Iron Deficiency in Blood Donors

A US perspective based on the NHLBI sponsored REDS-II RISE study

presented by Dr. Steven Kleinman
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Acknowledgements

- This lecture is based on work previously presented by the RISE principal investigators and study team members.
- It also includes perspectives from a November 2011 FDA public workshop: “Hemoglobin standards and maintaining adequate iron stores in blood donors.”
- Finally, I have added some personal perspectives.
Background information
## Donation eligibility standards vary internationally

<table>
<thead>
<tr>
<th>COUNTRY</th>
<th>HB (g/dL)</th>
<th>Interval (weeks) / (annual freq)</th>
</tr>
</thead>
<tbody>
<tr>
<td>USA</td>
<td>12.5</td>
<td>8 weeks</td>
</tr>
<tr>
<td>Canada</td>
<td>12.5</td>
<td>8 weeks</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>12.5 F, 13.5 M</td>
<td>16 weeks</td>
</tr>
<tr>
<td>Germany</td>
<td>12.5 F, 13.5 M</td>
<td>8 weeks/(4F, 6M)</td>
</tr>
<tr>
<td>Switzerland</td>
<td>12.5</td>
<td>(3F, 4M)</td>
</tr>
<tr>
<td>Australia</td>
<td>12.0 F, 13.0 M</td>
<td>12 weeks</td>
</tr>
<tr>
<td>Hong Kong</td>
<td>11.5 F, 13.0 M</td>
<td>(3F, 4M)</td>
</tr>
</tbody>
</table>

*Karp & King, Transfusion 2010;50:507-13.*
Iron loss during blood donation

- Male stores
- Female stores
- Iron lost in one donation

Iron lost in one donation:

- Male stores: 1000 mg Iron
- Female stores: 300 mg Iron
- Iron lost in one donation: 100 mg Iron
Daily dietary iron absorption

![Bar chart showing daily dietary iron absorption for Males, Females, HFE, Maximum, and Superdonor categories.]
Iron depletion as a consequence of blood donation is long-established

- Ferritin <12 ng/mL:
  - 8% in male donors
  - 16% - 25% in female donors depending on menstrual status
- Lifetime donations (cumulative iron loss) and frequency of donation predictors of iron status
- Hemoglobin level not associated with lifetime donations

Summary of knowledge concerning iron deficiency

- Frequent donors have a high frequency of iron deficiency
- Ferritin measurement is the best way to test for iron deficiency
- Hemoglobin is a poor measure of iron deficiency, so relying on donor deferral to protect against the consequences of iron deficiency is an ineffective strategy

- What are the detrimental consequences of iron deficiency in the absence of anemia?
  - Should the answer to this question determine the need for action?
RISE data
REDS-II Donor Iron Status Evaluation study (RISE)

- Part of the Retrovirus Epidemiology and Donor Study – II
  - NHLBI sponsored study of blood safety and availability conducted from 2004-2011 at 6 US blood centers (collecting 8% of the US blood supply), a central lab, and a coordinating center

- RISE was a longitudinal study designed to evaluate the effects of blood donation intensity on iron and hemoglobin status.
  - Enrollment and follow-up visits from Dec 2007 – Dec 2009
  - Iron status and Hb deferral were characterized as function of donation intensity, interval since last red cell donation, demographic factors, HFE polymorphisms, and behavioral characteristics
RISE study design

- Two cohorts of blood donors were recruited and enrolled
  - A first time and reactivated donor cohort, no whole blood/red cell donations in the previous two years - 888 donors enrolled
  - A frequent donor cohort, with $\geq 2$ (F) or $\geq 3$ (M) donations in the past year - 1537 donors enrolled
  - 2425 total donors enrolled; included a small % with double RBCs
  - These cohorts were not meant to be representative of the REDS-II donor population

- Enrolled donors agreed to donate frequently for the 15-24 month study period.
  - All made a successful donation as a condition of enrollment and agreed to donate frequently during the next 2 years

- Analyses included rates and predictors of iron status and of Hb deferral and also the impact of changes in eligibility/deferral criteria on blood availability
RISE baseline enrollment data collection

- Hb (venous and fingerstick)
- Iron status markers
- Iron protein (HFE, transferrin) polymorphisms
- Recent donation history and demographics from blood center records
- Donor Questionnaire Data:
  - Donation History: lifetime; past 2 years
  - Smoking: lifetime, recent
  - Dietary iron consumption
  - Use of multivitamin/multiminerals and iron supplements
  - Aspirin use
  - Menstrual status/nature of periods; pregnancy history
Detection of iron deficiency in blood donors

- Ferritin
  - Measures storage (RE system) iron
  - Acute phase reactant, however inflammatory conditions should be rare in blood donors
- Soluble transferrin receptor (sTfR)
  - Measures functional iron: sTfR is released from RBC surface when there is reduced erythrocyte iron
- Log (sTfR/ferritin)
  - A derived variable that has been shown to be the most sensitive measure of iron deficient erythropoiesis (IDE)
- Low hemoglobin (anemia): A late manifestation of IDE
RISE Definitions: Absent Iron Stores (AIS) and Iron Deficient Erythropoiesis (IDE)

- Absent iron stores (**AIS**) defined as plasma ferritin < 12 ng/mL (ug/L)
  - This is a very specific finding, correlating in other studies with absent bone marrow iron stores

- Iron deficient erythropoiesis (**IDE**) defined as log(sTfR/ferritin) above the 97.5\textsuperscript{th} percentile for first time/reactivated male donors in RISE
  - These donors should have a negligible risk of IDE
  - This value was determined as log (sTfR/ferritin) ≥ 2.07
  - In subsequent analyses, ferritin < 26 ng/mL was found to have comparable sensitivity and specificity
Effect of previous 12 month RBC donation frequency on plasma ferritin at enrollment
Effect of previous 12 month RBC donation frequency on donor HB at enrollment

![Graph showing the effect of previous 12 month RBC donation frequency on donor HB at enrollment.]
## Donors with Absent Iron Stores (AIS) and/or Iron Deficient Erythropoiesis (IDE) at enrollment

<table>
<thead>
<tr>
<th>Gender</th>
<th>Donor Status</th>
<th>AIS % ferritin &lt;12 µg/L</th>
<th>IDE % Log (sTfR/F) ≥ 2.07</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females FT/RA (n= 481)</td>
<td>6.5</td>
<td>24.6</td>
<td></td>
</tr>
<tr>
<td>Fqnt (n= 769)</td>
<td>27.0</td>
<td>66.1</td>
<td></td>
</tr>
<tr>
<td>Males FT/RA (n= 407)</td>
<td>0</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>Fqnt (n= 768)</td>
<td>16.4</td>
<td>48.7</td>
<td></td>
</tr>
</tbody>
</table>
# Adjusted ORs for AIS and IDE at enrollment by 2 year red cell donation frequency

<table>
<thead>
<tr>
<th>Number of RBC units in 24 months prior to enrollment</th>
<th>AIS Ferritin&lt;12 Adjusted ORs (95% CI)</th>
<th>IDE Log (R/F) ≥ 2.07 Adjusted ORs (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>p value</em></td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FT: 0 donations</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>RA: 0 Donations</td>
<td>0.5 (0.2-1.2)</td>
<td>1.5 (0.9-2.4)</td>
</tr>
<tr>
<td>R: ≤ 4 donations</td>
<td>5.3 (2.8-10.1)</td>
<td>14.0 (8.6-22.7)</td>
</tr>
<tr>
<td>R: 5-6 donations</td>
<td>12.5 (6.4-24.6)</td>
<td>24.0 (14.3-40.5)</td>
</tr>
<tr>
<td>R: 7-9 donations</td>
<td>13.5 (6.8-26.6)</td>
<td>32.3 (19.2-54.5)</td>
</tr>
<tr>
<td>R: 10+ donations</td>
<td>18.9 (9.0-39.6)</td>
<td>50.5 (28.4-89.9)</td>
</tr>
</tbody>
</table>

*Model includes previous RBC donations, gender, age, blood center, weight, smoking, iron supplementation, HFE polymorphisms, menstrual status, pregnancy history*
### Adjusted ORs for AIS and IDE at enrollment by age

<table>
<thead>
<tr>
<th>Age (in yrs)</th>
<th>AIS Ferritin&lt;12 Adjusted* ORs (95% CI)</th>
<th>IDE Log (R/F) ≥ 2.07 Adjusted* ORs (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td><strong>p value</strong></td>
<td>0.31</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>&lt;20</td>
<td>0.4 (0.0-3.3)</td>
<td>3.1 (1.0-9.6)</td>
</tr>
<tr>
<td>20-29</td>
<td>1.8 (0.8-4.2)</td>
<td>3.9 (2.0-7.6)</td>
</tr>
<tr>
<td>30-39</td>
<td>1.5 (0.7-3.2)</td>
<td>1.6 (0.9-3.0)</td>
</tr>
<tr>
<td>40-49</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>50-59</td>
<td>1.6 (0.9-2.9)</td>
<td>1.0 (0.6-1.7)</td>
</tr>
<tr>
<td>60+</td>
<td>0.9 (0.5-1.9)</td>
<td>0.7 (0.3-1.3)</td>
</tr>
</tbody>
</table>

Model includes previous RBC donations, gender, age, blood center, weight, smoking, iron supplementation, HFE polymorphisms, menstrual status, pregnancy history.
RISE longitudinal study design

- Full evaluation of venous HB and iron status at enrollment and at the end of study (final visit)
- Less extensive data collection at interim visits due to budget and logistic constraints
  - Interim visit occurred whenever the donor returned to donate
  - Hb deferral status tracked on all these visits
  - Venous Hb obtained on most of these visits (e.g., when a sample was collected)
  - Additional iron measures on visits from selected donors:
    - All first time/reactivated donors
    - All donors with hemoglobin deferrals
    - Selected female repeat donors at high risk of IDE at 3 centers
RISE longitudinal study accrual

- 12,695 total valid visits from 2,425 donors
- 2,425 enrollment visits
- 9,901 subsequent visits
  - 8,567 interim visits
  - 1,334 final visits (55% of enrollees)
  - 945 of these 9,901 visits (9.5%) had Hb deferral; (84% of these in females)
  - 369 additional returns excluded (e.g., deferral for other reasons)
- 6,449 visits (51%) with iron testing
  - All enrollment and final visits and 30% of interim visits
RISE longitudinal data –
Frequency of return by cohort

<table>
<thead>
<tr>
<th></th>
<th>FT/RA Females</th>
<th>FT/RA Males</th>
<th>Frequent Females</th>
<th>Frequent Males</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enrolled at Baseline</td>
<td>481</td>
<td>407</td>
<td>769</td>
<td>768</td>
</tr>
<tr>
<td>Donors w/one or more follow-up visits*</td>
<td>379 (79%)</td>
<td>288 (71%)</td>
<td>739 (96%)</td>
<td>749 (98%)</td>
</tr>
<tr>
<td>Mean return RBC donations per donor</td>
<td>2.6</td>
<td>2.9</td>
<td>4.4</td>
<td>5.2</td>
</tr>
<tr>
<td>Mean time in study</td>
<td>61 wks</td>
<td>63 wks</td>
<td>74 wks</td>
<td>75 wks</td>
</tr>
<tr>
<td>Annualized donations by returning donors</td>
<td>2.2</td>
<td>2.4</td>
<td>3.1</td>
<td>3.6</td>
</tr>
</tbody>
</table>

* 55% of all donors completed the final visit
Statistical Methods - Modeling

- Repeated measures logistic regression models were developed to predict AIS and IDE using data from all visits
  - These models account for multiple measurements on the same donor
- Similar models were developed to predict hemoglobin deferral at any return visit
  - Enrollment visits not included in deferral model since only accepted donors were enrolled
Predictors of AIS and IDE at any visit

<table>
<thead>
<tr>
<th>Significant Variables</th>
<th>Minimally or NS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female gender and younger age in women*</td>
<td>Race*</td>
</tr>
<tr>
<td>Time since last RBC donation*</td>
<td>Weight</td>
</tr>
<tr>
<td>Number of RBC units in previous 24 months</td>
<td>Menstrual status</td>
</tr>
<tr>
<td>Smoking (protective)</td>
<td>Previous donation type</td>
</tr>
<tr>
<td>Taking Iron (protective)</td>
<td>(WB vs double red cell)</td>
</tr>
<tr>
<td>Blood Center*</td>
<td>HFE status</td>
</tr>
</tbody>
</table>

* These items were also predictors for Hb deferral
### Significant variables predicting AIS: all visits

<table>
<thead>
<tr>
<th></th>
<th>Adjusted Odds Ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RBCs donated in last 2 yrs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;4 donations vs none</td>
<td>2.5</td>
<td>1.6 - 3.9</td>
</tr>
<tr>
<td>4-6 donations vs none</td>
<td>9.2</td>
<td>5.6-15.0</td>
</tr>
<tr>
<td>7-9 donations vs none</td>
<td>6.4</td>
<td>4.0-10.1</td>
</tr>
<tr>
<td>10+ donations vs none</td>
<td>8.6</td>
<td>5.3-14.0</td>
</tr>
<tr>
<td><strong>Iron Supplements</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Takes supplemental iron vs. None</td>
<td>0.6</td>
<td>0.5-0.7</td>
</tr>
<tr>
<td><strong>Smoking</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current vs. Never</td>
<td>0.6</td>
<td>0.4-0.9</td>
</tr>
<tr>
<td>Past vs. Never</td>
<td>0.8</td>
<td>0.6-1.0</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female vs. Male</td>
<td>2.9</td>
<td>1.9-4.7</td>
</tr>
</tbody>
</table>
Adjusted ORs: Effect of donation interval on AIS

- Mean ORs for all donations (vs 26 weeks) are represented by Gray squares and CI bars.
- Blue diamonds represent mean ORs for female donations only; insufficient males to analyze
Self-motivated iron use in RISE donors

<table>
<thead>
<tr>
<th>Cohort</th>
<th>N</th>
<th>% iron at enrollment</th>
<th>N completing</th>
<th>% iron at completion</th>
</tr>
</thead>
<tbody>
<tr>
<td>FT Female</td>
<td>481</td>
<td>43%</td>
<td>187</td>
<td>34%</td>
</tr>
<tr>
<td>FT Male</td>
<td>407</td>
<td>25%</td>
<td>149</td>
<td>21%</td>
</tr>
<tr>
<td>RPT Female</td>
<td>769</td>
<td>52%</td>
<td>486</td>
<td>46%</td>
</tr>
<tr>
<td>RPT Male</td>
<td>768</td>
<td>32%</td>
<td>512</td>
<td>25%</td>
</tr>
<tr>
<td>Total</td>
<td>2425</td>
<td>39%</td>
<td>1334</td>
<td>34%</td>
</tr>
</tbody>
</table>

At enrollment, 35% of donors reported use of multivitamins with iron and an additional 4% reported separate use of iron supplements. Of those completing the study, 76-84% continued the same status.
Donors with Absent Iron Stores (AIS) and/or Iron Deficient Erythropoiesis (IDE) at final visit

<table>
<thead>
<tr>
<th>Gender</th>
<th>Donor Status</th>
<th>AIS % ferritin &lt;12 µg/L</th>
<th>IDE % Log (sTfR/F) ≥ 2.07</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females</td>
<td>FT/RA (n=181)</td>
<td>20</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>Fqnt (n=492)</td>
<td>27</td>
<td>62</td>
</tr>
<tr>
<td>Males</td>
<td>FT/RA (n=143)</td>
<td>8</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Fqnt (n=518)</td>
<td>18</td>
<td>47</td>
</tr>
</tbody>
</table>
RISE summary: rates of AIS/IDE at study beginning and end

- FT/RA donors - after a mean of 15 months of frequent donation (3-3.5 total donations including index):
  - AIS/IDE rates in first time female donors essentially the same as frequent female donors
  - AIS/IDE rates in first time male donor rates about 50% that of frequent male donors
- Frequent donors - after a mean of 17 months continued donation at a rate similar to pre-RISE enrollment:
  - AIS/IDE rates at study end were similar to those at enrollment
Consequences of iron deficiency:

Data are suggestive but not definitive, and interpretation is somewhat controversial.
Iron deficiency may cause:

- Fatigue/Decreased exercise capacity
- Decreased cognitive function
- Restless leg syndrome
Iron and cognitive function

- Iron is an important element for brain function.
- Iron deficiency anemia in children and adolescents results in impaired cognitive function.
- Less clear if this is the case with IDE in the absence of anemia and in young adults.
- However, there is evidence that brain maturation and development still occurs in young adults who are old enough to donate.
What to do?
Donor education

- It is necessary to inform donors of the potential occurrence of iron deficiency with increasing frequency of donation
  - Enhance educational materials
  - Incorporate into informed consent?
- Should we advise some or all donors to consider replacing iron either through an iron-rich diet or through taking over the counter vitamin supplements which contain iron or specific iron supplements?
Possible interventions to mitigate iron deficiency in blood donors

- **Donor education**
  - Dietary and/or iron supplement recommendations

- **Modify interdonation interval/frequency**
  - Lengthen interval or restrict number of annual donations

- **Measure ferritin levels**
  - Could measure in donors at highest risk (demographics, freq of donation, Hb level)

- **Iron replacement**
  - Could be based on ferritin or frequency of donation or demographics (females below age 50)

- **Potential target populations**
  - All donors
  - Women of childbearing age
  - Donors with Hb 12.5-13.5 g/dL
  - Donors with iron deficiency (low ferritin)
  - Frequent donors
Iron replacement programs

- Multiple studies indicate that oral iron replacement works to improve iron stores if donors are compliant
  - Germany, Australia, NIH blood bank
- Questions as to optimal iron preparations, dosage, duration of replacement, and who should be included
- Concern about how blood programs can administer and manage these programs
- Suggested that pilot programs should be established and that comparative effectiveness research (CER) be done
  - CER should include analysis of alternate interventions (other than iron replacement) such as changed interdonation intervals
RISE publications

- Cable RG, Steele WR, Melmed RS, et al. The difference between fingerstick and venous hemoglobin/hematocrit varies by gender and iron stores. Transfusion 2012; 52: 1031-40
- Bahrami SH, Guiltinan AM, Schlumpf KS et al. Donation frequency of blood donors participating in a prospective cohort study of iron status. Transfusion 2011; 51: 1207-12
Ongoing NHLBI funded studies on iron in donors

- Ongoing REDS-II/RISE modeling analysis: uses 3 years of REDS-II donation data on frequency of presentation, fingerstick Hb values, and Hb based deferrals
  - Ferritin values simulated from RISE data
- STRIDE Study (Strategies to Reduce Iron Deficiency)
  - NHLBI grant to BCW includes 3 centers (BCW, ITxM New England Red Cross) and one DCC (Westat)
  - Goal is to develop and test methods for replacement of iron lost during donation that can be readily implemented in community blood centers
  - Evaluating informing donors of ferritin level and also evaluating iron replacement at daily doses of 19 and 38 mg of elemental iron
- REDS-III – HEIRS (Hemoglobin and Iron Recovery Study): study of hemoglobin recovery with and without iron replacement
  - 200 subjects with frequent ferritin and Hb measurements up to 24 weeks following donation