Advance Targeted Transfusion in Anemic Cardiac Surgical Patients for Kidney Protection

An Unblinded Randomized Pilot Clinical Trial

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ABSTRACT

Introduction: Acute kidney injury (AKI) is a serious complication of cardiac surgery, and preoperative anemia and perioperative erythrocyte transfusion are important risk factors. Prophylactic erythrocyte transfusion in anemic patients may, therefore, protect against AKI.

Methods: In this unblinded, parallel-group, randomized pilot trial, 60 anemic patients (hemoglobin 10–12 g/dL) undergoing cardiac surgery with cardiopulmonary bypass were randomized (1:1) to prophylactic transfusion (2 units of erythrocytes transfused 1 to 2 days before surgery (n = 29) or standard of care (transfusions as indicated; n = 31). Between-group differences in severity of perioperative anemia, transfusion, and AKI (more than 25% drop in estimated glomerular filtration rate) were measured. The relationships between transfusion, iron levels, and AKI were also measured.

Results: Perioperative anemia and erythrocyte transfusions were lower in the prophylactic transfusion group – median (25th, 75th percentiles) for nadir hemoglobin was 8.3 (7.9, 9.1) *versus* 7.6 (6.9, 8.2) g/dL ($P = 0.0008$) and for transfusion was 0 (0, 2) *versus* 2 (1, 4) units ($P = 0.0002$) – but between-group AKI rates were comparable (11 patients per group). In 35 patients with iron studies, perioperative transfusions were directly related to postoperative transferrin saturation (correlation coefficient 0.6; $P = 0.0002$), and high (more than 80%) transferrin saturation was associated with AKI (5/5 *vs.* 8/30; $P = 0.005$), implicating transfusion-related iron overload as a cause of AKI.

What We Already Know about This Topic

- Preoperative anemia and perioperative transfusion of erythrocytes have been demonstrated to increase the risk of acute kidney injury (AKI) after cardiac surgery
- The impact of preoperative transfusion on perioperative anemia and AKI is unknown

What This Article Tells Us That Is New

- Prophylactic erythrocyte transfusion decreased perioperative anemia and erythrocyte transfusions, and may reduce plasma iron levels
- High transferrin saturation was associated with AKI, and this finding may implicate perioperative transfusion-related iron overload as a cause of AKI

◇ This article is featured in "This Month in Anesthesiology." Please see this issue of ANESTHESIOLOGY, page 9A.

◆ This article is accompanied by an Editorial View. Please see: Vincent J-L, Lelubre C: Preoperative transfusions to limit the deleterious effects of blood transfusions. ANESTHESIOLOGY 2012; 116:513–4.

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Conclusions: In anemic patients, prophylactic erythrocyte transfusion reduces perioperative anemia and erythrocyte transfusions, and may reduce plasma iron levels. Adequately powered studies assessing the effect of this intervention on AKI are warranted.

ACUTE kidney injury (AKI) is a common and prognostically important complication of cardiac surgery.[1] It occurs, to various degrees of severity, in nearly one-third of cases that require the use of cardiopulmonary bypass (CPB),[2] and even when mild, is independently associated with markedly worse short- and long-term outcomes.[3–10] To mitigate this complication, numerous therapies have been tested but none have proven effective.[2,11] In the absence of effective therapies, risk factor modification may be one reasonable means for reducing AKI after cardiac surgery.[7]

Two important risk factors are preoperative anemia and perioperative erythrocyte transfusion.[7,12–16] In one study, moderate preoperative anemia (hemoglobin 10–12 g/dL) conferred a 60% increase and perioperative erythrocyte transfusions conferred an 8% per-unit increase in the odds of developing AKI.[7] In another study, the deleterious effects of perioperative transfusions were found to be more pronounced in patients with preexisting anemia than in those without anemia.[17] These two risk factors occur commonly and often in tandem: approximately one-third of patients undergoing nonemergent cardiac surgery are anemic before surgery,[12] and about 80% of them receive one or more erythrocyte transfusions.[12,18] Thus, interventions aimed at avoiding these risk factors may reduce the risk of AKI after cardiac surgery.

We postulated that prophylactically transfusing anemic patients with 2 units of erythrocytes 1 to 2 days before surgery would reduce the risk of AKI by reducing the severity of perioperative anemia and the need for erythrocyte transfusions. Moreover, it would allow time for the transfused erythrocytes to recover from the deleterious changes that they undergo during storage and the kidneys to recuperate from the harmful effects of the transfused erythrocytes before they are exposed to the myriad of surgical stressors that occur during cardiac surgery. Here we report the results of the pilot trial that was conducted to primarily assess the efficacy of this intervention in reducing the severity of perioperative anemia and the need for erythrocyte transfusions.

Materials and Methods

Ethics and Registration

This study was approved by the Research Ethics Board of the University Health Network (Toronto, Ontario, Canada), and written informed consent was obtained from all patients. The trial was registered at ClinicalTrials.gov (identifier: NCT00861822).

Trial Design and Participants

This was a single-center, unblinded, parallel-group study, with stratified randomization (according to baseline renal function) into intervention and standard-of-care arms (1:1).

Adult (older than 18 yr) patients who were undergoing cardiac surgery with CPB (coronary artery bypass grafting, valve repair or replacement, or both) and had a baseline hemoglobin concentration between 10–12 g/dL were eligible for inclusion in the study. Patients were not eligible if they were in congestive heart failure at assessment or had severe left ventricular dysfunction (ejection fraction less than 20%) to reduce the risk of transfusion-associated circulatory overload; if they had significant kidney disease (estimated glomerular filtration rate [eGFR] $<$30 ml/min) or liver disease (liver enzymes more than twofold higher than upper limit of normal); if they had received erythrocyte transfusions within 4 weeks of surgery, had donated blood for perioperative autologous transfusion, had adverse reactions to previous erythrocyte transfusions, or refused erythrocyte transfusions; if they had active bleeding or infection; or had undergone previous organ transplantation.

Intervention

Patients randomized to the intervention arm received two units of erythrocytes 1 to 2 days before surgery (same-day admit patients were transfused as outpatients in the medical day unit). Patients randomized to the standard-of-care arm received erythrocyte transfusions during or after surgery at the discretion of the clinical team, according to standard guidelines.[19] All other aspects of care were according to routine clinical management.

Clinical Practice

All patients received tranexamic acid (Pharmacia & UpJohn, Inc., Mississauga, Ontario, Canada) 30 mg/kg loading dose followed by a 15 $mg \cdot kg^{-1} \cdot h^{-1}$ infusion until chest closure. Management of CPB included intravenous heparin administration to achieve an activated clotting time greater than 480 s, systemic temperature drift to 32–34°C, α-stat pH management, targeted mean perfusion pressure between 50–70 mmHg, and pump flow rates of 2.0–2.5 $l \cdot min^{-1} \cdot m^{-2}$. Myocardial protection was achieved with intermittent antegrade and, occasionally, retrograde blood cardioplegia. During CPB, shed pericardial blood was salvaged into the cardiotomy suction reservoir and reinfused *via* the CPB circuit for as long as patients were anticoagulated. Patients generally received erythrocyte transfusions if their hemoglobin dropped below 7 to 8 g/dL. The CPB circuit was primed with 1 or 2 units of erythrocytes if standard calculations (based on weight and preoperative hemoglobin) showed that the hemoglobin would drop below 7 g/dL after hemodilution by CPB prime.

Outcomes

Outcomes aimed at our primary objective, which was to measure the efficacy and safety of the intervention, included:

change in hemoglobin concentration from before the intervention to before surgery, intraoperative nadir hemoglobin concentration (measured every 15 min during CPB and every 30 min during the remainder of surgery), incidence of profound intraoperative anemia (hemoglobin less than 7.0 g/dL), number of intraoperative erythrocyte transfusions (efficacy outcomes) and adverse events from initiation of prophylactic transfusions to initiation of surgery in the intervention arm, and total number of erythrocytes and other blood products transfused until hospital discharge in both arms (safety outcomes).

Other outcomes analyzed included measures of AKI, major adverse in-hospital postoperative events and estimated blood loss (using a previously described formula that incorporates change in hemoglobin, chest tube drainage, and amount of erythrocytes administered).[20] Creatinine values, measured before the intervention, before surgery, upon admission to the intensive care unit (ICU), and daily thereafter for 7 days, were used to calculate the patients' eGFR using the Cockcroft-Gault formula.[21] The maximum percent drop in eGFR (from preoperative to the lowest postoperative value up to postoperative day 7) was calculated, and patients with a more than 25% drop were classified as having had AKI (this corresponds to the "risk" category of the consensus-based RIFLE [Risk, Injury, Failure, Loss, and End stage kidney disease] classification criteria for AKI,[22] and has been shown to be prognostically important).[7]

To explore the relationship of iron status with intraoperative erythrocyte transfusion and AKI, plasma iron levels, transferrin concentration, and percent transferrin saturation were measured before the intervention, before surgery, and upon admission to the ICU. These measures were initiated after 25 patients had already been studied, and therefore are only available in the last 35 patients.

Sample Size

The sample size estimate was based on the expected efficacy of the intervention in reducing the need for erythrocyte transfusion during surgery from 80% to 36% (estimates based on the prestudy rates in anemic and nonanemic patients).[17] A sample size of 50 patients was deemed to be adequate to detect this effect size (power = 0.8; $\alpha = 0.05$). To allow for dropouts after randomization, the sample size was increased to 60 patients.

Randomization and Blinding

A restricted stratified randomization scheme was used for patient allocation. Stratification was by baseline kidney function (eGFR less than or equal to, or greater than, 60 ml/min). In each stratum, patients were randomized in randomly permuted blocks of four or six patients. The assignments were computer generated and maintained in sequentially numbered, opaque, sealed envelopes.

Research assistants enrolled patients, obtained consent, and revealed the group assignment at least 1 day before surgery. This was an unblinded study. Elective patients were assessed during their preadmission visit usually about 1 week before the scheduled surgery. Nonelective patients were assessed in the hospital usually 1 day before the scheduled surgery. Final eligibility was determined by remeasuring the hemoglobin before the intervention, and patients were excluded if their hemoglobin was greater than 12 g/dL.

Statistics

Patients who were excluded after randomization and did not receive the study intervention were not included in the analyses. For most of the analyses, all included patients were analyzed according to their randomization, irrespective of the timing of transfusions. For some of the analysis, two patients in the intervention arm who because of logistical problems did not complete their prophylactic transfusions until about 2 h before the start of their surgery were included with control patients (per-protocol analysis). For continuous outcomes, the Wilcoxon signed-rank test was used to assess the change in preoperative hemoglobin concentration in the intervention arm, and the Student *t* test or Mann–Whitney U test were used to assess between-group differences in other continuous outcomes. For categorical outcomes, Fisher exact test was used to assess between-group differences. In an exploratory per-protocol analysis, the association between the intervention and AKI was determined using multivariable logistic regression to adjust for important baseline differences.

In patients who had iron studies performed, the relationship between iron status and erythrocyte transfusion in the entire group was measured by the Spearman rank correlation coefficient. Based on evidence that transferrin saturation more than 80% is associated with the presence of significant amounts of free iron,[23] the Fisher exact test was used to assess the relationship between high (more than 80%) postoperative transferrin saturation levels and AKI (more than 25% drop in eGFR). Between-group differences in iron status were measured by the Mann–Whitney U test. Missing values were not imputed.

SAS™ version 9.1.3 (SAS Institute, Inc., Cary, NC) was used for the statistical analyses.

Results

From July 2009 to April 2011, 112 patients were screened and 72 were randomized, 12 of whom were excluded for various reasons (fig. 1). Of the 60 patients included in the analysis, 29 were assigned to the intervention arm and 31 to the control arm. Patient characteristics are shown in table 1. The groups had several important imbalances, most notably in sex and recent cardiac catheterization.

All of the patients in the intervention arm received 2 units of erythrocytes before surgery, and none of them suffered any adverse events from the time of transfusion until surgery. Except for two patients, all of the patients in the intervention arm completed their transfusions at least 1 day before surgery. In those two patients, because of logistical problems,

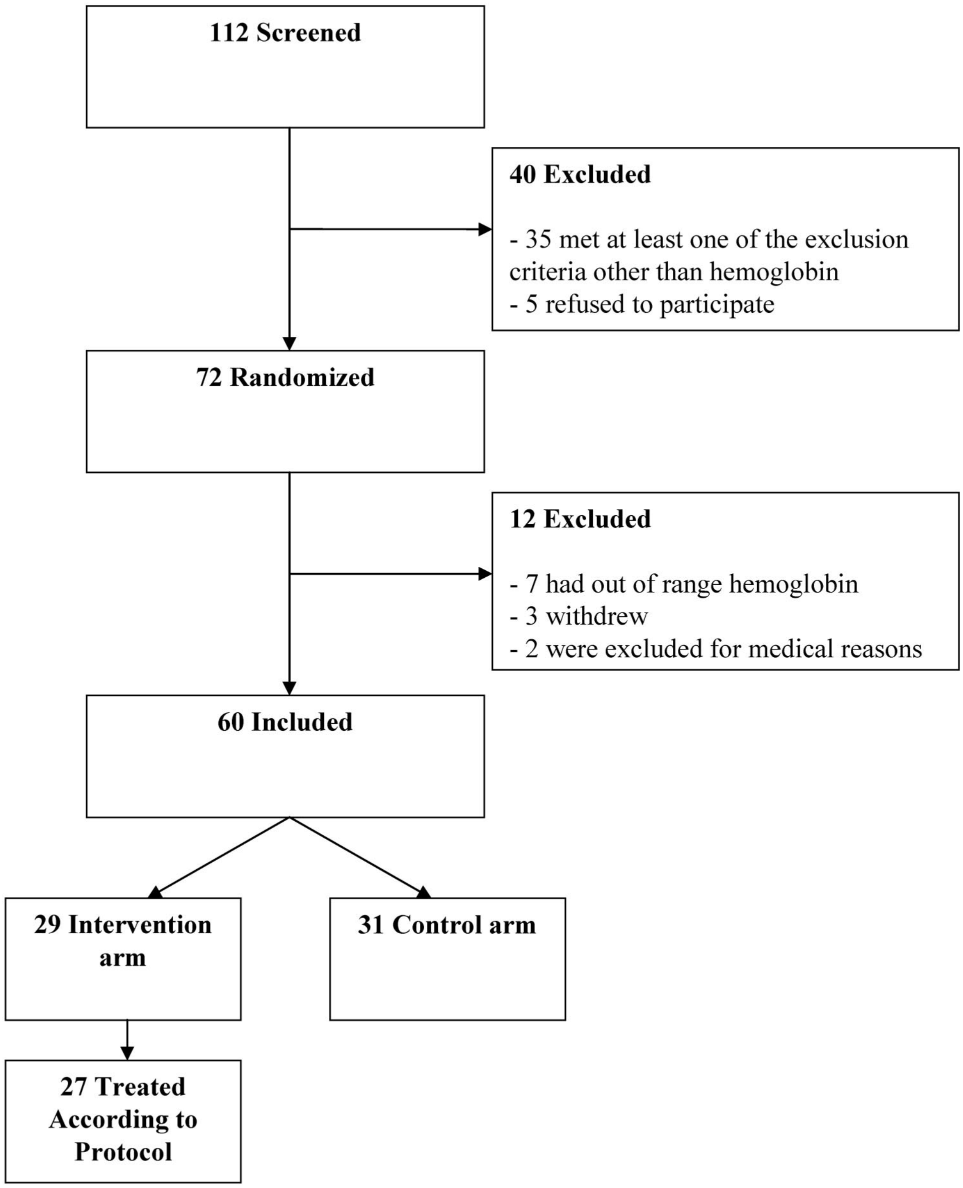

Fig. 1. Patient flow diagram.

the transfusions did not start until about 8 h before surgery and did not finish until about 2 h before start of surgery. In the intervention arm, the hemoglobin concentration increased from 11.1 ± 0.5 g/dL (mean ± SD) before the intervention to 12.7 ± 0.8 g/dL before surgery (paired *t* test $P < 0.0001$). As can be seen in table 2, patients in the intervention arm were less anemic during CPB (even though the protocol allowed for priming the CPB circuit with erythrocytes), and received substantially fewer intraoperative erythrocyte transfusions than the control arm. The two groups had similar overall exposure to blood products. In the control arm, except for one patient who received 1 unit of erythrocytes, all patients received at least 2 units of erythrocytes during their hospital stay.

Serious adverse clinical outcomes were comparable between the two arms: there were two deaths (one in each arm), two myocardial infarctions (one in each arm), 19 atrial fibrillations (12 control, seven intervention; $P = 0.3$), and nine infections (six control, three intervention; $P = 0.5$). The median (25th, 75th percentiles) maximum drop in eGFR was 18% (7%, 29%) in the control arm and 19% (10%, 33%) in the intervention arm ($P = 0.5$), and 11 patients in each group had AKI (37% overall incidence). One patient in each group required dialysis. In the exploratory per-protocol analysis (table 3), in which the two patients in whom prophylactic transfusions were not completed until just before surgery were analyzed as part of the control arm and baseline differences in gender and recent catheterization were controlled for by logistic regression (all variables forced in), the odds ratio for AKI in the intervention arm was 0.57 (95% CI 0.17–1.95; $P = 0.4$).

Iron studies were available in 35 patients and the results are shown in table 4 for the entire group and in table 5

Table 1. Patient Characteristics

Variables	Control (n = 31)	Treatment (n = 29)
Baseline variables	—	—
Age (years)	71 (62, 79)	73 (65, 75)
Female	71% (22)	28% (8)
Weight (kg)	70 (61, 80)	70 (63, 80)
Hypertension	77% (24)	76% (22)
Diabetes mellitus (type I or II)	35% (11)	41% (12)
History of stroke or transient ischemic attacks	3% (1)	17% (5)
Atrial fibrillation	16% (5)	21% (6)
Recent cardiac catheterization (within 3 days of surgery)	3% (1)	13% (4)
Estimated glomerular filtration rate (mL/min)	57 (47, 91)	61 (46, 76)
Hemoglobin concentration (g/dL)	10.8 (10.3, 11.7)	11.2 (10.9, 11.4)
Platelet count ($\times 10^9$/L)	206 (181, 262)	224 (168, 294)
International normalized ratio of prothrombin time	1.04 (0.99, 1.13)	1.05 (0.98, 1.09)
Operative variables	—	—
Procedure	—	—
Isolated CABG	29% (9)	31% (9)
Any valve replacement or repair	19% (6)	24% (7)
Combined procedures (CABG plus one or more valves)	52% (16)	45% (13)
Cardiopulmonary bypass duration (min)	99 (67, 132)	103 (81, 132)
Age of intraoperative transfused erythrocytes (days)	24 (20, 28)	26 (22, 29)

All data are presented as median (25th, 75th percentiles) or percentages (n).
CABG = coronary artery bypass grafting.

according to treatment group (per protocol). In the entire group (table 4), iron levels increased and transferrin levels decreased after surgery, leading to a marked increase in transferrin saturation levels. As can be seen in table 5, the increase in postoperative iron and transferrin saturation levels were more pronounced in the control arm. In the entire group, the ICU transferrin saturation was directly correlated with the number of erythrocyte transfusions from morning of surgery to ICU admission (Spearman correlation coefficient 0.6; $P = 0.0002$). The ICU transferrin saturation was more than 80% in five patients (three were in the control arm and two were those in the intervention arm who did not complete their prophylactic transfusions until just before surgery), all of whom developed AKI. In comparison, only 8 of the remaining 30 patients developed AKI ($P = 0.005$) (fig. 2).

Discussion

In this pilot study, we found that more than one-third of anemic patients undergoing cardiac surgery with CPB develop AKI. We also found that prophylactic transfusion of 2 units of erythrocytes at least 1 day before surgery safely re-

Table 2. Transfusion, Blood Loss, and Hemoglobin Outcomes

Variables	Control (n = 31)	Treatment (n = 29)	*P* Value
Erythrocyte transfusions (units)	—	—	—
Before surgery	0 (0, 0)	2 (2, 2)	<0.0001
During CPB	2 (0, 2)	0 (0, 0)	<0.0001
During surgery	2 (1, 4)	0 (0, 2)	0.0002
From surgery to discharge	4 (2, 5)	2 (1, 4)	0.01
Total	4 (2, 5)	4 (3, 6)	0.3
Plasma transfusions (units)	0 (0, 4)	2 (0, 4)	0.1
Platelet transfusions (units)	0 (0, 4)	0 (0, 4)	0.3
Estimated blood loss (mL)	850 (380, 1,430)	940 (447, 1947)	0.5
Re-exploration	0% (0)	10% (3)	0.1
Hemoglobin concentration (g/dL)	—	—	—
Before intervention	10.8 (10.3, 11.7)	11.2 (10.9, 11.4)	0.3
Before surgery	10.8 (10.3, 11.7)	12.6 (12.2, 13.3)	<0.0001
Lowest during CPB	7.6 (6.9, 8.2)	8.3 (7.9, 9.1)	0.0008
After CPB	8.4 (7.7, 9.2)	8.7 (7.9, 9.4)	0.6
ICU admission	9.4 (8.7, 10.2)	9.0 (8.5, 10.2)	0.8
Nadir hemoglobin <7 g/dL	29% (9)	3% (1)	0.01

All data are presented as median (25th, 75th percentiles) or percentages (n).
CPB = cardiopulmonary bypass; ICU = intensive care unit.

Table 3. Exploratory Multivariable Analysis Assessing the Effect of the Intervention on Acute Kidney Injury

Variables Included in the Model	(Standard Error)	(95% Confidence Interval)	*P* Value
Intervention*	−0.55 (0.62)	0.57 (0.17, 1.95)	0.4
Female sex	−0.49 (0.61)	0.61 (0.18, 2.02)	0.4
Recent cardiac catheterization (within 3 days of surgery)	1.10 (0.97)	3.01 (0.44, 20.3)	0.3

* Two patients in whom prophylactic transfusions were not completed until just before surgery were analyzed as part of the control arm.

duces the incidence of intraoperative erythrocyte transfusion and profound anemia without increasing overall transfusions. In addition, we found that perioperative erythrocyte transfusions are strongly correlated with transferrin saturation levels after surgery, and high (more than 80%) transferrin saturation levels are associated with AKI. Finally, in exploratory per-protocol analyses, we found an association between prophylactic erythrocyte transfusions and lower perioperative transferrin saturation levels and a trend toward lower AKI rates after surgery. These findings suggest that a large-scale randomized trial of prophylactic erythrocyte transfusion in anemic patients undergoing cardiac surgery is feasible and warranted.

AKI is thought to occur when a combination of several insults or stressors that induce renal hypoxia, inflammation, and oxidative stress occur in susceptible patients.[1,2,24–26] Cardiac surgery and CPB instigate multiple stressors including: reduced renal oxygen delivery because of hypotension, hemodilution, impaired renal blood flow, and embolism; systemic inflammatory response because of operative trauma, contact of the blood components with the artificial surface of the cardiopulmonary bypass circuit, ischemia-reperfusion injury, and endotoxemia; and oxidative stress because of the generation of free hemoglobin and iron from hemolysis that occurs during CPB.[1,2,7,25]

Several factors may explain why anemic patients seem to be a susceptible group for developing AKI after cardiac surgery. Preoperative anemia predisposes patients to severe intraoperative anemia,[12] which can cause tissue hypoxia. The

Table 4. Iron Studies, Both Groups Combined (n = 35)

Variables	Baseline	Admission to ICU	*P* Value
Iron (μM)	8 (5, 11)	16 (13, 21)	<0.0001
Transferrin (g/L)	2.4 (2.2, 2.8)	1.6 (1.4, 1.9)	<0.0001
Transferrin saturation (%)	14 (9, 17)	37 (28, 57)	<0.0001
Ferritin (μg/L)	89 (61, 224)	108 (74, 317)	0.2

All data are presented as median (25th, 75th percentiles).

ICU = Intensive care unit.

Table 5. Iron Studies in the Control and Intervention Arms (per Protocol)*

Variables	Control (n = 19)*	Treatment (n = 16)*	*P* Value
Baseline	—	—	—
Iron (μM)	9 (6, 11)	8 (5, 10)	0.5
Transferrin (g/L)	2.3 (2.2, 2.6)	2.5 (2.3, 2.9)	0.1
Transferrin saturation (%)	15 (10, 18)	12 (9, 16)	0.4
Ferritin (μg/L)	89 (72, 248)	81 (57, 203)	0.6
Admission to ICU	—	—	—
Iron (μM)	18 (14, 23)	15 (9, 19)	0.04
Transferrin (g/L)	1.6 (1.0, 1.8)	1.6 (1.4, 2.2)	0.3
Transferrin saturation (%)	45 (31, 89)	33 (22, 44)	0.04
Ferritin (μg/L)	108 (92, 286)	96 (74, 470)	0.7

All data are presented as median (25th, 75th percentiles).

* Two patients in whom prophylactic transfusions were not completed until just before surgery were analyzed as part of the control arm.

ICU = Intensive care unit.

kidneys are known to be highly vulnerable to hypoxic injury in the setting of reduced oxygen delivery because of both chronic and acute anemia.[27,28] In addition, despite having normal creatinine values, many anemic patients have subclinical kidney disease characterized by increased renal tubular oxygen consumption and oxidative stress,[29,30] predisposing them to acute-on-chronic kidney injury. Finally, anemic patients are at risk for oxidative stress and injury because of abnormal iron metabolism.[31,32]

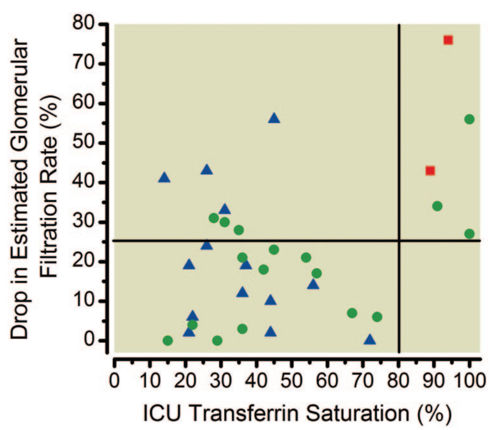

Fig. 2. Plot of serum transferrin saturation on admission to the intensive care unit against drop in renal function. Drop in renal function measured by change in estimated glomerular filtration rate from preoperative to lowest value during the first postoperative week. Two patients in the control arm had missing transferrin saturation values and are not included; they had 0% and 34% drops in estimated glomerular filtration rate. *Circles* represent patients in the control arm; *triangles* represent patients in the intervention arm treated according to protocol; *squares* represent the two patients in the intervention arm who did not complete their prophylactic transfusions until about 2 h before the start of their surgery. ICU = intensive care unit.

Transfused erythrocytes may cause kidney injury because of the many functional and structural changes that they undergo during storage, which collectively are referred to as the storage lesion.[33] These include depletion of adenosine triphosphate and 2,3-diphosphoglycerate, loss of ability to generate nitric oxide, increased adhesiveness to vascular endothelium, release of procoagulant phospholipids, and accumulation of proinflammatory molecules as well as free hemoglobin and iron.[33–37] In addition, erythrocytes undergo progressive structural alterations during storage that leads a substantial proportion (up to 30%) of them being quickly removed from the circulation by macrophages,[38] which may then release some of the scavenged hemoglobin-iron into the circulation unbound to transferrin.[39,40] As a result, stored erythrocytes may, at least for a few hours after they are transfused, paradoxically impair tissue oxygen delivery, stimulate the inflammatory cascade, and exacerbate tissue oxidative stress.[33–35,37] The physiologic derangements caused by transfusion of stored erythrocytes seem to be mostly resolved within 24–48 h after transfusion. The majority of damaged erythrocytes, for example, are removed from the circulation within an hour of transfusion,[38] and the resultant surge in nontransferrin-bound iron is resolved by 24 h after transfusion.[39] Moreover, erythrocytes that remain in circulation are quickly rejuvenated after transfusion: 2,3-DPG levels, for example, are 80% recovered by 24 h.[41]

Since, as noted above, the development of AKI likely requires the occurrence of a combination of stressors occurring together in susceptible patients, it seems reasonable to expect the proposed intervention – transfusion of 2 units of erythrocytes at least 24 h before surgery – to reduce the risk of AKI by allowing time for the transfused erythrocytes to recover their function before surgery, for the kidney to recuperate from the harmful effects of the transfused erythrocytes before they are exposed to the operative stressors, and by reducing the severity of anemia and the need for further erythrocyte transfusions during surgery. Although this pilot study did confirm the feasibility of the proposed intervention and its ability to reduce perioperative anemia and transfusions, it was not equipped to determine the ability of the intervention to protect against AKI. To make this determination, a much larger study is required. Specifically, based on the data garnered from this study, we estimate that such a study will require about 1000 patients (assuming a 40% incidence of AKI in the control arm and a 30% incidence in the intervention arm, and using a two-sided α level of 0.05 and power of 0.9).

In addition, the results of this pilot study help elucidate the pathogenesis of AKI. The observed relationships between perioperative erythrocyte transfusion, increased transferrin saturation levels, and AKI provide some support for the hypothesis that free or nontransferrin-bound plasma iron plays an important role in the pathogenesis of postcardiac surgery AKI in anemic patients, and that erythrocyte transfusions can be an important source of free iron.[39,40,42,43] Free-iron is a highly potent contributor to oxidative stress, and its concentration is normally tightly regulated to prevent oxidative organ injury.[44] Most of the body's iron is contained in the hemoglobin of erythrocytes, and it is the job of macrophages to phagocytose aged or damaged erythrocytes, extract and sequester the hemoglobin-iron, and then release it back into plasma, safely bound to transferrin.[44] Normally, transferrin is only about 30% saturated with iron, which provides for a considerable reserve in iron-binding capacity.[45] This reserve capacity, however, is largely exhausted when transferrin iron saturation reaches about 80%, beyond which free-iron is readily detectable in plasma.[23] This can occur if the ability of macrophages to sequester hemoglobin-iron is impaired, if macrophages are presented with an excessive amount of aged or damaged erythrocytes, or if the plasma transferrin concentration falls.[44] All of these events can occur in anemic patients undergoing cardiac surgery: 1) the ability of macrophages to sequester iron is promoted by the hormone hepcidin, which is inhibited in the setting of anemia[44]; 2) the conduct of CPB causes substantial damage to erythrocytes,[46] increasing the burden on macrophages, and as shown in this study and by others,[47,48] reduces transferrin concentrations; and 3) CPB-induced hemodilution often necessitates the transfusion of 1 or more units of erythrocytes during surgery, which, as noted above, acutely presents a large number of damaged erythrocytes to the macrophages. As we did not measure free-iron levels in this study, we could not directly determine if transfusion-related free iron toxicity is a cause of postcardiac surgery AKI in anemic patients. Our finding that transferrin saturation levels in anemic patients are increased after surgery in direct proportion to the number of erythrocytes transfused during surgery, and that high transferrin saturation levels after surgery are associated with AKI, however, does provide indirect support for this mechanism.

Our study had several important limitations. Most importantly, as noted above, this was a pilot study powered to detect the efficacy of the intervention in preventing perioperative anemia and transfusions, not clinical outcomes such as AKI. Moreover, we conducted multiple comparisons without adjusting the significance threshold, which increases the potential for a type I error. In addition, because of the study's small sample size, randomization failed to balance the groups for important confounders such as sex. Such imbalances threaten the validity of clinical inferences (*e.g.*, effect of the intervention on AKI), but there is no indication that they had a significant effect on our measures of efficacy. Other important limitations of the study were that it was an unblinded, pragmatic study with postrandomization dropouts and important protocol deviations (*i.e.*, delayed transfusions in the intervention arm). Proper blinding of the intervention, in our opinion, is not feasible. To minimize bias, future studies will need to minimize postrandomization dropouts, standardize perioperative transfusion practice, use objective outcomes and blinded assessors, and analyze their results by the intention-to-treat principle. Because of its limitations, it would be inappropriate to modify clinical practice based on the results of this pilot study.

In summary, this pilot study showed that in anemic cardiac surgical patients, prophylactic transfusion of 2 units of erythrocytes 1 to 2 days before surgery safely reduces perioperative anemia and erythrocyte transfusions, and may reduce plasma iron levels. Large multicenter trials adequately powered to determine if this intervention reduces postoperative AKI are warranted.

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