Donor Iron Deficiency Study (DIDS): protocol of a study to test whether iron deficiency in blood donors affects red blood cell recovery after transfusion

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Background. Despite fulfilling all requirements for blood donation, a large proportion of regular blood donors are iron deficient. Red blood cells (RBC) from iron-deficient donors may be particularly susceptible to damage induced by standard refrigerated storage. Herein, we present a study protocol for testing whether correcting iron deficiency in donors with iron-deficient erythropoiesis will improve the quality of their refrigerator-stored RBC.

Materials and methods. This is a randomised, controlled, double-blind clinical trial. Sixty healthy regular donors who meet donation standards, while exhibiting iron-deficient erythropoiesis by laboratory testing criteria, will donate a single standard RBC unit that will be leucoreduced and stored in a refrigerator under standard conditions for 40-42 days. A ^{51}Cr-radiolabelled 24-hour RBC recovery study will be performed and then these donors will be randomised to receive, in a double-blinded fashion, either intravenous saline, as a control, or low-molecular weight iron dextran (1 g), to provide total iron repletion. Four to six months later, they will donate a second RBC unit, which will be similarly stored, and autologous ^{51}Cr-labelled 24-hour post-transfusion RBC recovery will again be determined.

Results. The primary endpoint will be the change in 24-hour post-transfusion recovery from the first to the second donation. The primary outcome will be the group mean difference in the primary endpoints between the group receiving intravenous saline and the group receiving intravenous iron dextran. Secondary outcomes will be quality of life, fatigue, and emotional health, assessed by surveys.

Conclusion. This study will provide definitive evidence as to whether donor iron deficiency affects the quality of the blood supply and will assess the severity of symptoms affecting iron-deficient blood donors.

Keywords: post-transfusion recovery, iron deficiency, blood donation, red blood cells.

Introduction

Iron deficiency is common among regular blood donors. In the United States, 69% of the donors who provided the ~15.7 million units of red blood cells (RBC) that were collected in 2011 were repeat donors[1]. In Canada, approximately 90% of RBC units collected for transfusion are provided by repeat donors[2]. Although iron deficiency is surprisingly prevalent in first-time donors[3,4], its prevalence is even higher in the particularly altruistic frequent donors, especially among women of childbearing age[5,6]. In the Recipient Epidemiology Donor Evaluation Study (REDS)-II Donor Iron Status Evaluation (RISE) study[7], up to 49% and 66% of male and female frequent donors, respectively, had either iron depletion (i.e., absent iron stores) or iron-deficient erythropoiesis. Similar frequencies of iron deficiency were also reported in Canadian[2], Austrian[8], Danish[9], and Dutch[10] populations.

RBC from individuals with iron-deficiency anaemia have decreased levels of endogenous anti-oxidants[11,12], have evidence of oxidative damage[13,14], and are more sensitive to oxidative stress[11,14] and low pH[12]; the latter, in particular, decreases progressively during RBC storage[15]. In a recent study by the REDS-III group, RBC collected from frequent donors with low ferritin were found to have altered susceptibility to *in*

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vitro haemolysis[16]. Furthermore, refrigerated storage induces oxidative stress in donor RBC and inhibits their oxidative stress defence mechanisms[13,17-22]. Oxidative damage *per se* also impairs RBC deformability[23] and impaired deformability was seen in humans[14], rats[14], and rabbits with iron-deficiency anaemia[24] and in stored RBC from healthy human donors[25]. Rigid RBC are less able to pass through previously negotiable microcirculatory beds and are more prone to undergo extravascular haemolysis in the spleen[26]. Indeed, circulatory RBC lifespan is decreased in humans with iron-deficiency anemia[12,27-29] and in relevant animal models[24,30]. In humans, decreased circulatory lifespan is most likely due to extravascular haemolysis in the spleen[27-29] and is corrected by iron repletion[12,28].

Remarkably, in several older studies[12,27,31], RBC obtained from donors with iron-deficiency anaemia were transfused into healthy recipients, without prior refrigerated storage. In each study, the transfused iron-deficient RBC had a decreased circulatory lifespan/recovery, most likely due to splenic clearance. Indeed, when RBC obtained from healthy donors were transfused into recipients with iron-deficiency anaemia, the transfused RBC had a normal lifespan, suggesting that the iron deficiency-induced defect was intrinsic to the RBC and not due to enhanced clearance mechanisms[31,32].

We used a mouse model to test whether iron deficiency is associated with decreased RBC recovery[33]. Three donor cohorts were prepared: iron-replete mice, mice with iron-deficient erythropoiesis, and mice with iron deficiency anaemia. Similar to prior studies[34], refrigerator-stored, transfused RBC from iron-replete donors had a normal 24-hour post-transfusion recovery (mean 77.1%). In addition, as expected from other publications[12,27,31], the 24-hour post-transfusion recovery was poor using RBC from donors with severe iron-deficiency anaemia (mean 46.7%; $p<0.001$). In contrast, results using donors with iron-deficient erythropoiesis were subnormal (mean 66.5%; $p<0.05$) and would be less than the minimum mandated by the United States Food & Drug Administration (FDA). Taken together, these data support our hypothesis that, after refrigerated storage, RBC from donors with iron-deficient erythropoiesis without anaemia are suboptimal.

Materials and methods

Study design

This is a randomised, controlled, double-blind clinical trial (Figure 1). Sixty healthy regular donors who meet donation standards, while exhibiting iron-deficient erythropoiesis by laboratory test criteria, will donate a single standard RBC unit that will be leucoreduced and stored in a refrigerator under standard conditions in

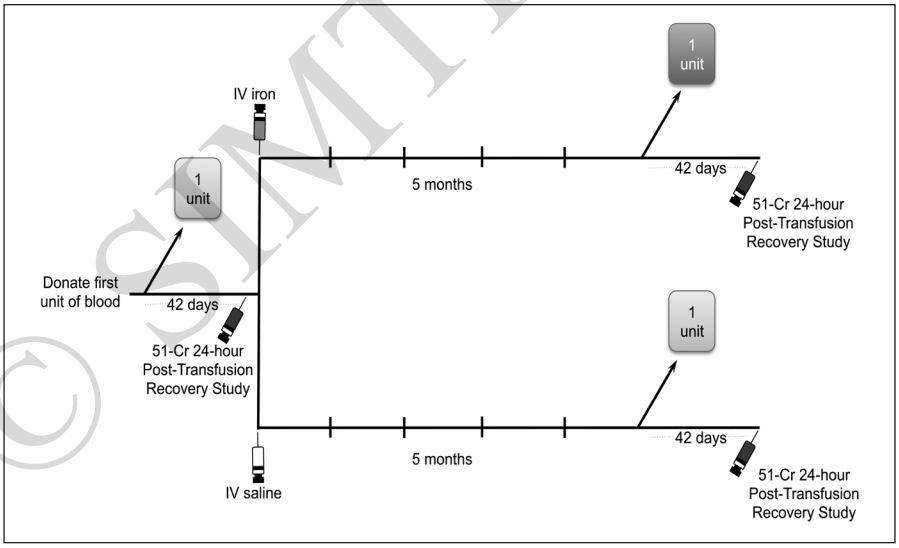

Figure 1 - Study schema.
Sixty frequent blood donors with iron-deficient erythropoiesis will donate one unit of red blood cells (RBC) and a ^{51}Cr-labelled post-transfusion RBC recovery study will be performed after 40-42 days of refrigerated storage. Each volunteer will then be randomised to receive placebo (intravenous saline) or iron repletion (1 g intravenous low molecular weight iron-dextran) within 4 weeks of completing the post-transfusion RBC recovery study. Five months ± 4 weeks later they will donate another RBC unit. Then 40-42 days after the second blood donation, another ^{51}Cr-labelled post-transfusion RBC recovery study will be performed and the result will be compared to that of the first ^{51}Cr-labelled post-transfusion RBC recovery study.
IV: intravenous.

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AS-3 for up to 42 days. After 40-42 days of storage, a ^{51}Cr-radiolabelled 24-hour RBC recovery study will be performed. Thus, a small aliquot of the donated RBC will be radiolabelled and injected into the volunteers according to a standardised protocol[35]. RBC recovery will be calculated from samples obtained at 5 min, 7.5 min, 10 min, 12.5 min, 15 min, 30 min, 1 hour and 24 hours after autologous infusion. In a prospective, randomised, double-blind manner, these donors will then receive either intravenous saline or low-molecular weight iron dextran (INFeD; 1 g) from 1 day to 4 weeks (target, 1 day) after the first post-transfusion recovery study. Five months later, they will donate a second RBC unit, similarly stored for 40-42 days (target same as first recovery study), and autologous ^{51}Cr-labelled 24-hour post-transfusion RBC recoveries will again be determined. The primary endpoint will be the change in 24-hour post-transfusion recovery from the first to the second donation. The primary outcome will be the group mean difference in the primary endpoints between the group receiving intravenous saline and the group receiving intravenous iron dextran.

The secondary outcome measures are listed in Table I. These secondary outcomes include serum markers of iron status, quality of life surveys, and mental wellbeing questionnaires. These measures will be administered at each donation and post-transfusion recovery study (i.e., a total of four times throughout the study). Markers of iron status will be examined to determine the efficacy of treatment. The quality of life surveys and mental wellbeing questionnaires were chosen based on prior associations with iron deficiency or on their use in prior studies in blood donors[36-44].

Table I - Secondary outcome measures of the donor iron deficiency study.

Serum ferritin
Haemoglobin
Zinc protoporphyrin
Soluble transferrin receptor
Hepcidin
Transferrin saturation
SF-36 Physical functioning score
SF-36 Role functioning/physical score
SF-36 Role functioning/emotional score
SF-36 Energy/fatigue score
SF-36 Emotional well-being score
SF-36 Social functioning score
SF-36 Pain score
SF-36 General health score
SF-36 Health change score
Beck Depression Inventory (BDI) II score
Beck Anxiety Inventory (BAI) score
Global Fatigue Index (GFI) score
Restless Legs Syndrome (RLS) Rating Scale

SF-36: Short Form 36 Health Survey

Screening

The New York Blood Center study staff will send a recruitment letter/e-mail to potential subjects, 18-75 years old, who are frequent blood donors. For the purposes of this study frequent blood donors are defined as men who have donated the equivalent of at least two, and women who have donated the equivalent of at least one, RBC units in the preceding year. Volunteers responding to the recruitment request will be screened for eligibility to participation in the study by telephone or e-mail and then invited for a screening visit to confirm eligibility and provide informed consent (see Table II for inclusion/exclusion criteria). Those subjects who meet the criteria of ferritin <15 ng/mL and zinc protoporphyrin >60 µmol/mol haem, but do not qualify because of anaemia may be re-screened between 2 weeks to 3 months later.

Table II - Inclusion/exclusion criteria.

Inclusion criteria
18-75 years old
Healthy (by self-report)
Body weight >110 lbs (~50 kg)
Female haematocrit >38% Male haematocrit >39%
Frequent blood donor (male ≥2 and female ≥1 RBC unit donations in past year)
Ferritin <15 ng/mL
Zinc protoporphyrin >60 µmol/mol haem
Exclusion criteria
Ineligible for donation based on the New York Blood Center autologous donor questionnaire
C-reactive protein >10 mg/L
Sickle cell trait (by self-report)
Systolic blood pressure >180 or <90 mm Hg, diastolic blood pressure >100 or <50 mm Hg
Heart rate <50 or >100 bpm
Temperature >99.5 °F (37.5 °C) prior to donation (attempts will be made to reschedule donation, if possible)
Temperature >100.4 °F (38 °C) or subjective feeling of illness prior to ^{51}Cr-labelled 24-hour RBC recovery study
Positive results on standard blood donor infectious disease testing
Pregnancy
Taking, or planning to take, iron supplements and not willing to stop for duration of study
History of severe asthma requiring hospitalisation, allergic eczema (atopic dermatitis) or other atopic allergy associated with anaphylaxis

RBC: red blood cells

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Post-transfusion recovery studies

A urine pregnancy test will be performed on all female participants <55 years old on the day of infusion. A positive pregnancy test will result in exclusion from the study. Two intravenous lines will be placed in contralateral arms. A 30 mL aliquot of the autologous blood unit donated 6 weeks previously will be removed into a syringe by a licensed radiopharmacist using a sterile technique. The radiolabelling will be performed using 20 mCi of sodium chromate (^{51}Cr) based on the methods of Moroff *et al.*[35] and the recommendations from the International Committee for Standardization in Haematology[45]. Although there are potential measurement errors associated with the 24-hour post-transfusion recovery study[46], it is the current FDA gold-standard test. Furthermore, other assessments of RBC clearance involving transfusion of a full unit of RBC followed by the measurement of markers of haemolysis (e.g., serum iron, indirect bilirubin) were not considered because they would partially iron replete subjects randomised to the placebo group. The ^{51}Cr-labelled RBC will be washed with saline and then infused intravenously (over 1 min through one intravenous line) into the volunteer. In order to calculate counts per minute per millilitre of RBC, a blood sample (10 mL) will be taken from the contralateral arm at time zero (T0) and 5, 7.5, 10, 12.5, 15, and 30 min, and 1 and 24 hours after the infusion.

Low molecular weight iron dextran or placebo infusion

Recent studies support the convenience, safety, and efficacy of a single infusion of 1 g of low molecular weight iron dextran as therapy for iron deficiency in adults[47], along with the efficacy of 1 g of iron to counteract iron deficiency in blood donors[48]. To this end, one peripheral intravenous line will be inserted. A research pharmacist will provide the placebo (intravenous [IV] saline) or treatment (IV low molecular weight iron dextran; INFeD) and test dose (25 mg INFeD, 12.5 mL of a 2 mg/mL solution of iron dextran diluted in normal saline) in tinted infusion bags and tubing specifically designed to maintain blinding in clinical research studies (Medipak, Winchester, VA, USA). In addition, the pharmacy will provide methylprednisolone 125 mg IV to be given both before and after the infusion, acetaminophen (paracetamol) 650 mg PO and diphenhydramine 25 mg PO to be administered before the infusion. After the intravenous test dose (25 mg of INFeD, infused over 20 min), patients will be observed for side effects for 40 min (1 hour from the start of infusion); if no adverse effects are observed, then the entire dose diluted in 500 mL normal saline (i.e., 2 mg/mL of INFeD) will be infused over a period of 2-6 hours as tolerated (target, 2 hours). Adverse events will be identified by observation, direct inquiry, and physical examination of each volunteer. Vital signs will be measured before, during (after 15 min and then hourly), and after each infusion. Resuscitation equipment and personnel trained in detecting and treating anaphylactic-type reactions will be readily available during drug administration.

Laboratory measures

Laboratory measures of iron status and inflammation will be determined from blood samples obtained at the screening visit and then at each of the two study blood donations and two post-transfusion recovery studies (i.e., once before randomisation and once following randomisation). Zinc protoporphyrin, serum iron, total iron binding capacity, ferritin, C-reactive protein, soluble transferrin receptor, and complete blood counts, including reticulocytes, will be measured using clinically validated instruments. Hepcidin will be measured using an enzyme-linked immunosorbent assay (ELISA) kit following the manufacturer's instructions (Intrinsic LifeSciences, La Jolla, CA, USA).

Surveys

Surveys assessing health status and quality of life will be administered in printed format at each of the two study blood donations and two post-transfusion recovery studies (i.e., twice before randomisation and twice following randomisation). The Short Form 36 Health Survey (SF-36), a 36-item, patient-reported survey, assesses overall health status and quality of life within the preceding 4 weeks[49]. The Multidimensional Assessment of Fatigue (MAF) 16-item scale is used to measure fatigue according to four dimensions: degree/severity, distress that it causes, timing of fatigue, and its impact on activities of daily living within the preceding week[50]. The Beck Depression Inventory-II (BDI-II) 21-item, self-report, multiple choice inventory is used to assess for depression experienced within the preceding 2 weeks[51]. The Beck Anxiety Inventory (BAI) 21-question, multiple-choice, self-report inventory, which is used to measure how the subject has been feeling in the preceding month, focuses primarily on somatic symptoms associated with anxiety[52].

Randomisation

The study subjects will be randomised using a computerised system with equal allocation (1:1) to iron repletion or placebo. Randomisation will be stratified by gender; randomly permuted block sizes of 4, 6, or 8 will be used. The moment of randomisation will be recorded and will occur only after successful completion of the first post-transfusion RBC recovery study.

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Blinding

The study will be double-blind. Thus, the randomised group will only be known to the Columbia University Irving Medical Center Research Pharmacy. A research pharmacist will provide the placebo (IV saline) or treatment (IV INFeD), and the test dose of iron/placebo, in tinted infusion bags with tubing specifically designed to maintain blinding in clinical research studies (Medipak). A research nurse unaffiliated with the study team will be responsible for the test infusion and total dose iron infusion. With this design, volunteers and study investigators will be blinded to whether volunteers receive the active intervention or placebo. Subjects in both groups will receive similar discharge instructions as if they had received low molecular weight iron-dextran. Scheduling and logistic communications with volunteers will be made by the study coordinator, who will also be blinded to the treatment group.

Situations may arise in which breaking the blinding earlier would be in the best interest of the volunteer. In any situation in which a physician or the subject asks to be un-blinded to study treatment, the research pharmacy can be reached on an emergency basis to provide this information. Finally, any un-blinding that occurs will be reported to the Data Safety Monitoring Board (DSMB) and ultimately reported in the resulting publication.

Statistical analysis plan

The primary null hypothesis will be tested in an intent-to-treat analysis using a *t*-test, or non-parametric equivalent, of the between-group difference in means of the within-subject change in the post-transfusion RBC recovery from the initial study under conditions of iron-deficient erythropoiesis and the subsequent study performed after randomisation to iron repletion or placebo. We will also examine pre-specified demographic variables (gender [male/female], race [white/not white], age [<50/≥50 years]) to see if we can identify one or more variables that may be effect modifiers. These factors will be considered for inclusion in an adjusted model. The specific criteria for inclusion are: (i) difference by treatment group significant at α=0.10 two-sided, and (ii) related to outcome at level α=0.10 two-sided. If any of the pre-specified covariates meet the criteria for inclusion, they will be incorporated in an adjusted model, and that model will become the primary analysis. Otherwise, the simple model will be primary.

The secondary outcome null hypotheses will be tested in an intent-to-treat analysis using mixed-effect models to compare the differences in the iron repletion and placebo group temporal course at the four defined time points on the secondary outcome measures (Table I). We will also examine pre-specified demographic variables, as above, to see if we can identify one or more that may be effect modifiers.

Furthermore, because we expect variable responses to iron repletion in the experimental group, and some crossover in the placebo group, we will also perform a tertiary analysis to explore the effect of iron status on post-transfusion RBC recovery. We will use multiple regression to assess whether RBC zinc protoporphyrin level increases the R^2 of a model of post-transfusion RBC recovery predicted by treatment group membership. With 60 subjects, we will have 80% power to detect a partial correlation coefficient increase of 0.33 for the unique contribution of RBC zinc protoporphyrin level[53].

Sample size estimate

Based on preliminary data from prior human ^{51}Cr RBC recovery studies, the standard deviation of the measure in our single site is 5.0%. Furthermore, the expected mean difference in post-transfusion RBC recovery between iron-replete mice and mice with iron-deficient erythropoiesis is 10.6%. If the difference were this large in humans, we would require fewer than six subjects (α=0.05, two-sided, power=0.80). However, we expect the difference to be less dramatic in humans than in inbred mice. Thus, we will power the study to detect a clinically relevant difference in post-transfusion recovery of 4%. Under this assumption, the calculated sample size required for each arm is 26 (α=0.05, two-sided, power=0.80). Furthermore, to allow for a dropout rate of up to 15%, we plan to randomise 30 subjects per arm for a total sample size of 60 subjects.

Interim analyses

Interim analysis will be performed twice (after every 20 subjects have completed study participation) in addition to the final analysis. The DSMB will conduct the analyses using a two-sided asymmetric Lan-DeMets α-spending approach with an O'Brien-Fleming two-sided symmetric stopping boundary and overall α=0.05. The DSMB criteria for early stopping will include: (i) the Z-score at an interim analysis lying outside of the group sequential boundaries as calculated (Table III); (ii) major safety violations; and (iii) convincing evidence of futility in the context of adverse events. Interim boundaries together with terminal criteria (Z-scores and associated p-values) calculated using the WinLD version 2 programme (Microsoft, Redmond, WA, USA) are provided in Table III.

Table III - Lan-DeMets group sequential boundaries calculations.

Volunteers completed	Lower boundary	Upper boundary	Nominal upper alpha	Cumulative alpha
20	−3.7103	3.7103	0.00010	0.00021
40	−2.5114	2.5114	0.00601	0.01210
60	−1.9930	1.9930	0.02313	0.05000

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Study approval and registration

The study will be conducted according to the Declaration of Helsinki and in accordance with good clinical practice guidelines. The Columbia University Irving Medical Center and New York Blood Center Institutional Review Boards approved the protocol. All research participants will provide written informed consent prior to study participation. The study was registered prior to initial enrolment in ClinicalTrials.gov with the identifier #NCT02889133.

Conclusions

This study will provide definitive evidence as to whether donor iron deficiency affects the quality of the blood supply and whether iron repletion influences the severity of symptoms of iron-deficient blood donors. The proposed research offers a new approach to improve the quality of RBC units obtained from volunteer donors by providing definitive evidence for an unrecognised source of substandard post-transfusion recovery. Current FDA guidelines, which permit up to six blood donations per year with a minimum haemoglobin of 12.5 g/dL, have the consequence that most regular volunteer donors become iron deficient[54]. Beyond the potential adverse effects of iron deficiency for these committed blood donors, the quality of the RBC units that they altruistically donate has not been rigorously examined. This proposed prospective, randomised, double-blind, placebo-controlled trial will decisively determine whether donor iron-deficient erythropoiesis significantly and substantially decreases 24-hour post-transfusion RBC recovery of refrigerator-stored donor RBC. Because RBC that do not circulate cannot deliver oxygen, measures to improve post-transfusion RBC recovery could produce sustained improvements in patients' outcomes. Nonetheless, regardless of the study outcome, conducting this carefully-designed trial will yield clinically important information that will help refine guidelines for safe blood donation frequency and blood product approval.

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Authorship contributions

Contributions: ZCB and EAH drafted the first version of the manuscript. All Authors reviewed and edited the manuscript.

Disclosure of Conflicts of Interest

SLS is on the scientific advisory board of Hemanext, Inc. and is a consultant for Tioma, Inc. The other Authors declare that they have no competing financial interests.

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