Published in final edited form as:

Clin Lab Med. 2015 March ; 35(1): 73–91. doi:10.1016/j.cll.2014.10.011.

Laboratory and Genetic Assessment of Iron Deficiency in Blood Donors

Joseph E. Kiss, M. D.

Division of Hematology-Oncology, Department of Medicine, The University of Pittsburgh and The Institute for Transfusion Medicine, Pittsburgh, PA, United States

Synopsis

Over 9 million individuals donate blood annually in the US. Between 200 to 250 mg of iron is removed with each whole blood donation, reflecting losses from the hemoglobin in red blood cells. This amount represents approximately 25% of the average iron stores in men and almost 75% of the iron stores in women. Replenishment of iron stores takes many months, leading to a high rate of iron depletion, especially in frequent blood donors (e. g., more than 2 times per year). In large epidemiologic studies, donation frequency, female gender, and younger age (reflecting menstrual status), are particularly associated with iron depletion. Currently, a minimum capillary hemoglobin of 12.5 gm/dl is the sole requirement for donor qualification in the US as far as iron levels are concerned, yet it is known that hemoglobin level is a poor surrogate for low iron. In an effort to better identify and prevent iron deficiency, blood collection centers are now considering various strategies to manage donor iron loss, including changes in acceptable hemoglobin level, donation interval, donation frequency, testing of iron status, and iron supplementation. This chapter highlights laboratory and genetic tests to assess the iron status of blood donors and their applicability as screening tests for blood donation.

Keywords

Blood donation; iron deficiency; ferritin; soluble transferrin receptor; reticulocyte hemoglobin content; hypochromic mature red blood cells; MCV; hereditary hemochromatosis (HFE); TMPRSS6

Blood Donors and Iron Depletion

Blood donors and the red blood cells they provide serve as a vital link in the delivery of healthcare worldwide. Over 9 million volunteer blood donors donate each year in the US.¹ Nearly 70% are repeat donors, many of whom become iron deficient as a result of regular blood donation.^{2,3} Moreover, nearly 7% of presenting donors are deferred from donating

© 2014 Elsevier Inc. All rights reserved.

Corresponding Author: Joseph E. Kiss, M.D. The Institute for Transfusion Medicine, 3636 Boulevard of the Allies, Pittsburgh, Pennsylvania 15213, jkiss@itxm.org.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

because they cannot meet the minimum hemoglobin standard of 12.5 gm/dl obtained by fingerstick testing. The US Food and Drug Administration(FDA) defines this as the minimum hemoglobin value in both men and women to determine donor eligibility, but no requirement currently exists for determining iron levels. Low hemoglobin, a late consequence of iron deficiency, represents the largest category of blood donor deferral and occurs more frequently in women. In addition, women are three times more likely to be iron deficient than men.⁴ Even in the absence of anemia, iron depletion has been associated with a number of conditions arising from the key role iron plays in central nervous system and neuromuscular function⁵, including fatigue⁶, decreased exercise capacity⁷, neurocognitive changes⁸, pica (the compulsive ingestion of non-nutritive substances, such as ice), and restless legs syndrome.^{9, 10}

Overall, 35% of the blood donor population in the US is estimated to be iron deficient.⁴ The large number of affected blood donors and recognition of the potential health consequences has prompted the main blood banking organization, AABB, to recommend that measures be adopted to identify and to prevent iron deficiency in all or in selected high risk individuals. As a result, blood collection centers worldwide are examining potential strategies to manage donor iron loss, including changes in acceptable hemoglobin level, donation interval, donation frequency, testing of iron status, and iron supplementation. This review will consider the relative merits of different laboratory and genetic tests to assess the iron status of blood donors and their suitability as screening tests for blood donation.

Brief Review of Iron physiology in men and women

Iron is an essential element in many physiologic processes. In association with heme, it participates in the reversible binding of oxygen by red blood cells and is also a key constituent in the myoglobin of muscle and mitochondrial cytochromes. Non-heme iron plays a key role in the activity of many enzymatic reactions. Iron may also be toxic when present in excess: Absorption is tightly regulated because there is no active mechanism for excretion. Dietary iron is absorbed in the proximal small intestine. Iron from animal sources (heme iron) has greater bioavailability (~30% absorbed) than nonheme (i.e., plant products) iron (~10% absorbed).¹¹ Men normally absorb ~ 1 mg/day, equaling basal losses primarily from the gastrointestinal tract. Iron absorption in premenopausal women is greater, ~ 1.3-1.5 mg/day, because of additional losses from menstruation. Absorption capacity increases proportionate to the level of iron deficiency (i. e., ferritin level), to an average “maximum” of 4-5 mg/day in very active blood donors.¹² The average iron absorption is more in the range of 2-3 mg/day.¹¹ Absorbed iron binds to transferrin and is transported to transferrin receptors located on early erythroid and all other nucleated cells. Iron not directly utilized in physiologic pathways is stored intracellularly as ferritin; small amounts present in blood are in equilibrium with intracellular ferritin, the serum level of which is considered a reliable indicator of available storage iron in the absence of inflammation.

Total body iron content in men averages approximately 50 mg /kg (~3500 mg) whereas in women, who have lower hemoglobin levels and less blood volume in proportion to weight, the average is closer to 35 mg/kg (~2100 mg). Seventy to eighty percent of body iron exists in red blood cells in the form of hemoglobin. Cook, et al., estimated average tissue iron

stores of only 776 ± 313 mg in men and 309 ± 346 mg in women.¹³ Thus, it should not be surprising that the loss of approximately 230 mg iron with each whole blood donation along with the limited capacity for absorption leads to a high incidence of iron deficiency in regular donors, especially women.

With losses in excess of absorption iron deficiency occurs progressively, beginning with the gradual loss of storage iron, followed by the development of iron-deficient erythropoiesis (IDE), and culminating in iron-deficiency anemia (IDA).¹⁴ Measurement of hemoglobin is a poor indicator of iron depletion and iron deficient erythropoiesis (IDE) because anemia occurs as the last stage in this sequence. In the clinical setting, the laboratory evaluation of iron deficiency is usually undertaken in the setting of anemia, and begins by examining the complete blood count. Microcytic red blood cells (MCV < 80 fl) and reduced mean cellular hemoglobin (MCH) are often present and a reticulocyte count is decreased (absolute < 50,000/ul). The usual biochemical panel includes a serum iron and a total iron binding capacity (TIBC). The serum iron level is the least reliable test since it has a diurnal fluctuation and is affected by inflammation and infectious stimuli. The TIBC assesses the iron binding capacity of the transport protein (transferrin). In IDE, the serum iron is low and the TIBC is high reflecting unsaturated transferrin. The normal saturation of transferrin (Fe/TIBC) is 20-50% with values <15% being characteristic of IDE. Ferritin, a measurement of storage iron, is also commonly performed. In IDE, stores are low; however, ferritin becomes elevated during inflammation due to release from storage sites (eg, liver) making this test less reliable. A laboratory test that can be used to avoid the confounding effects of inflammation on ferritin is the serum soluble transferrin receptor (sTfR), a transmembrane receptor which is elevated in IDE because of shedding into blood by iron-depleted erythroid cells.¹⁵ Finally, the gold standard concerning the presence or absence of storage iron is an assessment of a sample of bone marrow stained for the presence of iron (Prussian blue). Examination of bone marrow material solely to assess iron stores is not routinely done due to the discomfort and invasiveness of the procedure.

Molecular defects have been described that impact iron homeostasis. In hereditary hemochromatosis, the protein that normally down regulates iron absorption is defective, leading to excessive accumulation of iron in the body. In addition, a common transferrin polymorphism (G277S mutation) has been elucidated that predisposes individuals to the development of iron deficiency.

Tests to Identify Iron Depletion and Iron Deficient Erythropoiesis in Blood Donors

In one important sense, blood donors are quite different from clinical patients who often have inflammatory, infectious, or neoplastic disorders that alter the diagnostic variables used to define iron deficiency. Serum iron and, to a lesser degree, transferrin levels decrease in response to inflammation, and ferritin levels increase, irrespective of iron stores. These alterations describe a clinical state termed iron-restricted erythropoiesis in which iron is not depleted *per se* but is sequestered in storage sites and is also less able to be absorbed, resulting in functional iron deficiency. We now know that the primary regulator of iron homeostasis, hepcidin, mediates this state through inhibition of the transmembrane iron-

chaperone receptor, ferroportin.^{16,17} Hepcidin is increased in inflammatory states, which leads to iron restricted erythropoiesis and “chronic disease” anemia by this and other mechanisms. On the other hand, blood donors behave more like a normal control population. Before they are accepted for donation, they must affirmatively answer several questions regarding their physical well-being, starting with: “Are you feeling well and healthy today?” and must have normal vital signs including body temperature. This screening approach selects against those with inflammatory and or infectious disease. Thus, studies that have evaluated acute phase proteins such as C-reactive protein(CRP) in blood donors have found low levels.¹⁵ As a result, serum ferritin levels in blood donors are less influenced by acute phase changes and provide a more reliable indication of true iron status than in clinical medicine.

Ferritin has been used alone and in combination with other biochemical tests to assess iron status in blood donors. In addition, both standard red blood cell measurements (eg, MCV, MCHC) and more specialized hematology analyzer indices (e.g., % hypochromic mature cells or HYPOM and reticulocyte hemoglobin content, or CHr) have been used. It is important to again emphasize that as a practical matter, hemoglobin is the only point-of-care test currently used to qualify the donor. Therefore, in addition to accurately detecting iron depletion on the current donation it may be important that any proposed test be evaluated in the context of its ability to identify subsequent (that is, next visit) blood donor iron status and or low hemoglobin deferral.

Serum Iron, TIBC, and % Transferrin Saturation (% Sat)

Box1 lists representative iron assays that have been investigated in blood donors. Transferrin is the major iron transport protein in plasma, binding Fe³⁺ ions and is measured as the Total Iron Binding capacity (TIBC). Normally one-third of the binding sites are occupied (20-50% saturated, abbreviated as %Sat). Use of %Sat has been limited to historical studies. It has not been used without other biochemical assays such as ferritin because the %Sat level has a relatively low sensitivity in detecting iron depletion.¹⁸ For example, in the study by Simon using ferritin 12ng/ml to define iron *depletion* the overall frequency in repeat blood donors was 8% in males and 23% in females.³ Iron *deficiency* (as defined by both a low ferritin and transferrin saturation of < 16% - the latter indicating decreased iron availability for transport into red blood cells) was found in a smaller subset of those with low ferritin, 2% of male and 13% of female donors. Defining iron deficiency is relevant to the diagnosis of clinical anemia and associated symptoms, whereas, identification of iron depletion and ability to tolerate additional iron loss (and prevention of frank iron deficiency) is a more important goal in the management of blood donors.¹⁹

Serum (or plasma) Ferritin

Ferritin can be measured in either serum or plasma (using EDTA plasma ferritin concentration is approximately 5% lower than serum²⁰) and is considered to reflect the level of tissue iron stores, at least in blood donors who generally have reduced iron stores compared to epidemiologically “normal” populations. The level in blood results from equilibration or “leakage” from cellular or tissue sources. Each ng/ml of ferritin in blood corresponds to 8-10 mg of iron in the storage compartment.^{15,21} As mentioned, in contrast to

the situation in blood donors, ferritin is an acute phase protein with variable levels that imperfectly measure iron stores in clinical medicine. The “classic” cutoff value, 12 ng/ml was originally based on a US population survey performed before the international standard for ferritin was established in 1985.²² Since then, it has been adopted as a specific but insensitive indicator of absent iron stores.²³ Bone marrow analysis for the presence of iron in iron deficient and replete women revealed that a serum ferritin cutoff of 15 ng/ml correctly classified 98% iron replete and 75% iron-deficient subjects.²⁴ A systematic review of high methodologic quality studies (bone marrow iron determined in over half) found ferritin levels to be superior to several other measurements (MCV, %Sat, zinc protoporphyrin), and ferritin < 15 ng/ml “confirms the diagnosis” of IDA.²⁵ Other studies suggest higher levels (22-40 ug/L) more sensitively reflect iron-deficient erythropoiesis.^{14, 25, 26} Patients with chronic renal disease have shown responses to intravenous iron at even higher ferritin levels.¹⁸

In blood donors, the original description by Finch et al. used the ferritin assay to show that blood donation was associated with a significant drop in ferritin levels, which was related to the intensity of blood donation over the previous 4-5 years.² Studies consistently show the same predictable effect of donation intensity on iron stores.^{3, 27, 28} (Figure 1) Simon, et al. in an observational study of blood donors showed the frequency of iron depletion as measured by serum ferritin 12 ng/ml was <3% in male first time donors, but 12% in female first time donors, reflecting the impact of menstrual blood loss.³ Each successive lifetime donation reduced the mean ferritin values, particularly in men so that both sexes reached apparent iron equilibrium after approximately 5-6 lifetime donations. Post-menopausal women had higher ferritin levels than pre-menopausal women but lower values than male donors. Pre-menopausal women taking iron supplements had improved iron stores approaching those of male donors. No change in hemoglobin levels were seen with blood donation in this retrospective study.

O'Meara, et al conducted a large single center longitudinal study to determine the value of routine ferritin measurement in blood donors.²⁹ A total of 160,612 donations in 23,557 donors from 1996-2009 were assessed for serum ferritin at each blood donation starting in 2004. Values below 10ng/ml were identified as iron depleted, and resulted in medical counseling by a blood bank physician to assess other potential medical reasons for low iron or to be referred to their physician, and to consider steps to improve iron balance such as decreasing donation frequency and/or taking iron supplements. The major results comparing groups before and after the intervention revealed: 1) Hemoglobin levels increased by 0.26 g/dl in women and 0.19 g/dl in men; 2) The prevalence of anemia, defined as hemoglobin <12 g/dl (women) or 13g/dl (men) declined from 3.6% to 2.2% in women, and from 0.7% to 0.5% in men; 3) Hemoglobin deferrals (cutoff for women < 12.3g/dl, men < 13.3 g/dl) also declined, from 2.8% to 1.9% in women; and 4) Donor return rate decreased from 72-75% to 60-64% after institution of ferritin screening. It can be seen from this study that ferritin monitoring has a positive impact on anemia and hemoglobin deferral, while some donors stop donating entirely.

As in clinical studies, it is clear that a ferritin cutoff of 12 ng/ml is a specific indicator of iron depletion in blood donors, but also lacks sensitivity.³⁰ One study found that this cutoff

failed to identify iron depletion in over 1/3 of cases in blood donors.³¹ The investigators found a higher ferritin level, 22 ng/ml, more indicative of functional iron depletion. These investigations were not based on the “gold standard” bone marrow iron stains or a hematologic response to iron, but relied instead on serum soluble transfer receptor measurements to indicate functional iron deficiency.

Soluble Transferrin Receptor (sTfR) and Soluble Transferrin Receptor/Ferritin ratio (“R/F” ratio)

The soluble Transferrin Receptor (sTfR) is a truncated form of the transferrin receptor that is shed when early erythroid cells are deprived of iron or if there is increased erythropoietic proliferative activity (eg, hemolytic anemia, thalassemia). Serum levels are also affected by race (~9% higher in African-American subjects) and altitude (~ 9% higher).¹⁵ Until recently, there has been no uniform reference standard. However, there is now agreement on a source standard that promises to enhance the reliability of the assay³¹ in combination with ferritin to define IDE as a more sensitive measure of iron status. Increased sTfR levels reflect the functional iron compartment and have been shown to correlate with depleted iron stores in bone marrow preparations.²³ Soluble TfR levels also show excellent correspondence to oral iron treatment in otherwise healthy anemic females.¹⁴ The transferrin receptor is expressed primarily on the surface of erythroid cells. Reduced iron levels lead to increased TfR synthesis and shedding into circulating blood. Levels greater than the normal reference range (95% CI) have been used to suggest tissue iron deficiency. In addition, ferritin measurements (which reflect storage iron) and sTfR values (which reflect functional iron) have been combined into a ratio, $\log(sTfR/ferritin)$, as a derived measurement. Experience to date in blood donors using the two inversely related measurements has shown efficacy in assessing iron status.^{31a, 32, 52} A (sTfR/log ferritin) “index” has been advocated as another way to discriminate iron deficient erythropoiesis from storage iron depletion.^{14, 23, 33}

Using the $\log(R/F)$ ratio, however, carries with it another important advantage because it can be used to assess iron stores quantitatively. Based on the work of Skikne and Cook^{13, 14a} we know there is a log linear relationship between $[sTfR/ferritin]$ and storage Fe, as expressed by the formula:

$$\text{Body Iron(mg/kg)} = - [\log(R/F\text{ratio}) - 2.8229] / 0.1207$$

Use of this equation allowed the estimation of the tissue iron stores in men as described above, as well as the ability to quantify the iron deficit because sTfR continues to increase as ferritin levels reach the lower limit of detection. Body iron can be expressed as the iron surplus in stores or the iron deficit in tissues. In addition, quantifying iron stores in this manner in serial follow up studies permits an estimation of iron absorption in blood donors.³⁴

The US REDS-II Iron Status Evaluation (RISE) group conducted a multicenter, prospective longitudinal study of iron status in over 2400 first time and frequent (men who donated

3×/year and women 2×/year) blood donors who were followed for 15-24 months while continuing to donate.^{4,27} Two measures of iron depletion were studied: Absent Iron Stores, defined as ferritin < 12 ng/ml, and Iron Deficient Erythropoiesis (IDE), defined as log (sTfR/ferritin) > 2.07 (97.5% of the upper limit of the reference range in first time iron-replete male donors). In RISE, half of the repeat male donors and two-thirds of the repeat female donors demonstrated evidence of IDE at enrollment. (Table 1) By the time of the final visit, the first time donors had developed similar levels of iron depletion as the repeat donors at enrollment and the repeat donors continued to maintain a similar levels of AIS and IDE, highlighting the impact of donation frequency on iron losses. The RISE study identified donation intensity, female sex, and younger age as the most important factors increasing the likelihood of iron deficiency. Donor self-reported iron supplement intake was beneficial. Of the 9.9% of donors deferred for low hemoglobin, 77% had evidence of IDE. As expected, female sex was an important predictor of hemoglobin deferral. In addition, inter-donation intervals at less than 14 weeks were found to be associated with increased hemoglobin deferral (OR ~2-2.5) and iron deficiency (OR ~3-4.4 for ferritin < 12ug/L).

In the RISE analysis plasma ferritin was highly correlated with IDE, $R^2 = 0.96$, whereas sTfR was less so: $R^2 = 0.54$.³² Multivariate regression analysis revealed that log (sTfR/ferritin) of 2.07 (97.5% of the upper limit of the reference range) equated to a ferritin level of 26.7 ug/L, indicating evidence of iron-deficient erythropoiesis. Ferritin at this cutoff value had 95.1% sensitivity and 89.6% specificity in detecting IDE. The investigators concluded that this ferritin threshold had optimal sensitivity and specificity to detect early iron depletion in blood donors and that sTfR added little diagnostic information. A ferritin of 26.7 ng/ml is in the range that has been proposed for detection of iron depletion in other studies discussed above.^{3, 11}

Zinc Protoporphyrin (ZPP)

Zinc protoporphyrin, also called Free Erythrocyte Protoporphyrin (FEP), is measured using a portable hematofluorometer as a point of care test using capillary (finger stick) samples. Zinc is chelated by protoporphyrin IX if iron is not available during last step of porphyrin ring synthesis, resulting in elevated erythrocyte zinc protoporphyrin ZnPP/mole Heme (ferrous protoporphyrin).³⁵ The normal reference range (method specific) is <60 umole ZnPP /mol Heme. Among conditions that have a low prevalence in blood donors, such as lead intoxication, values are also increased in thalassemia, occurring in as many as 51% subjects with beta-thalassemia trait, and 20% with alpha-thalassemia trait.³⁶ Limited studies in blood donors show early detection of iron deficiency and correlation with hemoglobin deferral. Erythrocyte zinc protoporphyrin (ZPP) was measured in 102 women to evaluate iron deficiency anemia and hemoglobin deferral. Women with increased ZPP values all had low serum ferritin concentrations (< 12 ug/L). The positive predictive value of an increased ZPP in predicting deferral of the donor after one or two donations was 75%, whereas a serum ferritin concentration < 12 ug/L predicted deferral in 26% of the donors.³⁷ Another group of investigators found in a multivariable analysis that elevated ZPP levels (using venous blood) aided in the prediction of future hemoglobin deferral when added to other variables including previous hemoglobin value, age, gender, time since previous visit, and total number of blood donations over a two year period (OR ~ 2.0 and 2.2 in men and

women, respectively).³⁸ However, they were unable to validate the model using ZPP in a different donor population in which different hemoglobin eligibility criteria were in place.³⁹ Additional trials using ZPP as a point of care screening test for detecting and managing iron deficiency in blood donors are in progress.⁴⁰

Red Blood Cell Indices for Assessment of Functional Iron status

As discussed previously, the conventional morphologic hallmarks of iron deficiency anemia -- low MCV, MCH, and MCHC -- occur relatively late in the development of iron depletion. Alexander, et al., found a significant trend correlating lower MCV and MCH and donation frequency (as a risk factor for low iron) in men and women, however the strength of the correlation was weak (R^2 only minus 0.08-0.17).⁴¹ Insufficient sensitivity is also a limitation in predicting the likelihood of low hemoglobin deferral. For example, Stern et al found RBC parameters including Mean Corpuscular Hemoglobin Concentration (MCHC < 330 g/l) ($R^2=0.12$) and MCV < 80 fl ($R^2=0.00$) to be inferior to hemoglobin ($R^2=0.63$) in predicting subsequent hemoglobin deferral.⁴² In line with results of other studies, ferritin level was similarly non-predictive ($R^2=0.07$), highlighting the fact that most iron-depleted donors continue to meet donor eligibility criteria for donating blood despite iron depletion. Their analysis prompted a change in blood donor management strategy (reported above²⁹) that was originally based on serum ferritin determination in all donations, to one focused on annual serum ferritin as a routine check and then only in donors with hemoglobin values within 0.5 g/dl of the hemoglobin deferral threshold in Switzerland (< 12.3 gm/dl in women and 13.3 g/dl in men).

Technologic advances in the assessment of functional iron status by measuring red blood cell indices using a “next” generation of hematology analyzers (ADVIA® 120, Siemens Healthcare Diagnostics, Deerfield, IL, USA and Sysmex XE-5000, Kobe, Japan)⁴³ have also been reported to be useful in monitoring iron status in blood donors. (Table 2) In functional iron deficiency, a reduction in hemoglobin content of red blood cells results from an imbalance between iron supply and iron requirements of erythropoiesis, which is increased in blood donors. Analysis of the fraction of individual red blood cells with deficient hemoglobinization using laser scatter techniques reflects changes in the availability of iron during erythropoiesis, which may be comparable to and possibly better than biochemical markers.⁴⁴ CHr (reticulocyte hemoglobin content) reflects iron incorporation to developing reticulocytes (i.e., iron availability during formation of reticulocytes that can be detected within the 3 day lifespan of these cells), whereas the proportion of HYPOM (hypochromic mature red blood cells) is a time-averaged marker of iron availability that is detected within the 3 month lifespan of mature RBCs. These measurements have been likened to monitoring glucose levels and HbA1c levels in diabetics, respectively.

RBC Indices in Clinical Populations

The RBC indices have been found to be more sensitive indicators of functional iron deficiency than biochemical iron tests in renal failure patients who are treated with erythroid stimulating agents, in pregnancy, and in pediatric patients with iron deficiency.⁴⁵⁻⁴⁸ Adequate iron stores are essential for optimizing the treatment response to rHuEpo. For example, a state of functional iron deficiency as detected by decreased CHr can be induced

with rHuEpo administration in normal individuals when ferritin levels are below 100 ng/ml.⁴⁹ (Figure 2) Functional iron deficiency is frequently seen at even higher ferritin values in renal disease patients and other patient populations, including malignancy, infection, and in collagen vascular diseases where serum ferritin is an acute phase response protein that is an unreliable indicator of iron stores. Another RBC index marker, the percentage of hypochromic red cells [%HYPO; red blood cells with a mean corpuscular Hb concentration (MCHC) < 28 g/dl] has been shown to indicate functional iron lack in dialysis patients and in normal individuals treated with r-HuEPO.^{50, 51} (Figure 3) Additionally, HYPOM was found to have a high degree of correlation with sTfR (Area under Receiver Operating Characteristic (ROC) curve = 0.98) in female students with iron deficiency anemia, in whom the increased %HYPO returned to normal after oral iron therapy.⁴⁵ This suggests that measurement of RBC indices may be efficacious for assessing iron depletion in normal otherwise “healthy” individuals as represented by blood donors.

RBC Indices in Blood Donor Populations

Since biochemical measures such as sTfR and the hematologic indices CHr and HYPOM reflect iron currently available for erythropoiesis, normal values may be seen in a blood donor with absent iron stores, who is maintaining sufficient dietary iron intake. Thus, the potential value of these tests may be in assessing the ability of some donors who continue to successfully donate (without deferral) or in predicting future donor deferrals (at risk donors). In a study employing the ADVIA® 120 analyzer in the evaluation of blood donor iron status, Radtke, et al, found reasonable sensitivity of CHr at 32 pg cutoff and HYPOM at 0.3% cutoff individually (57.5% for both measures), and combined (69%) in the identification of iron-deficient erythropoiesis, with high specificity (~90%).³¹ In a smaller study Nadarajan, et al., found 81% sensitivity and 89% specificity for a HYPOM equivalent parameter, RBC-Y and lower sensitivity, 69%, v. 93% specificity for CHr at 28 pg cutoff.⁵² Both investigators used the log [sTfR/ferritin] ratio as the “gold standard” to define iron deficiency. These investigators reported greater sensitivity/specificity using serum ferritin over RBC parameters: 88%/92% at ferritin cutoff 20 ng/ml³¹, and 100%/90% (AUC 0.99) at cutoff of 15 ng/ml.⁵² However, the analyses were methodologically biased in ascribing these results to ferritin, since ferritin was compared to log [sTfR/ferritin] in the ROC analysis.³² The investigators concluded that despite logistical issues in obtaining RBC indices, they were superior to hemoglobin values in assessing blood donor iron status.

In the multicenter RISE analysis, plasma ferritin and sTfR were compared to red blood cell indices including reticulocyte hemoglobin content and the %hypochromic red cells to characterize AIS and IDE in blood donors.³² The RBC index that performed the best overall was %HYPOM. (Figure 4) At a %HYPOM cutoff value of 0.55%, sens/spec was 85%/57% for AIS and 72%/68% for IDE. Comparative values for CHr at 32.6 pg (optimized according to ROC curve analysis) were 81%/49% for AIS and 69%/53% for IDE, with poorer results at the conventional cutoff of 28 pg. CHr had lower diagnostic usefulness than HYPOM or several other indices including proportion of reticulocyte hypochromic RBCs (HYPOr) or hemoglobin content of mature cells (CHCMm). The RBC assays were better (greater AUC) at identifying more severe iron depletion, ie, AIS, or ferritin < 12 ng/ml. At 28 pg for CHr there was extremely poor sensitivity (7%), no better than MCV of 80 fl. In recent single

center study in blood donors, CHr using a cut off value of 28 pg was reported to have excellent specificity with slightly higher sensitivity for detection of AIS (defined in their study as sTfR/log ferritin > 97.5th percentile, or 1.5): 27.3% (males) and 40.8% (females), however, the authors also expressed reservations that CHr did not identify a considerable proportion of “latent” iron depleted donors.³⁹

Technical issues affecting some of the measured RBC parameters are critical to the accuracy of RBC index tests. The manufacturer recommends testing within 6 hours because cell swelling may affect the accuracy of some measured parameters including HYPOM, HYPOR, CHCM, and MCV but not CHr.^{43,32} Whether these sample preparation /storage issues affected the reported differences in results between HYPOM and CHr are not clear. Some studies report better results using CHr,^{33, 53} whereas others report that HYPOM is superior. For example, HYPOM was found to have better sensitivity/specificity than CHr in two hemodialysis/erythropoietin treatment trials^{55,55a} and a study of iron deficient young women or patients hospitalized with anemia.⁴⁷ Mitsuiki, et al., found that a higher cutoff for CHr, 32 pg, improved the sensitivity and specificity of this test in the diagnosis of iron deficiency.⁵⁴ In particular, because of the immediate uptake of iron into developing immature red blood cells, reticulocyte variables (CHr, HYPOR) may be more indicative of recent or “real time” hematinic therapy: HYPOR and CHr have been shown to have more rapid correction than HYPOM after iron replacement in anemic young women.⁴³ CHr ranked low as an indicator of AIS and IDE in the RISE study.³² The RISE investigators theorized that the reticulocyte hemoglobin compartment may be preserved because regular blood donors may be able to compensate as they either consciously or unconsciously consume more iron-rich foods and/or iron supplements leading up to their scheduled donation, thus replenishing the reticulocyte hemoglobin compartment while remaining chronically iron depleted.

In conclusion, the RISE study found that RBC indices had only a modest value in assessing the iron status of blood donors. While clearly superior to hemoglobin in detecting iron depletion, using HYPOM or CHr detected IDE in about 70% of “true positive” donors while falsely classifying ~32% (HYPOM) and ~47% (CHr). A plasma ferritin value of 26.7 ug/L (comparable serum level of ~28 ug/L) provided the simplest and best discriminatory value overall used in conjunction with the 12.5 gm/dl capillary hemoglobin standard. It would be advised that blood centers considering using RBC indices to screen for iron depletion in blood donors should rigorously validate their results, especially with regard to ROC curve-based threshold values, in comparison to ferritin levels. Future research would benefit greatly from well-designed prospective studies using RBC indices in donor management.

Genetic Assessment of Iron Status in Blood Donors

A growing body of research is beginning to uncover genetic variations involved in iron metabolism and hemoglobin production, many affecting the actions of the central regulator of iron metabolism, hepcidin.(Table 3) Indeed, a new term has been coined--- “ironomics”--- to emphasize the interplay between genomics and iron pathways.⁵⁶ Current guidelines that govern blood donation are fairly uniform for all donors despite considerable individual differences in the ability to donate without being deferred for low hemoglobin. For example, there are subsets of very frequent/high intensity donors that appear to be less susceptible to

iron deficiency and hemoglobin deferral. Mast, et al. have characterized a group of female “super-donors” with six donations in the previous 12 months that had less than one-half the odds for low hemoglobin deferral than those with only one donation.^{57,58} Data such as this, at the “extremes” of donor tolerance of iron losses suggest there may be individuals that can respond by more efficient dietary iron absorption. At the other end of the spectrum are donors who are deferred after they donate once or only a few times, suggesting a vulnerability to iron loss from blood donation.

HFE Polymorphisms

Hereditary hemochromatosis is associated with two major polymorphisms affecting the HFE gene, 845 G → A (C282Y) and 187 C → G (H63D). Clinical iron overload occurs in individuals who are either homozygous for C282Y or are C282Y/H63D compound heterozygotes⁵⁹, who cannot upregulate hepcidin to decrease iron absorption. As a result, individuals with hemochromatosis have inappropriately low hepcidin for the amount of iron stores they possess. It is reasonable to think that individuals with HFE polymorphisms might be ideal blood donors, and in fact the Food and Drug Administration has approved a program that allows them to donate more frequently than regular donors.⁶⁰ In a large population survey, Beutler found a significantly lower prevalence of iron depletion without anemia (defined as a transferrin saturation of <16%, serum ferritin < 21 ng/ml and hemoglobin concentration < 12 g/dl) in female C282Y carriers compared with controls: 1.9% vs 4.1% in all women; 4.1% vs 9.2% in women under 50 years of age.⁶¹ No protective effect of HFE polymorphisms was observed on the frequency of iron deficiency anemia (which affected only 3.3% of women between 25-50 years of age). These results are consistent with a relatively modest effect stemming from defective hepcidin regulatory function as a consequence of HFE mutations, leading to relatively increased iron levels compared to control individuals.

HFE polymorphisms are quite common, being found in ~ 34 % (10% C282Y and 24% H63D) of the US Caucasian population.⁶² Similar or only slightly higher prevalence rates have been found in blood donors (Table 3), even among high intensity donors who might be at an advantage to absorb more iron to compensate for the physiological stress of repeated iron losses and avoid the development of iron deficiency anemia. Could HFE polymorphisms be protective of iron balance and hemoglobin levels in regular blood donors? Mast, et al genotyped first-time and repeat blood donors participating in the REDSIII-RISE study, who were assessed for iron and hemoglobin status at each donation up to 24 months. Among first time donors the proportion of those without HFE mutations was lower than a large population database reported previously, 66.7% vs. 76.0% ($p=0.0006$)⁶³, suggesting blood donors (or those that volunteer participate in clinical trials) may be “enriched” for HFE. There was no difference overall between first time and repeat donors in HFE at enrollment, except the frequency of H63D was found to be higher in black repeat donors compared to first-time donors, 15.8% vs. 3.8% ($p=0.01$). First time donors with two HFE polymorphisms had significantly higher ferritin, CHr, and hemoglobin levels initially, and carriers had slightly higher levels. However, after donating blood a few times the changes in iron and hemoglobin status decreased in parallel to donors who did not have these polymorphisms. Laboratory measures that were found to affect iron and hemoglobin

changes longitudinally included ferritin and CHr, which were suggested as better indicators of iron status and ability to donate repeatedly than HFE status. Similar analysis of another genetic polymorphism, the transferrin (G227S) gene was also performed. This mutation is associated with a reduction in TIBC and predisposes menstruating women to iron deficiency⁶⁴. However, blood donors in the RISE study with this variant gene did not show increased risk of iron depletion or iron deficiency anemia greater than controls.⁶³

TMPRSS6 and other Genomic Studies

With the recognition of hepcidin as the central iron regulatory hormone that controls body iron,⁶⁵ other molecules involved in the regulation of hepcidin biosynthesis have been identified along with polymorphisms that alter hemoglobin and iron status in recent genome wide association studies (GWAS).⁶⁶ TMPRSS6 is a membrane associated serine protease that decreases hepcidin biosynthesis. TMPRSS6 knockout mice have iron deficiency and microcytic anemia.⁶⁷ SNPs in the TMPRSS6 gene are associated with changes in hemoglobin concentration.⁶⁸ One of the SNPs, rs855791, encoding a valine to alanine amino acid change, was strongly associated with hemoglobin levels and changes in MCV and it appears that it affects TMPRSS6 mediated changes in hepcidin expression, thereby influencing iron metabolism.⁶⁹ Mast, et al., analyzed the A736V TMPRSS6 polymorphism and found approximately 40% AA, 40% AV, and 20% VV variants in a random sample.⁷⁰ For females, average hemoglobin was 0.74 and 0.53 g/dL higher in AA ($p<0.0001$) and A/V ($p=0.0057$) than in VV, respectively. This correlated with average ferritin, which was 77% and 54% higher in AA ($p=0.0024$) and A/V ($p=0.022$) than in VV donors. The female AA donors maintained 0.35-0.50 g/dL significantly higher hemoglobin levels over the course of successive donations. Although preliminary, these investigations have identified TMPRSS6 genotype as a common polymorphism of iron status that may influence individual responses to blood donation.

There is another example of a high prevalence polymorphic gene that impacts hemoglobin levels and tolerance to repeated blood donation.⁷¹ Hypoxia inducible factor [HIF]-1- α , is involved in iron homeostasis by increasing erythropoietin, and suppressing hepcidin expression in the liver under conditions of hypoxia. Men who were carriers of the *HIF-1 α* P-582-S polymorphism (affecting 25-30% of reported subjects) were found to have higher hemoglobin and ferritin levels than individuals homozygous for the wild-type allele. In addition, the HIF-1 α polymorphism protected blood donors from developing iron deficiency and was shown to reduce low hemoglobin and low ferritin deferrals, allowing more intensive blood donation activity.

These seminal studies utilizing genomic analysis make it likely that there are additional polymorphisms in other iron regulatory proteins that account for either high intensity donation success or vulnerability in becoming anemic and being deferred. Careful comparison with phenotypic data including longitudinal studies using measures of hemoglobin and iron status will need to be performed to determine their overall usefulness. Along these lines, the NHLBI-sponsored REDSIII group has initiated a study called “RBC-Omics”⁷² which will examine the hypothesis that genetic variation underlies the different ability of individuals to repeatedly donate blood without low hemoglobin deferral, or to

develop adverse consequences associated with iron depletion, including pica (the compulsive eating of non-nutritional substances such as ice) and Restless Legs Syndrome (involuntary spastic leg movements, occurring typically at night), which are reported in 10-20% of iron depleted blood donors. Investigations will include exome sequencing, genome-wide association study (GWAS) analyses, and metabolomic studies. This and similar studies are expected to impact transfusion medicine by providing genomic markers of a wide range of red cell parameters including hemoglobin production and iron metabolism, ultimately leading to a better understanding of the determinants of pica and RLS. Increasingly available genetic information from this study and others may one day allow customized recommendations that enhance donor safety and reduce adverse effects of those who voluntarily participate in the blood donation experience.

References

1. Whitaker, BI.; Hinkins, S. The 2011 National Blood Collection and Utilization Survey Report. The United States Department of Health and Human Services; Available at: <http://www.hhs.gov/ash/bloodsafety/2011-nbcus.pdf>
2. Finch CA, Cook JD, Labbe RF, Culala M. Effect of blood donation on iron stores as evaluated by serum ferritin. *Blood*. 1977; 50:441–447. [PubMed: 884321]
3. Simon TL, Garry PJ, Hooper EM. Iron stores in blood donors. *JAMA*. 1981; 245:2038–2043. [PubMed: 7230400]
4. Cable RG, Glynn SA, Kiss JE, Mast AE, Steele WR, Murphy EL, Wright DJ, Sacher RA, Gottschall JL, Tobler LH, Simon TL. Iron deficiency in blood donors: the REDS-II Donor Iron Status Evaluation (RISE) study. *Transfusion*. 2011 Epub ahead of print. 10.1111/j.1537-2995.2011.03401.x
5. Jahanshad N, Kohannim O, Hibar DP, et al. Brain structure in healthy adults is related to serum transferrin and the H63D polymorphism in the HFE gene. *Proc Natl Acad Sci USA*. 2012; 109:e851–e859. [PubMed: 22232660]
6. Kravenbuehl PA, Battegay E, Breymann C, et al. Intravenous iron for the treatment of fatigue in nonanemic, premenopausal women with low serum ferritin concentration. *Blood*. 2011; 118:3222–7. [PubMed: 21705493]
7. Brownlie T 4th, Utermohlen V, Hinton PS, Haas JD. Tissue iron deficiency without anemia impairs adaptation in endurance capacity after aerobic training in previously untrained women. *Am J Clin Nutr*. 2004; 79:437–43. [PubMed: 14985219]
8. Murray-Kolb LE, Beard JL. Iron treatment normalizes cognitive functioning in young women. *Am J Clin Nutr*. 2007 Mar; 85(3):778–87. [PubMed: 17344500]
9. Bryant BJ, Yau YY, Arceo SM, Hopkins JA, Leitman SF. Ascertainment of iron deficiency and depletion in blood donors through screening questions for pica and restless leg syndrome. *Transfusion*. 2013; 53:1637–44. [PubMed: 23305102]
10. Spencer BR, Kleinman S, Wright DJ, et al. Restless legs syndrome, pica, and iron status in blood donors. *Transfusion*. 2013 Aug; 53(8):1645–52. [PubMed: 23763445]
11. Hallberg L, Hulthen L, Gramatkovski E. Iron absorption from the whole diet in men: how effective is the regulation of iron absorption? *Am J Clin Nutr*. 1997; 66:347–56. [PubMed: 9250114]
12. Garry PJ, Koehler KM, Simon TL. Iron stores and iron absorption: effects of repeated blood donations. *Am J Clin Nutr*. 1995; 62:611–20. [PubMed: 7661124]
13. Cook JD, Flowers CH, Skikne BS. The quantitative assessment of body iron. *Blood*. 2003; 101(9):3359–64. [PubMed: 12521995]
14. Suominen P, Punnonen K, Rajamäki A, Irjala K. Serum transferrin receptor and transferrin receptor ferritin index identify healthy subjects with subclinical iron deficits. *Blood*. 1998; 92(8):2934–9. [PubMed: 9763580]

- 14a. Skikne BS, Flowers CH, Cook JD. Serum transferrin receptor: a quantitative measure of tissue iron deficiency. *Blood*. 1990; 75:1870–6. [PubMed: 2331526]
15. Worwood M. Serum transferrin receptor assays and their application. *Ann Clin Biochem*. 2002; 39:221–30. [PubMed: 12038596]
16. Ganz T, Nemeth E. Regulation of iron acquisition and iron distribution in mammals. *Biochim Biophys Acta*. 2006; 1763:690–699. [PubMed: 16790283]
17. Goodnough LT, Nemeth E, Ganz T. Detection, evaluation, and management of iron-restricted erythropoiesis. *Blood*. 2010; 116:4754–61. [PubMed: 20826717]
18. Thomas DW, Hinchliffe RF, Briggs C, Macdougall IC, Littlewood T, Cavill I. on behalf of the British Committee for Standards in Haematology. Guideline for the laboratory diagnosis of functional iron deficiency. *BJH*. 2013; 161:639–48. [PubMed: 23573815]
19. Farrugia A. Iron and blood donation - an under-recognized safety issue. *Advances in Transfusion Safety – Volume IV. Dev Biol*. 2007; 127:137–46.
20. Package Insert, Ferritin. Advia Centaur Assay Manual. 111353 Rev J, 2005-02. 1998 Bayer HealthCare LLC
21. Cook JD, Skikne BS. Serum ferritin: a possible model for the assessment of nutrient stores. *American Journal of Clinical Nutrition*. 1982; 35:S1180–S1185.
22. Cook JD. Serum ferritin as a measure of iron stores in normal subjects. *Am J Clin Nutrition*. 1974; 27:681–87. [PubMed: 4472911]
23. Punnonen K, Irjala K, Rajamäki A. Serum transferrin receptor and its ratio to serum ferritin in the diagnosis of iron-deficiency anemia. *Blood*. 1997; 89:1052–57. [PubMed: 9028338]
24. Hallberg L, Bengtsson C, Lapidus L, Lindstedt G, Scree Lundberg PA, Hultén L. Screening for iron deficiency: an analysis based on bone-marrow examinations and serum ferritin determinations in a population sample of women. *Br J Haematol*. 85:787–798. [PubMed: 7918045]
25. Guyatt GH, Oxman AD, Ali M, Willan A, McIlroy W, Patterson C. Laboratory diagnosis of iron-deficiency anemia: an overview. *J Gen Intern Med*. 1992; 7:145–53. [PubMed: 1487761]
26. Mast AE, Blinder MA, Gronowski AM, Chumley C, Cline Scott MG. Clinical utility of the soluble transferrin receptor and comparison with serum ferritin in several populations. *Clin Chem*. 1998; 44(1):45–51. [PubMed: 9550557]
27. Cable RG, Glynn SA, Kiss JE, Mast AE, Steele WR, Murphy EL, Wright DJ, Sacher RA, Gottschall JL, Tobler LH, Simon TL. Iron deficiency in blood donors: analysis of enrollment data from the REDS-II Donor Iron Status Evaluation (RISE) study. *Transfusion*. 2011; 51:511–22. [PubMed: 20804527]
28. Rosvik AS, Ulvik RJ, Wentzel-Larsen T, Hervig T. The effect of blood donation frequency on iron status. *Transf Apheresis Science*. 2009; 41:165–69.
29. O'Meara A, Infanti L, Stebler C, et al. The value of routine ferritin measurement in blood donors. *Transfusion*. 2011; 51:2183–8. [PubMed: 21517893]
30. Punnonen K, Rajamäki A. Evaluation of iron status of Finnish blood donors using serum transferrin receptor. *Transfusion Medicine*. 1999; 9:131–4. [PubMed: 10354382]
31. Thorpe SJ, Heath A, Sharp G, Cook J, Ellis R, Worwood M. A WHO reference reagent for the Serum Transferrin Receptor (sTfR): international collaborative study to evaluate a recombinant transferrin receptor preparation. *Clinical Chemistry Laboratory Medicine*. 2010; 48:815–820. [PubMed: 20446759]
- 31a. Radtke H, Meyer T, Kalus U, Röcker L, Abdulgabar S, Kiesewetter H, Latza R. Rapid identification of iron deficiency in blood donors with red cell indexes provided by Advia 120. *Transfusion*. 2005; 45:5–10. [PubMed: 15647011]
32. Kiss JE, Steele WR, Wright DJ, Mast AE, Carey PM, Murphy EL, Gottschall JL, Simon TL, Cable RG. NHLBI Retrovirus Epidemiology Donor Study-II (REDS-II). Laboratory variables for assessing iron deficiency in REDS-II Iron Status Evaluation (RISE) blood donors. *Transfusion*. 2013; 53:2766–75. 2013. [PubMed: 23617531]
33. Semmelrock MJ, Raggam RB, Amrein K, Avian A, Schallmoser K, Lanzer G, Semmelrock HJ, Pruessler F, Berghold A, Rohde E. Reticulocyte hemoglobin content allows early and reliable detection of functional iron deficiency in blood donors. *Clin Chim Acta*. 2011; 10.1016/j.cca.2011.12.006

34. Kiss JE, Cable RG, Steele WR, Wright DJ, Bethel J, Birch RJ. Quantification of iron stores and iron absorption in REDS-II iron evaluation study (RISE) donors. *Transfusion*. 2013; 53:S47–030G.
35. Labbe RF, Vreman HJ, Stevenson DK. Zinc Protoporphyrin: A Metabolite with a Mission. *Clinical Chemistry*. 1999; 45:2060–2072. [PubMed: 10585337]
36. Graham EA, Felgenhauer J, Detter JC, Labbe RF. Elevated zinc protoporphyrin associated with thalassemia trait and hemoglobin. *E J Pediatr*. 1996; 129:105–10.
37. Harthoorn-Lasthuizen EJ, Lindemans J, Langenhuijsen MM. Zinc protoporphyrin as screening test in female blood donors. *Clin Chem*. 1998; 44:800–04. [PubMed: 9554491]
38. Baart AM, deKort WL, Moons KG, Atsma F, Vergouwe Y. Zinc protoporphyrin levels have - added value in the prediction of low hemoglobin deferral in whole blood donors. *Transfusion*. 2013; 53:1661–9. [PubMed: 23176250]
39. Baart, AM.; deKort, WL.; Moons, KG.; Atsma, F.; Vergouwe, Y.; de Kort, WL. External validation and updating of a Dutch prediction model for low hemoglobin deferral in Irish whole blood donors. *Transfusion*. 2013. <http://dx.doi.org/10.1111/trf.12211>
40. Gorlin J. Commentary: Iron man pentathlon or “we have met the enemy and they is us!”. *Transfusion*. 2014; 54:747–749. [PubMed: 24617626]
41. Alexander HD, Sherlock JP, Bharucha C. Red cell indices as predictors of iron depletion in blood donors. *Clin Lab Haem*. 2000; 22:253–258.
42. Stern M, O'Meara A, Infanti L, Sigle JP, Buser A. Prognostic value of red blood cell parameters and ferritin in predicting deferral due to low hemoglobin in whole blood donors. *Ann Hematol*. 2012; 91:775–780.10.1007/s00277-011-1371-4 [PubMed: 22147004]
43. Harris N, Kunicka J, Kratz A. The ADVIA 2120 hematology system: flow cytometry-based analysis of blood and body fluids in the routine hematology laboratory. *Lab Hematol*. 2005; 11:47–61. [PubMed: 15790553]
44. Thomas C, Thomas L. Biochemical markers and hematologic indices in the diagnosis of functional iron deficiency. *Clinical Chemistry*. 2002; 48:1066–76. [PubMed: 12089176]
45. Kotisaari S, Romppanen J, Penttilä I, Punnonen K. The Advia 120 red blood cell and reticulocyte indices are useful in diagnosis of iron-deficiency anemia. *Eur J Haematol*. 2002; 68:150–6. [PubMed: 12068795]
46. Kotisaari S, Romppanen J, Agren U, Eskelinen S, Punnonen K. Reticulocyte indices rapidly reflect an increase in iron availability for erythropoiesis. *Haematologica*. 2003; 88:1422–3. [PubMed: 14687997]
47. Ervasti M, Kotisaari S, Heinonen S, Punnonen K. Use of advanced red blood cell and reticulocyte indices improves the accuracy in diagnosing iron deficiency in pregnant women at term. *Eur J Haematol*. 2007; 79:539–45. Erratum in: *Eur J Haematol*. 2008;80:92. [PubMed: 17976190]
48. Buttarello M, Pajola R, Novello E, Rebeschini M, Cantaro S, Oliosi F, Naso A, Plebani M. Diagnosis of iron deficiency in patients undergoing hemodialysis. *Hematopathology Am J Clin Pathol*. 2010; 133:949–54.
49. Brugnara C, Zurakowski D, DiCanzio J, Boyd T, Platt O. Reticulocyte hemoglobin content to diagnose iron deficiency in children. *JAMA*. 1999; 281:2225–30. [PubMed: 10376576]
50. Maccougall IC, Cavill I, Hulme B, Bain B, McGregor E, McKay P, et al. Detection of functional iron deficiency during erythropoietin treatment: a new approach. *BMJ*. 1992; 304:225–6. [PubMed: 1739797]
51. Brugnara C. Iron deficiency and erythropoiesis: new diagnostic approaches. *Clinical Chemistry*. 2003; 49(10):1573–8. [PubMed: 14500582]
52. Nadarajan V, Sthaneshwar P, Eow GI. Use of red blood cell indices for the identification of Iron deficiency among blood donors. *Transfusion Medicine*. 2008; 18:184–9. [PubMed: 18598281]
53. Mast AE, Blinder MA, Lu Q, Flax S, Dietzen DJ. Clinical utility of the reticulocyte hemoglobin content in the diagnosis of iron deficiency. *Blood*. 2002; 99:1489–91. [PubMed: 11830506]
54. Mitsui K, Harada A, Miyata Y. Assessment of iron deficiency in chronic hemodialysis patients: investigation of cutoff values for reticulocyte hemoglobin content. *Clin Exp Nephrol*. 2003; 7:52–7.

55. Bovy C, Gothot A, Delanaye P, Xavier W, et al. Mature erythrocyte parameters as new markers of functional iron deficiency in haemodialysis: sensitivity and specificity. *Nephrol Dial Transplant*. 2007; 22:1156–62. [PubMed: 17237481]
- 55a. Tessitore N, Solero GP, Lippi G, Bassi A, et al. The role of iron status markers in predicting response to intravenous iron in haemodialysis patients on maintenance erythropoietin. *Nephrology Dialysis Transplantation*. 2001; 16:1416–23.
56. Waldvogel-Abramovski S, Waeber G, Gassner C, Buser A, Frey BM, Favrat B, Tissot JD. Iron and transfusion medicine. *Blood Reviews*. 2014; 27:289–95. [PubMed: 24148756]
57. Mast AE, Foster TM, Pinder HL, Beczkiewicz CA, Bellissimo DB, Murphy AT, Kovacevic S, Wroblewski VJ, Witcher DR. Behavioral, biochemical, and genetic analysis of iron metabolism in high-intensity blood donors. *Transfusion*. 2008 Oct; 48(10):2197–204. [PubMed: 18657084]
58. Mast AE, Schlumpf KS, Wright DJ, Custer B, Spencer B, Murphy EL, Simon TL. Demographic correlates of low hemoglobin deferral among prospective whole blood donors. *Transfusion* 2010; *Haematol*. 1993; 85(4):787–98.
59. Feder JN, Gnirke A, Thomas W, Tsuchihashi Z, Ruddy DA, Basava A, Dormishian F, Domingo R, Ellis MC, Fullan A, et al. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. *Nat Genet*. 1996 Aug; 13(4):399–408. [PubMed: 8696333]
60. US Food and Drug Administration. Guidance for Industry: Variances for Blood Collection from Individuals with Hereditary Hemochromatosis. Available at: www.fda.gov/BiologicsBloodVaccines
61. Beutler E, Felitti V, Gelbart T, Waalen J. Haematological effects of the C282Y HFE mutation in homozygous and heterozygous states among subjects of northern and southern European ancestry. *Br J Haematol*. 2003; 120:887–93. [PubMed: 12614226]
62. Adams PC, Reboussin DM, Barton JC, McLaren CE, Eckfeldt JH, McLaren GD, Dawkins FW, Acton RT, Harris EL, Gordeuk VR, et al. Hemochromatosis and iron-overload screening in a racially diverse population. *N Engl J Med*. 2005; 352(17):1769–78. [PubMed: 15858186]
63. Mast AE, Lee TH, Schlumpf KS, Wright DJ, Johnson B, Carrick DM, Cable RG, Kiss JE, Glynn SA, Steele WR, Murphy EL, Sacher R, Busch MP. for the NHLBI Retrovirus Epidemiology Donor Study-II (RES-II). The impact of HFE mutations on hemoglobin and iron status in individuals undergoing repeated iron loss through blood donation. *Br J Haematol*. 2012; 156:388–401. [PubMed: 22118647]
64. Lee PL, Halloran C, Trevino R, Felitti V, Beutler E. Human transferrin G277S mutation for iron deficiency anemia. *Br J Haematol*. 2001; 115:329–33. [PubMed: 11703331]
65. Nemeth E, Ganz T. Regulation of Iron Metabolism by Hepcidin. *Annual Review of Nutrition*. 2006; 26:323–42.
66. Ganesh SK, Zakai NA, van Rooij FJ, Soranzo N, Smith AV, Nalls MA, Chen MH, Kottgen A, Glazer NL, Dehghan A, et al. Multiple loci influence erythrocyte phenotypes in the CHARGE Consortium. *Nat Genet*. 2009 Nov; 41(11):1191–8. [PubMed: 19862010]
67. Du X, She E, Gelbart T, et al. The serine protease TMPRSS6 is required to sense iron deficiency. *Science*. 2008; 320:1088–1092. [PubMed: 18451267]
68. Chambers JC, Zhang W, Li Y, Sehmi J, Wass MN, Zabaneh D, Hoggart C, Bayele H, McCarthy MI, Peltonen L, et al. Genome-wide association study identifies variants in TMPRSS6 associated with hemoglobin levels. *Nat Genet*. 2009 Nov; 41(11):1170–2. [PubMed: 19820698]
69. Benyamin B, Ferreira MA, Willemsen G, Gordon S, Middelberg RP, McEvoy BP, Hottenga JJ, Henders AK, Campbell MJ, Wallace L, et al. Common variants in TMPRSS6 are associated with iron status and erythrocyte volume. *Nat Genet*. 2009 Nov; 41(11):1173–5. [PubMed: 19820699]
70. Mast, AE.; Langer, JC.; Bialkowski, W., et al. The TMPRSS6 Ala736 Val polymorphism is associated with decreased hemoglobin and iron status in females undergoing repeated phlebotomy. Available at: <https://ash.confex.com/ash/2013/webprogram/Paper58169.html>
71. Kleinman S, Busch MP, Murphy EL, et al. The National Heart, Lung, and Blood Institute Recipient Epidemiology and Donor Evaluation Study (REDS-III): a research program striving to improve blood donor and transfusion recipient outcomes. *Transfusion*. 2014 Mar; 54(3 Pt 2):942–55. Epub. 10.1111/trf.12468 [PubMed: 24188564]

Acronyms

CHCM_m	hemoglobin content of mature cells
CHr	reticulocyte hemoglobin content
CRP	C-reactive protein
FDA	Food and Drug Administration
FEP	Free Erythrocyte Protoporphyrin
GWAS	genome wide association studies
HYPOM	hypochromic mature red blood cells
HYPOR	reticulocyte hypochromic red blood cells
IDA	iron-deficiency anemia
IDE	iron-deficient erythropoiesis
MCH	mean cellular hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
R/F	ratio Receptor/Ferritin ratio
RISE	group REDS-II Iron Status Evaluation group
ROC	Receiver Operating Characteristic
sTfR	serum soluble transferrin receptor
TIBC	total iron binding capacity
ZPP	Zinc Protoporphyrin

Box1**Measurements of iron status in blood donors**

- Serum iron/transferrin (% Transferrin Saturation)
- Ferritin
- Soluble transferrin receptor (sTfR)
- Soluble transferrin receptor/ferritin ratio
- Zinc (Free Erythrocyte) Protoporphyrin
- Red blood cell indices (% hypochromic RBC, etc.)

Key points

- Iron depletion is common in blood donors, especially women and frequent donors
- Hemoglobin level is useful to detect anemia but has limited value in assessing blood donor iron status
- Red blood cell indices, such the percentage of hypochromic mature RBC (%HYPOm) and reticulocyte hemoglobin content (CHr) can improve the assessment of iron status over hemoglobin alone
- Current studies suggest that measurement of ferritin at a level of 26-30 ng/ml optimally identifies donors who are iron depleted
- Genetic assessment of iron pathways may reveal new approaches for selecting individuals who are more or less able to donate blood on a regular basis

Figure 1. RISE Study: Effect of previous RBC donation frequency on plasma ferritin level at enrollment

Figure 2. Reticulocyte Hemoglobin Content (CHr): Response in CHr (blue) to iron dextran in a patient unresponsive to oral iron therapy. Hemoglobin increased concomitantly from 7.8 g/dl at Day 0 (red) to 11.2 g/dl at Day 13

Percent Hypochromic Red Cells (%HYPO)

- **Flow cytometry with 2 detectors**
 - High angle for Hb content
 - Low angle for cell size
 - Allows construction of a histogram for Hb content

Figure 3. The effect of depleted iron stores or ESA therapy on flow cytometry detection of %HYPO

Figure 4. RBC Indices for Detection of Iron Deficiency in RISE Donors

Table 1
Findings from REDS-II Donor Iron Status Evaluation (RISE) study: Iron status at enrollment and final visit

	Enrollment Visit		Final Visit(15 m)	
	% IDE	% AIS	% IDE	% AIS
FT Fern	25%	6.6%	51%	20%
RPT Fern	67%	28%	62%	27%
FT Male	2.5%	0%	20%	8%
RPT Male	49%	16%	47%	18%

IDE Iron deficient erythropoiesis log[sTfR/ferritin] 2.07

AIS: Absent Iron Stores, Ferritin 12 ng/ml

Data from Cable RG, Glynn SA, Kiss JE, Mast AE, Steele WR, Murphy EL, Wright DJ, Sacher RA, Gottschall JL, Tobler LH, Simon TL. Iron deficiency in blood donors: the REDS-II Donor Iron Status Evaluation (RISE) study. *Transfusion*. 2012;52:702-11.

Table 2
RBC Indices Definitions

Channel	Parameter*	Cell Population
Mature – reflects Fe incorporation during 3 month lifespan of mature RBCs	HYPOM	% of mature RBC population with HGB less than 280 g/L
	CHCMm	Mean corpuscular Hgb concentration in mature cells (g/L)
Retic – reflects Fe incorporation into 3 day lifespan of reticulocytes	HYPOR	% of retics with HGB less than 280 g/L
	CHr	Cellular Hgb content of reticulocytes (pg)

* ADVIA 120 Hematology Analyzer (Siemens Corp) terminology

Sysmex XE2100 utilizes similar technology: RET-Y (compares with CHr) & RBC-Y (compares with HYPOM)

From ADVIA® 120 Hematology System Operator's Guide: Glossary Version 1.02.00, Copyright © 1997, 2002 Bayer Corporation; with permission.

Table 3
Genetic Alterations and Iron Status in Blood Donors

Genetic Polymorphism	Iron Pathway Effect
HFE: C282Y, H63D	Hepcidin regulation(<i>increased</i> Fe absorption)
TF G277S	Transferrin mutation (<i>decreased</i> iron transport and iron deficiency anemia)
Hypoxia Inducible Factor 1 α (HIF-1 α)	Erythropoietin and hepcidin regulation (<i>increased</i> RBC production and iron absorption)
TMPRSS6	Hepcidin regulation (<i>decreased</i> iron absorption)

Genotype Data Rise Study [Data from Mast AE, Lee TH, Schlumpf KS, Wright DJ, Johnson B, Carrick DM, Cable RG, Kiss JE, Glynn SA, Steele WR, Murphy EL, Sacher R, Busch MP, for the NHLBI Retrovirus Epidemiology Donor Study-II (RES-II). The impact of HFE mutations on hemoglobin and iron status in individuals undergoing repeated iron loss through blood donation. Br J Haematol. 2012; 156: 388-401.].

HFE genotype: Wild Type 1,568 (64.7%)

Heterozygous H63D 573 (23.6%)

Heterozygous C282Y 194 (8.0%)

Homozygous H63D 39 (1.6%)

Homozygous C282Y 7 (0.3%)

Double Mutation 41 (1.7%)

Missing 3 (0.1%)

TF G277S Genotype: Wild Type 2,107 (86.9%)

Hetero- or Homozygous 254 (10.5%)

Missing 64 (2.6%)