Advance Targeted Transfusion in Anemic Cardiac Surgical Patients for Kidney Protection
An Unblinded Randomized Pilot Clinical Trial

Keyvan Karkouti, M.D.,* Duminda N. Wijeysundera, Ph.D.,† Terrence M. Yau, M.D.,‡ Stuart A. McCluskey, Ph.D.,§ Christopher T. Chan, M.D.,¶ Pui-Yuen Wong, Ph.D.,|| Mark A. Crowther, M.D.,** Siroos Hozhabri, M.D.,†† W. Scott Beattie, Ph.D.‡‡

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

* Associate Professor, Department of Anesthesia and Institute of Health Policy, Management, and Evaluation, University of Toronto, Toronto, Ontario, Canada, and Scientist, Toronto General Research Institute, University Health Network, Toronto, Ontario, Canada. † Assistant Professor, Department of Anesthesia and Institute of Health Policy, Management, and Evaluation, University of Toronto, and Keenan Research Centre, Li Ka Shing Knowledge Institute of St. Michael’s Hospital, Toronto, Ontario, Canada. ‡ Professor, Toronto General Research Institute, University Health Network, and Division of Cardiovascular Surgery, Department of Surgery, Peter Munk Cardiac Centre, University of Toronto, Toronto, Ontario, Canada. § Assistant Professor, †† Research Coordinator, ‡‡ Professor and R. Fraser Elliott Chair in Cardiac Anesthesia, Department of Anesthesia, Toronto General Hospital, University Health Network, University of Toronto. # Associate Professor, Division of Nephrology, Department of Medicine, University Health Network, University of Toronto. † Professor, Department of Laboratory Medicine and Pathobiology, University Health Network, University of Toronto. ** Professor, Department of Medicine, Division of Hematology, McMaster University, Hamilton, Ontario, Canada.

Received from the Department of Anesthesia, Toronto General Hospital, University Health Network, University of Toronto, Ontario, Canada. Submitted for publication June 3, 2011. Accepted for publication November 1, 2011. Supported by the Heart and Stroke Foundation (Ontario, Canada). Drs. Karkouti, Wijeysundera, and Beattie are supported in part by merit awards from the Department of Anesthesia, University of Toronto (Toronto, Ontario, Canada). Dr. Wijeysundera is also supported by a Clinician-Scientist Award from the Canadian Institutes for Health Research, Canada. Dr Crowther holds a Career Investigator Award from the Heart and Stroke Foundation of Canada.

Address correspondence to Dr. Karkouti: Department of Anesthesia, Toronto General Hospital, 200 Elizabeth Street, EN 3-402, Toronto, Ontario, Canada M5G 2C4. keyvan.karkouti@uhn.ca. This article may be accessed for personal use at no charge on the Journal Web site, www.anesthesiology.org.

Copyright © 2012, the American Society of Anesthesiologists, Inc. Lippincott Williams & Wilkins. Anesthesiology 2012; 116:613-21

Anesthesiology, V 116 • No 3

Copyright © by the American Society of Anesthesiologists. Unauthorized reproduction of this article is prohibited.
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. It occurs, to various degrees of severity, in nearly one-third of cases that require the use of cardiopulmonary bypass (CPB), and even when mild, is independently associated with markedly worse short- and long-term outcomes. To mitigate this complication, numerous therapies have been tested but none have proven effective. In the absence of effective therapies, risk factor modification may be one reasonable means for reducing AKI after cardiac surgery.

Two important risk factors are preoperative anemia and perioperative erythrocyte transfusion. 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. 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. These two risk factors occur commonly and often in tandem: approximately one-third of patients undergoing nonemergent cardiac surgery are anemic before surgery, and about 80% of them receive one or more erythrocyte transfusions. 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. All other aspects of care were according to routine clinical management.

Clinical Practice

All patients received tranexamic acid (Pharmacia & Upjohn, Mississauga, Ontario, Canada) 30 mg/kg loading dose followed by a 15 mg kg⁻¹ 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 min⁻¹ 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 inter-
vention to before surgery, intraoperative nadir hemoglobin
concentration (measured every 15 min during CPB and ev-
ery 30 min during the remainder of surgery), incidence of
profound intraoperative anemia (hemoglobin less than 7.0
g/dL), number of intraoperative erythrocyte transfusions (ef-
cicacy outcomes) and adverse events from initiation of pro-
phylactic transfusions to initiation of surgery in the interven-
tion 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, ma-
ajor adverse in-hospital postoperative events and estimated
blood loss (using a previously described formula that incor-
porates change in hemoglobin, chest tube drainage, and
amount of erythrocytes administered).20 Creatinine values,
measured before the intervention, before surgery, upon ad-
mission 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
classification criteria for AKI,22 and has been shown to
be prognostically important].7

To explore the relationship of iron status with intraoper-
ative 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 pa-
tients).17 A sample size of 50 patients was deemed to be
adequate to detect this effect size (power = 0.8; α = 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 func-
tion (eGFR less than or equal to, or greater than, 60 ml/min).
In each stratum, patients were randomized in randomly per-
muted blocks of four or six patients. The assignments were
computer generated and maintained in sequentially num-
ered, opaque, sealed envelopes.

Research assistants enrolled patients, obtained consent,
and revealed the group assignment at least 1 day before sur-
gery. This was an unblinded study. Elective patients were
assessed during their preadmission visit usually about 1 week
before the scheduled surgery. Nonelective patients were as-
sessed in the hospital usually 1 day before the scheduled
surgery. Final eligibility was determined by remeasuring the
hemoglobin before the intervention, and patients were ex-
cluded 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 anal-
yses. 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 explor-
atory per-protocol analysis, the association between the inter-
vention and AKI was determined using multivariable logistic
regression to adjust for important baseline differences.

In patients who had iron studies performed, the relation-
ship between iron status and erythrocyte transfusion in the
total 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%) postopera-
tive 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 sur-
gery. In those two patients, because of logistical problems,
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...
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 1. Patient Characteristics

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

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

### Table 2. Transfusion, Blood Loss, and Hemoglobin Outcomes

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

All data are presented as median (25th, 75th percentiles) or percentages (n).
CPB = cardiopulmonary bypass; ICU = intensive care unit.
Transferrin (g/L) 2.4 (2.2, 2.8) 1.6 (1.4, 1.9)
Iron (g/L) 15 (10, 18) 12 (9, 16)
Transferrin saturation (%) 74 (57, 203) 78 (61, 177)
Transferrin (g/L) 1.6 (1.0, 1.8) 1.6 (1.4, 2.2)
Transferrin saturation (%) 45 (31, 89) 33 (22, 44)
Ferritin (µg/L) 108 (92, 286) 96 (74, 470)

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.

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

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

<table>
<thead>
<tr>
<th>Variables</th>
<th>Baseline</th>
<th>Admission to ICU</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron (µM)</td>
<td>9 (6, 11)</td>
<td>8 (5, 10)</td>
<td>0.5</td>
</tr>
<tr>
<td>Transferrin (g/L)</td>
<td>2.3 (2.2, 2.6)</td>
<td>2.5 (2.3, 2.9)</td>
<td>0.1</td>
</tr>
<tr>
<td>Transferrin saturation (%)</td>
<td>15 (10, 18)</td>
<td>12 (9, 16)</td>
<td>0.4</td>
</tr>
<tr>
<td>Ferritin (µg/L)</td>
<td>89 (72, 248)</td>
<td>81 (57, 203)</td>
<td>0.6</td>
</tr>
</tbody>
</table>

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

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

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

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

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control (n = 19)*</th>
<th>Treatment (n = 16)*</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Iron (µM)</td>
<td>9 (6, 11)</td>
<td>8 (5, 10)</td>
<td>0.5</td>
</tr>
<tr>
<td>Transferrin (g/L)</td>
<td>2.3 (2.2, 2.6)</td>
<td>2.5 (2.3, 2.9)</td>
<td>0.1</td>
</tr>
<tr>
<td>Transferrin saturation (%)</td>
<td>15 (10, 18)</td>
<td>12 (9, 16)</td>
<td>0.4</td>
</tr>
<tr>
<td>Ferritin (µg/L)</td>
<td>89 (72, 248)</td>
<td>81 (57, 203)</td>
<td>0.6</td>
</tr>
</tbody>
</table>

* 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.

**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 to 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.35 This reserve capacity, however, is largely exhausted when transferrin iron saturation reaches about 80%, beyond which free-iron is readily detectable in plasma.35 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.

References

33. van de Watering L: Red cell storage and prognosis. Vox Sang 2011; 100:36–45