammation.

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Reprints will not be ordered.

Authors' contributions: All authors contributed to the generation of clinical data or the laboratory data, or provided care of the patients described, as well in the editing of the manuscript. JMC, NB and AP initially conceived the study, and NB wrote the first draft of the manuscript and performed the data analysis. KFH organized and collected the laboratory data. JMC collected and organized the clinical data. RPP and SLS contributed to the laboratory data generation and interpretation.

Conflict of interest statement: Dr. Blumberg has served as a recent consultant to Alexion, and in the distant past as a consultant to and research grant recipient from manufacturers of leukoreduction filters (Terumo, Fenwal), RBC rejuvenation solutions (Biomet/Citra Labs) and cell washing devices (Terumo BCT) (Haemonetics). Terumo provided a small proportion of the cell washing sets for patients in the washed arm of the study. Drs. Blumberg and Refaai have received fees for medical services to donors from CSL Behring. No other author has any financial or personal relationship with other people or organizations that could influence his/her work.

Results—Higher peri-operative free hemoglobins (and lower haptoglobins) were associated with mortality, nosocomial infection, thrombosis, hours of intubation and inotropes, increased IL-6, peak serum lactate levels and lower nadir mean arterial pressures. The median free hemoglobin in patients without infection (30; interquartile range 24–52 mg/dL) was lower than in those who became infected (39; IQR 33–88 mg/dL) ($p=0.0046$). The median mechanical ventilation requirements were 19 hours (IQR 7–72) in patients with higher levels of haptoglobin vs. 48 (IQR 18–144) hours in patients with lower levels ($p=0.0047$). Transfusion dose, bypass duration and complexity of surgery were all significantly correlated with hemoglobin levels and haptoglobin levels. Multivariate analyses demonstrated that these variables independently and significantly were associated with outcomes.

Conclusions—Elevated pre- and post-operative levels of free hemoglobin and decreased levels of haptoglobin were associated with adverse clinical outcomes, inflammation and unfavorable physiologic metrics. There were inverse relationships between free hemoglobin and haptoglobin. Transfusion, RACHS score, and duration of bypass were associated with increased free hemoglobin and decreased haptoglobin. Further investigation of the role of hemolysis and haptoglobin as potential mediators/markers of outcomes is warranted.

Keywords

hemoglobin; haptoglobin; congenital heart disease; cardiac surgery; cardiopulmonary bypass; transfusion; nosocomial infection; thrombosis; RBC storage

INTRODUCTION

It has been postulated that free hemoglobin and its metabolic byproducts, free heme and iron, mediate organ injury and adverse clinical outcomes.^{1–3} Gladwin and colleagues have provided evidence that free hemoglobin at low levels may be significantly more toxic than previously appreciated. Hod, Spitalnik and colleagues have suggested a name for these observations specifically related to blood transfusions: “the iron hypothesis.” Increased circulating cell-free hemoglobin, heme and iron, and decreased haptoglobin concentrations have been associated with morbidity and mortality in both animal models and patients.^{4,5} There are *in vitro* and animal model data linking increased free hemoglobin, heme and iron to inflammation,⁶ infection,⁷ platelet activation,^{8,9} vasculopathy¹⁰ and thrombosis.¹¹ Therapeutically, use of hemoglobin or heme binding proteins such as haptoglobin and hemopexin can mitigate these effects in animal models.^{12–14}

In a recent study of children undergoing cardiac surgery randomized to washed versus standard RBCs (RBC), we observed that washed transfusions mitigated some of the pro-inflammatory effects of transfusion, as measured by post-operative IL-6 and C-reactive protein levels.¹⁵ Furthermore, patients receiving washed RBCs stored for 21 days or less had favorable clinical outcomes compared with patients receiving unwashed, standard RBCs.¹⁶ In contrast, patients receiving washed RBCs stored 28 days or longer had higher levels of inflammatory mediators and poorer outcomes than patients receiving standard unwashed RBCs stored for that duration.¹⁶ Since saline washing is capable of both removing supernatant free hemoglobin (potentially a benefit) and increasing hemolysis of

fragile long-storage RBCs (potentially a detriment), these data suggested hemolysis as a potential contributing factor to account for the results observed.

These findings led us to hypothesize that the plasma concentration of cell-free hemoglobin, whether transfusion-related or transfusion-independent, might be a biomarker for poor clinical outcomes. The rationale was that free hemoglobin (and related mediators such as heme and iron) has been shown in animal models and *in vitro* studies to cause end-organ damage (particularly to the kidney) and increase mortality (reviewed in references 1 and 3). Furthermore, experiments of nature that lead to increased levels of chronic hemolysis, such as sickle cell anemia and paroxysmal nocturnal hemoglobinuria, provide evidence that low levels of hemolysis may be harmful, and contribute to inflammation, thrombosis, vasculopathy and impaired host defenses against infection.^{1,11} Animal model data demonstrate that administration of scavengers of free hemoglobin and heme (for example, haptoglobin and hemopexin), can mitigate the toxicity of these mediators of organ injury.^{12–14}

We used archived pre- and post-operative specimens from pediatric cardiac surgery patients to assess the relationship of free hemoglobin and haptoglobin levels with biomarkers of inflammation, key physiologic measures and clinical outcomes. Clinical outcomes evaluated included post-operative infection, thrombosis, duration of mechanical ventilation and inotropic/vasopressor support, and mortality. We also investigated the relationship between free hemoglobin and haptoglobin levels and the key physiologic metrics of post-operative peak serum lactate level and nadir mean arterial blood pressure.

MATERIALS AND METHODS

Subjects

This cohort of transfused pediatric patients up to 18 years of age treated at the University of Rochester Medical Center (URMC) for cardiac surgical repair/palliation with cardiopulmonary bypass (CPB) has been previously described.¹⁵ The protocol was approved by the URMC Research Subjects Review board and registered with ClinicalTrials.gov (NCT00693498). Subjects were enrolled at their pre-anesthesia visit, with properly witnessed and documented informed consent. Once enrolled, subjects were block-randomized preoperatively by computer algorithm according to age and the presence of cyanosis to the unwashed or washed transfusion strategy maintained throughout their hospital admission.

Surgical Techniques

Peri-operative management including anesthesia, CPB technique, surgical repair and post-operative management was performed per our current standard of care. The cardiac surgeons (GMA, FG) were blinded to study assignment to prevent any potential bias on the surgical or CPB management. Obvious differences in packaging and labeling of washed blood products prevented blinding of the perfusionists and anesthesiologists. The Terumo RX05 oxygenator (Terumo Cardiovascular Systems, Ann Arbor, MI) was used for subjects weighing ≤ 10 kg, and the Terumo RX15 oxygenator for subjects >10 kg. RBCs were used in CPB prime for

infants weighing <10kg. Fresh frozen plasma (FFP), platelets and/or cryoprecipitate were never included in CPB prime, nor given routinely in the operating room. All patients were managed intra-operatively on CPB with unfractionated heparin adjusted according to activated clotting times and reversed with protamine. All patients received epsilon aminocaproic acid as an anti-fibrinolytic. Conventional ultrafiltration was employed for all study subjects. All subjects undergoing deep hypothermic circulatory arrest and children 6 months of age received intravenous corticosteroids on CPB (solumedrol 0.5 mg/kg). Vasoactive medications were utilized to maintain hemodynamics during transition off CPB at the discretion of the cardiac surgical team.

PCICU Management

All patients remained intubated and sedated for transfer to the pediatric cardiac intensive care unit (PCICU). Comprehensive clinical and transfusion details were collected. Subjects were weaned from mechanical ventilation as their cardiopulmonary status allowed. Crystalloid and/or 5% albumin were infused in 10–20mL/kg boluses for clinical findings of hypovolemia and poor cardiac output as needed to maintain hemodynamic stability. Vasoactive medications were adjusted to maintain hemodynamics and end-organ perfusion.

Transfusion of RBC, platelet and hemostatic products were based upon standard URM PCICU practice that takes into account: 1) the type of cardiac defect and repair; 2) the overall hemodynamic stability of the patient; and 3) the presence/absence of active bleeding/MT output, in addition to the hemoglobin, platelet or coagulation profile. RBC are transfused in 10 mL/kg volumes at a rate at the discretion of the prescribing physician. Subjects received anticoagulation and anti-platelet drugs (i.e., aspirin, enoxaparin, warfarin) as appropriate for the type of cardiac defect and surgical palliation/repair. A separate manuscript describing in detail the association between transfusion and free hemoglobin and haptoglobin levels, including transfusion dose, storage duration and washing vs. no washing is planned. The relationship between transfusion, transfusion dose, storage duration, washing and clinical outcomes has been described in previous publications (references 15 and 16).

Blood Bank Blood Component Therapy Procedures

All blood products were prestorage leukoreduced with a PALL BPF High Efficiency Leukocyte removal filter that removes leukocyte, platelets (from the RBC units) and microaggregates from the RBC or platelet units. All platelets and RBC were irradiated using a CIS-US IBL 437 Blood Irradiator delivering 2500 centigray. The “age” (number of stored days) of RBC and platelet products prepared for each group was recorded. All blood products were ABO identical.

Washing procedures: A COBE (Gambro/Caridian/Terumo, Lakewood, CO) 2991 Blood Cell Processor separated and removed supernatant plasma proteins, preservative agents and microaggregates from the RBC or platelet unit by centrifugation using a previously published method.¹⁷ After separation and concentration of the RBCs, the supernatant was expressed into a waste bag and two additional washing cycles were repeated by adding 0.9% saline solution.

Clinical Outcome Measures

Subjects were monitored daily for clinical complications including requirement of mechanical ventilation or inotropic support, re-operation, hemodynamically significant bleeding or arrhythmias, seizures, sepsis,¹⁸ venous thrombosis¹⁹ and/or nosocomial infection as defined below adapted from the CDC surveillance definitions for specific types of infections.²⁰ All information was based upon clinical observations in the medical record.

Nosocomial pneumonia was diagnosed by chest radiograph demonstrating new or progressive infiltrate or consolidation, and at least two of the following: fever $>38^{\circ}\text{C}$, leukocytosis ($\text{WBC} > 20,000/\mu\text{l}$), decline in oxygenation, new onset of purulent sputum or change in sputum character, isolation of an etiologic agent from a tracheal aspirate or isolation of virus or detection of viral antigen in respiratory secretions, or organism cultured from blood, and institution of antimicrobial therapy.

Mediastinitis was diagnosed if the patient had a fever ($>38^{\circ}\text{C}$) and at least one of the following: sternum instability, purulent discharge from the mediastinal area, organisms cultured from blood or mediastinum, spontaneous dehiscence or surgical opening by surgeon for localized signs and symptoms of sternal pain, tenderness, fluctuance and/or erythema, and institution of antimicrobial therapy.

Urinary tract infection was defined by fever $>38^{\circ}\text{C}$ in a patient with an existing or history of an indwelling urinary catheter, and either a positive urine culture or urinalysis with leukocyte esterase or nitrites or pyuria or micro-organisms on gram stain and institution of antimicrobial therapy. Bacteremia was defined when a recognized pathogen was identified in one or more cultures and the organism is not related to an infection at another site, in the setting of fever $>38^{\circ}\text{C}$ and institution of antimicrobial therapy. Necrotizing enterocolitis was diagnosed if the infant has at least two of the following signs or symptoms with no other recognized cause: abdominal distention, persistent vomiting, microscopic or gross blood in stools and at least one radiographic abnormality (pneumoperitoneum, pneumatosis intestinalis, unchanging “rigid” loops of small bowel), and institution of antimicrobial therapy. If the patient was described as having an infection, based upon culture or other laboratory findings, as well as clinical findings, we considered that patient as having an infection. Suspicion of infection without confirmation and treatment was considered “no infection.”

Blood Sampling and Mediator Measurement

Citrated whole blood was collected in the operating room before initiation of cardiopulmonary bypass (CPB); after protamine was administered at the conclusion of CPB; 6 hours after CPB; and 12 hours after CPB. Samples were immediately centrifuged and the plasma was stored at -80°C for later assays.

IL-6 and CD40L (CD154)—Measurement of these levels were determined by a Luminex bead multiplex assay per manufacturer’s (Millipore Corporation, Billerica, MA) instructions. These (and CRP) were chosen because of extensive evidence that they are biomarkers for adverse clinical outcomes.

High-Sensitivity C-Reactive Protein—C-reactive protein (CRP) was measured before and after CPB and on postoperative days (POD) 1–3. CRP testing was performed at the UPMC Clinical Laboratories, analyzed by immunoturbidimetric assay (ADVIA 2400 Chemistry System; Bayer Healthcare, Tarrytown, NY).

Hemoglobin—Cell-free hemoglobin levels were measured using QuantiChrom assay kits (BioAssay Systems, Hayward, CA).

Haptoglobin—Haptoglobin measurement was performed using a quantitative ELISA assay per manufacturer's protocol (Genway Biotech, Inc., San Diego, CA). While haptoglobin was employed as a continuous variable, it is significantly lower in neonates and infants. Adult levels vs. lower levels were employed in a categorical analysis based upon previously published data.²¹

Statistical Analysis

All subjects were randomized to their transfusion group pre-operatively to allow for preparation of the RBC units for the operating room blood cooler prior to surgery. Mann-Whitney or Wilcoxon tests were used for continuous outcome data, as appropriate. The chi-square or Fisher's exact test were used for categorical data, as appropriate. Linear regression and non-parametric correlations (Spearman's) was performed to ascertain correlations between continuous variables. We did not adjust for multiple comparisons as all analyses are considered exploratory in nature. JMP 10.0 (SAS Institute, Cary, NC) was used for all statistical analyses. A p value < 0.05 was considered statistically significant, although not as a strict criteria for inference. Limited multi-variate logistic and linear regression was performed to assess the relative contributions of pre-operative variables (RACHS score, free hemoglobin, haptoglobin) and post-operative clinical/physiologic outcomes (survival, infection, thrombosis, duration of mechanical ventilation, peak serum lactate, nadir mean arterial pressure). Similar analyses were performed for intra-operative (duration of cardiopulmonary bypass, transfusion dose) and early post-operative variables (12 hour free hemoglobin; 12 hour haptoglobin) and outcomes.

RESULTS

Subjects

Characteristics of the patients in this study have been previously published (references 15 and 16). In brief, median age was 7 months (range: 2 days to 17 years) and median weight was 6.75 kg (2.2–106.8kg). Thirty-six (23%) were neonates (age <31 days), 39 (24.1%) had single ventricle physiology, 78 (48%) had cyanotic cardiac lesions, and 31 (19%) had diagnosed chromosomal abnormalities or syndromes.

Blood transfusions

Of 162 subjects that completed study procedures, 34 (21%) did not receive any blood products, 17 in each group. Patients received a median of about 3 RBC transfusions, with the majority (58%) receiving 2. Fewer than 5% of subjects received platelets or plasma transfusions.

Mortality

There were eight (4.9%) deaths overall, with a higher mortality rate in the unwashed (6 patients; 9.4%) than the washed arm (2 patients; 3.1%) and no deaths in the non-transfused group. This difference was not statistically significant ($p=0.15$). There was no relationship between duration of RBC storage (oldest, mean, median) and mortality (data not shown). However, patients who died had significantly higher plasma cell-free hemoglobin and lower haptoglobin concentrations at all time points; pre-operatively and immediately postoperatively, 6 and 12 hours post-operatively (Fig. 1 shows the 12 hour post-operative data). Many patients with very high free hemoglobin levels did not die so this correlation is relatively weak ($r^2 < 0.05$). Mortality was substantially but not significantly lower in patients with pre-operative haptoglobins that were in the adult normal range than in patients with lower levels of pre-operative haptoglobin (Table 1; first row).

Infection

As previously reported, there was no association between average or median duration of RBC storage and rate of post-operative infection.¹⁶ The post-operative infection rate was significantly higher in children receiving the oldest blood (third tertile: 25–38 days) compared to those receiving the freshest RBCs (first tertile: 7–15 days); (34% v. 7%; $p = 0.004$). The types of infection have been previously reported in detail.

The median (and mean—data not shown) free hemoglobin levels pre-operatively and at all post-operative time points (immediately, 6 and 12 hours post-operatively) were significantly higher in those who went on to develop a proven infection (Figure 2 displays the 12 hour data). Many patients with very high free hemoglobin levels did not develop infection so this correlation is relatively weak ($r^2 < 0.1$). Adult normal levels of pre-operative haptoglobin were associated with a substantially, but not significantly lower rate of infection than lower levels (Table 1, second row).

Thrombosis

The median (and mean—data not shown) free hemoglobin levels pre-operatively and at all post-operative time points (immediately, 6 and 12 hours post-operatively) were higher ($p=0.057$) in those who later developed a proven thrombosis (Figure 3 displays the 12 hour data). Many patients with very high free hemoglobin levels did not develop thrombosis so this correlation is relatively weak ($r^2 < 0.1$). Adult normal levels of pre-operative haptoglobin were associated with a substantially and statistically significantly lower incidence of thrombosis than lower levels of haptoglobin (Table 1; row 3).

Duration of mechanical ventilation

There was a statistically significant relationship (all $p < 0.01$ by linear regression) between free hemoglobin levels pre-operatively and at all post-operative time points (immediately, 6 and 12 hours post-operatively) and hours of intubation post-operatively. Many patients with high free hemoglobin levels did not have prolonged duration of intubation so this correlation is relatively weak ($r^2 < 0.1$). Adult normal levels of pre-operative haptoglobin were associated with a substantially and significantly shorter duration of intubation than lower levels of haptoglobin (Figure 4).

Duration of inotropes

There was a statistically significant relationship (all $p < 0.01$ by linear regression) between free hemoglobin levels pre-operatively and at all post-operative time points (immediately, 6 and 12 hours post-operatively) and hours of inotropes required post-operatively. Many patients with high free hemoglobin levels did not have prolonged duration of inotropes so this correlation is relatively weak ($r^2 < 0.1$). Adult normal levels of pre-operative haptoglobin were associated with substantially and significantly shorter duration of inotropes than lower levels of haptoglobin (Figure 5).

Day of surgery peak serum lactate and nadir mean arterial pressures

There was a statistically significant relationship (inverse for nadir mean arterial pressure) between pre-operative free hemoglobin levels and higher peak blood lactate and nadir mean arterial pressure (data not shown—see haptoglobin data below).

Haptoglobin levels as predictors of post-operative inflammatory biomarkers, free hemoglobin, number and storage duration of transfusions, peak day 1 blood lactate and nadir mean arterial pressure

Table 1 displays the relationships between pre-operative haptoglobin levels and median levels of biomarkers of inflammation, as well as the relationship between pre-operative haptoglobin levels and median post-operative levels of free hemoglobin. Adult normal pre-operative haptoglobins were associated with statistically significantly higher median levels of inflammatory mediators post-operatively, including IL-6 and CRP, but not sCD40L. Adult normal pre-operative haptoglobins also predicted lower median pre-operative and post-operative free hemoglobins at every time point.

Pre-operative adult normal haptoglobin levels, as compared with lower pre-operative haptoglobin levels, were associated with significantly lower day one peak blood lactate levels and higher nadir mean arterial pressures on day one (Supplementary figures 1 and 2) as well as on the first and second days post-operatively (data not shown). The median peak lactate level was 2.6 (interquartile range 1.9 to 3.3) in patients with adult normal levels vs. 3.8 (IQR 2.3 to 6.1) hours in patients with low levels ($p < 0.0001$ by Wilcoxon test). The median nadir mean arterial pressure was 55 (interquartile range 50 to 66) in patients with adult normal levels vs. 48 (IQR 41 to 56) hours in patients with low levels ($p = 0.0001$ by Wilcoxon test). Finally, lower pre-operative haptoglobins were associated with greater numbers of RBC transfusions, but not with the median age of RBCs transfused nor with the median oldest RBC transfused (last three rows of the Table).

The Spearman's correlation coefficients for the association between dose of transfusion and 12 hour free hemoglobin (+0.45) and 12 hour haptoglobin (−0.26) were significant at $p < 0.0001$ and $p < 0.001$ respectively. Many of the key independent variables (RACHS score, duration of bypass, transfusion dose, pre and post-operative free hemoglobin and haptoglobin) were significantly correlated with each other. Multi-variate analysis of the small number of key clinical outcomes (mortality, infection, thrombosis) was conducted employing 3–4 key variables to avoid overfitting of the data, given the small number of patients who experienced these severe outcomes. For example, Spearman's correlation

coefficient between RACHS score and 12 hour post-operative free hemoglobin was +0.52 ($p<0.0001$). This was also true for transfusion dose and 12 hour free hemoglobin ($r=+0.45$; $p<0.0001$). Duration of bypass, transfusion dose, RACHS score and pre and post-operative free hemoglobin and haptoglobin were all highly associated (data not shown).

The only pre-operative variable independently and significantly associated with survival was RACHS score ($p=0.002$). No pre-operative variable predicted infection. The only pre-operative variable that independently predicted thrombosis was pre-operative haptoglobin level ($p=0.00002$). The only pre-operative variable that independently predicted hours of intubation was RACHS score ($p=0.0037$). Hours of inotropes were independently associated with RACHS score ($p=0.007$) and pre-operative free hemoglobin ($p=0.07$). Peak post-surgery lactate levels were significantly and independently associated with RACHS score ($p<0.0001$) and pre-operative free hemoglobin ($p=0.03$). Nadir post-surgery mean arterial pressure was independently and significantly associated with RACHS score ($p=0.0001$), pre-operative free hemoglobin ($p=0.049$) and haptoglobin ($p=0.035$).

Intraoperative variables (dose of transfusion and duration of bypass) and early post-operative variables (immediate post-operative free hemoglobin and haptoglobin) were examined by multi-variable regressions. No variable independently and significantly was associated with survival. Only bypass duration was associated with infection ($p=0.02$), and none were associated with thrombosis. Hours of intubation and inotropes were independently and significantly associated with duration of bypass ($p=0.0002$; $p<0.0001$) and transfusion dose ($p=0.02$; 0.01). Peak serum lactate and nadir mean arterial pressure were independently and significantly associated with bypass duration ($p<0.0001$ for both), transfusion dose ($p=0.016$; $p<0.0001$) and post-operative free hemoglobin ($p=0.0078$; 0.039).

The relationships between clinical and physiologic outcomes, and pre-/post-operative free hemoglobin and haptoglobin were investigated in more homogeneous patient subgroups, including only neonates, infants, patients with cyanotic lesions, patients with single ventricle physiology and patients with intermediate (2,3) RACHE scores and identical trends in associations to those in the entire cohort (data not shown) were generally present in some but not all subgroups.

DISCUSSION

Our findings in the complex setting of critically ill pediatric cardiac surgery patients demonstrate that higher levels of free hemoglobin and lower levels of haptoglobin are associated with serious post-operative clinical complications (infection, thrombosis, death), immunomodulation and inflammation. An even more striking association was observed between higher free hemoglobin levels, lower haptoglobin levels and key physiologic parameters such as post-operative peak lactate levels and nadir mean arterial pressures. Karkouti and colleagues recently reported that transfusions are associated with increases in post-operative free hemoglobin after cardiac surgery.²² Our results mirror theirs. These findings are consonant with the “iron hypothesis” which proposes that free iron, heme and hemoglobin contribute to an increased risk of nosocomial infection and other adverse events after surgery, and in hemolytic diseases, with or without allogeneic transfusions.^{1–4,23–25}

Novel advantages of this dataset include prospective, blinded data collection from consecutively enrolled subjects in a randomized clinical trial, exclusive transfusion of RBC (no platelet, plasma or cryoprecipitate transfusions) in >95% of patients, and receipt of RBCs from two or fewer unique blood donors in the majority of patients. These analyses on archived specimens are hypothesis-generating, justifying further investigation, and cannot prove causality. Free hemoglobin, both pre-operatively and post-operatively is a potential mediator of organ injury. This is consonant with previous findings in patients and in animal models.^{1-4,23-25} Haptoglobin, a major physiologic regulator of free hemoglobin levels, exhibits an inverse relationship for all outcomes with free hemoglobin, as might be predicted. Lower haptoglobin levels are associated with poor outcomes, immunomodulation and unfavorable changes in physiologic measures.^{12,13,26,27} However, the explanation for the somewhat contradictory finding of higher levels of CRP and IL-6 in patients with normal adult levels of haptoglobin is unexpected, and requires confirmation in a larger dataset.

Children undergoing open heart surgery experience a progressively increasing risk of post-operative infection, thrombosis, increasing need for mechanical ventilation and inotropes, increasing day one through two peak blood lactate and decreased nadir mean arterial pressure as the levels of free hemoglobin rise and the levels of haptoglobin decrease. These associations are not fully explained by transfusion, nor by the duration of storage of transfused RBCs nor by severity of anatomic lesion, nor surgical complexity as measured by duration of cardiopulmonary bypass. Additional research is required to determine why some patients have higher levels of free hemoglobin even before surgery and transfusion, the mechanisms by which transfusions contribute to increased free hemoglobin, and whether modifications of transfusions (e.g., washing) and infusion of exogenous hemoglobin binding proteins such as haptoglobin might be beneficial.

These findings support the further study of free hemoglobin and haptoglobin levels (and other biomarkers such as free heme, non-transferrin bound iron, etc.) as biomarkers of prognosis and potential mediators of outcomes. Free hemoglobin and its metabolic products are potential targets for intervention with therapeutic agents (e.g., haptoglobin or hemopexin) which might mitigate the hypothesized deleterious effects of free hemoglobin.

The strengths of this study relate to a patient population that is uniquely suited to analysis of the relationship between free hemoglobin and post-operative complications. Pediatric cardiac surgery patients are particularly vulnerable to post-operative morbidity because of the extensive and technically difficult surgery, and the intensity of required post-operative treatment. Moreover, nearly all patients require RBC transfusion at predictable and similar time points in their perioperative course (i.e. prime for bypass, in the operating room, and/or on the first post-operative day). Importantly, the dose of RBC (in mL) per kilogram for these small patients is strikingly large, and the free hemoglobin levels in recipients are commensurately high. An additional strength of the current study is that our patient sample was recruited prospectively and consecutively as part of a randomized clinical trial.

Our patient population is also a limitation, since these small patients received massive weight-based irradiated RBC transfusion dosages far exceeding those received by the vast majority of transfused adult or pediatric patients, thus constraining the generalizability of

these findings. Additional investigation of the role of transfusion in post-operative changes in free hemoglobin, heme, iron and haptoglobin will help illuminate the mechanisms by which transfusions might contribute to adverse outcomes. It is possible that free hemoglobin and haptoglobin may be merely surrogate measures of severity of co-morbidities or deranged anatomy, rather than causal physiologic mediators. It is also of interest that pre-operative haptoglobin levels were not predictive of outcomes in the non-transfused patients, since transfusion is a known source of increased free hemoglobin and decreased haptoglobin post-operatively. In the absence of transfusion, pre-operative haptoglobin was not a predictor of outcome, unlike the significant association in transfused patients. This supports the hypothesis that free hemoglobin and haptoglobin may have a causal role in outcomes in some patients.

The degree of confounding contributed by severity of congenital cardiac lesion and difficulty of surgery to free hemoglobin/haptoglobin levels is not determinable from our data. Single ventricle anatomy or cyanotic lesions may be deleterious in part because they lead to increased hemolysis. Hemolysis is associated with poor outcomes in various critically ill patient populations. Assessing the contributions of type of lesion, underlying comorbidities, surgical variables, transfusion dose, etc. is beyond the scope of this study, and the power of the data to illuminate these important issues. This study is also limited by its observational design, which ultimately can only demonstrate associations, not causality. However, in the context of what is known from animal models, in vitro studies and previously published clinical data, our findings support the hypothesis that free hemoglobin (and likely heme and iron) may mediate adverse clinical outcomes, and that higher levels of haptoglobin (and hemopexin and related molecules) might play a role in mitigating these effects.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Free hemoglobin at 12 hours post-operatively is shown as a boxplot (median and 10th, 25th, 75th, 90th percentiles) for patients who died (and for whom samples were available) (n=4) versus patients who survived (n=154). Two patients had no samples available or died before 12 hours. The graphs on the right are histograms displaying the relative number of patients in each range of free hemoglobins. The median free hemoglobin in survivors (31; interquartile range 24–63) was less than in those who died (93; IQR 43–170) (p=0.036 by Wilcoxon test).

Figure 2.

Free hemoglobin at 12 hours post-operatively are shown as a boxplot (median and 10th, 25th, 75th, 90th percentiles) for patients who developed infections (n=27) versus patients who remained uninfected during their hospital stay (n=131). Two patients had no sample or died prior to 12 hours. The graphs on the right are histograms displaying the relative number of infected patients in that range of free hemoglobin levels. The median free hemoglobin in patients without infection (30; interquartile range 24–52) was lower than in those who became infected (39; IQR 33–88) (p=0.0046 by Wilcoxon test).

Figure 3.

Free hemoglobin at 12 hours post-operatively are shown for patients who developed thrombosis (n=15) versus patients who had no thrombosis during their hospital stay (n=143) as a boxplot (median and 10th, 25th, 75th, 90th percentiles). Two patients had no sample or died prior to 12 hours. The graphs on the right are histograms displaying the relative number of patients with thrombosis at the specified range of free hemoglobin. The median free hemoglobin in patients without thrombosis (31; interquartile range 24–50) was less than in those who developed a thrombosis. (60; IQR 29–125) (p=0.057 by Wilcoxon test).

Figure 4.

Hours of intubation are shown as a boxplot (median and 10th, 25th, 75th, 90th percentiles) in patients with low pre-operative haptoglobins (n=67) versus patients with adult normal levels (60–200 mg/dl) (n=91). Two patients were missing data on pre-operative haptoglobin. The graphs on the right are histograms displaying the relative number of patients in each range of haptoglobin levels. The median hours of intubation were 19 (interquartile range 7 to 72) in patients with adult normal levels vs. 48 (IQR 18 to 144) hours in patients with low levels (p=0.0047 by Wilcoxon test).

Figure 5.

Hours of inotropes are shown as a boxplot (median and 10th, 25th, 75th, 90th percentiles) in patients with low pre-operative haptoglobins (n=67) versus patients with adult normal levels (60–200 mg/dl) (n=91). Two patients were missing data on pre-operative haptoglobin. The graphs on the right are histograms displaying the relative number of patients in each range of haptoglobin levels. The median hours of inotropes were 48 (interquartile range 7 to 96) in patients with adult normal levels vs. 72 (IQR 24 to 168) hours in patients with low levels (p=0.01 by Wilcoxon test).

Table 1

Pre-Operative haptoglobin levels and clinical and immunologic outcomes

Clinical Outcome or Biomarker	Adult Normal Pre-Operative Haptoglobin (n=92)	Low Pre-Operative Haptoglobin (n=68)	p value
Survival	2.2%	5.9%	0.40 *
Infection	12%	22%	0.13 *
Thrombosis	5.4%	15%	0.057 *
Median Day 1 CRP (95% CI)	45 (43–60)	25 (30–49)	0.0055 †
Median 6 hour IL-6 level (units)	147 (166–377)	96 (88–334)	0.01 †
Median 6 hour sCD40L pg/ml	289 (406–1008)	255 (42–748)	0.30 †
Median Post-Operative IL-10 level (units)	327 (472–868)	611 (788–1250)	0.018 †
Median Pre-operative free hemoglobin (mg/dl)	23 (24–40)	31 (46–64)	0.0004 †
Median Post-operative free hemoglobin (mg/dl)	59 (46–87)	84 (95–143)	0.0001 †
Median 6 Hour free hemoglobin (mg/dl)	31 (34–53)	52 (63–85)	0.0001 †
Median 12 Hour free hemoglobin (mg/dl)	28 (32–54)	44 (58–84)	0.0002 †
Median RBC Dose (ml/kg)	80 (53–162 interquartile range)	45 (33–130 interquartile range)	0.003 †
Median Oldest RBC Transfused (days)	19 (19–23)	21 (19–23)	0.91 †
Median Age of RBCs Transfused (days)	18 (17–20)	16 (16–19)	0.40 †

Hours are hours post-operative

† Wilcoxon

* Fisher's Exact test (2-sided)