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Iron Deficiency in Blood Donors

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Laboratory variables for assessing iron deficiency in REDS-II Iron Status Evaluation (RISE) blood donors

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Abstract

BACKGROUND—Iron deficiency is common in regular blood donors. We evaluated the diagnostic sensitivity and specificity of red blood cell (RBC) hematology analyzer indices to assess iron status as a part of donor management.

STUDY DESIGN AND METHODS—A total of 1659 male and female donors from the Retrovirus Epidemiology Donor Study-II (REDS-II) Donor Iron Status Evaluation (RISE) study who were either first-time/reactivated (FT/ RA; no donations for 2 years) or frequent donors were recruited into a longitudinal study of regular donation of RBCs. Of these, 1002 donors returned 15 to 24 months later for a final assessment. Absent iron stores (AIS) was defined as plasma ferritin level of less than 12 μg/L. Logarithm of the ratio of soluble transferrin receptor to ferritin of at least 2.07 (≥97.5% in FT/RA males) was used to define iron-deficient erythropoiesis (IDE). Receiver operating characteristics analysis was performed to assess selected RBC indices (e.g., percentage of hypochromic mature RBCs, proportion of hypochromic mature RBCs [HYPOm], and hemoglobin [Hb] content of reticulocytes [CHr]) in identifying AIS and IDE.

RESULTS—HYPOm and CHr detected IDE with comparable sensitivity, 72% versus 69%, but differed in specificity: HYPOm 68% and CHr 53%. For detecting AIS, sensitivity was improved to 85% for HYPOm and 81% for CHr but specificity was reduced for both. Venous Hb had high specificity but poor sensitivity for IDE and AIS. A plasma ferritin level of less than 26.7 u.g/L was a good surrogate for assessing IDE.

CONCLUSION—RBC indices correlate with AIS and IDE and are more informative than Hb measurement, but lack sufficient sensitivity and specificity to be used as diagnostic tools in blood donors at risk for iron deficiency.

Iron deficiency is a frequent condition that arises as a direct consequence of regular blood donation in both men and women, who lose approximately 230 mg of elemental iron with each whole blood donation. This amount represents approximately 25% of the average iron

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CONFLICT OF INTEREST

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stores in men and approximately 75% of the iron stores in women.[1] The impact of repeat blood donation on donor iron stores has been recognized for more than 30 years.[2,3] Iron depletion occurs in progressive stages beginning with the loss of storage iron, the development of iron-deficient erythropoiesis (IDE), and finally, iron-deficiency anemia.[4] The current minimum hemoglobin (Hb) requirement of 12.5 g/dL for blood donors in the United States is intended to prevent anemia but does not prevent the development of iron deficiency.

Older studies of iron status in blood donors used various biochemical markers including serum iron, serum transferrin, transferrin saturation, and serum ferritin levels.[2,3] In these studies, iron depletion was considered to be present if the ferritin concentration was below 12 μg/dL. This cutoff is a highly specific indicator of iron deficiency but lacks sensitivity,[5,6] with the result that studies in blood donors have failed to identify iron deficiency in over one-third of cases.[7,8] Other investigators have found that a higher ferritin concentration, between 22 and 40 μg/dL, better reflects functional iron depletion.[4,9,10] These findings were based on more sensitive measures of iron status such as serum (soluble) transferrin receptor (sTfR) levels, which reflect the functional iron compartment and have been shown to correlate with depleted iron stores in marrow preparations.[11] Moreover, ferritin measurements (which reflect storage iron) and sTfR values have been combined into a ratio, logarithm of the ratio of sTfR to ferritin (log[sTfR/ferritin]), as a derived measurement.[1,12] The combined use of the two reciprocally regulated measures appears to provide excellent discrimination of clinical iron deficiency, and early experience in blood donors suggests high sensitivity in the detection of iron depletion.[7]

As in other studies,[2,3,11,13,14] we have previously reported a high prevalence of iron deficiency in blood donors participating in the Retrovirus Epidemiology Donor Study-II (REDS-II) Donor Iron Status Evaluation (RISE) study.[8] The question has been raised as to whether laboratory monitoring can be effectively used to monitor and prevent iron depletion.[15,16] Although biochemical tests of iron status have been useful as part of investigational studies, they may not be well suited for blood donor screening because of their expense and the difficulty with quick turnaround in a blood center. Rapid assessment of functional iron status may be performed by measuring red blood cell (RBC) indices using certain types of hematology analyzers, for example, the ADVIA 120(Siemens Healthcare Diagnostics, Deerfield, IL) and the Sysmex XE-5000 (Kobe, Japan). In functional iron deficiency, a reduction in the Hb concentration of RBCs results from an imbalance between iron supply and iron requirements of erythropoiesis. Analysis of the fraction of individual RBCs with deficient Hb concentration by laser scatter reflects recent changes in erythropoiesis, which may be at least as good and possibly superior to biochemical markers.[17] Reticulocyte Hb content (CHr) is an indicator of iron availability for RBC production over the previous 4 days (lifespan of the reticulocyte in the marrow and circulation), whereas the proportion of hypochromic mature RBCs (HYPOm) is a time-averaged marker (iron availability within the 3-month lifespan of mature RBCs). In this study, we sought to determine the diagnostic value of these RBC indices in evaluating iron deficiency in newly active and repeat blood donors enrolled in the National Heart, Lung, and Blood Institute's (NHLBI) RISE study and to compare them to sensitive biochemical markers. We also evaluated the relationship between venous Hb and iron measurements.

MATERIALS AND METHODS

Study population and design

Data were obtained from the RISE study conducted from December 2007 until December 2009. Four of the six REDS-II blood centers were able to participate in this sub-study to evaluate selected RBC indices because of their access to the required hematology analyzers.

The four centers performing testing for RBC indices during the RISE enrollment and final visit study periods were: the American Red Cross New England Region (Dedham, MA); Blood Center of Wisconsin (Milwaukee, WI); Hoxworth Blood Center/University of Cincinnati Academic Health Center (Cincinnati, OH); and the Institute for Transfusion Medicine (Pittsburgh, PA). Details on the entire enrolled study population, eligibility requirements, and enrollment procedures have been reported previously.[8,18]

There were 1659 donors enrolled in RISE at the four centers participating in this analysis and of these donors, 1002 returned at least 15 months after their enrollment visit for a final study visit. There were four cohorts of donors in the RISE study: two first-time/reactivated (FT/ RA) donor cohorts each consisting of approximately equal numbers of men and women who had either never given blood before (FT) or had not given a donation in the 2 years before enrollment (RA), and two frequent donor cohorts consisting of approximately equal numbers of men and women who had given three or more whole blood donations in the past year (males) and two whole blood donations (female) in the past year or equivalent double RBC donations. At each study visit fingerstick Hb or hematocrit (Hct) was used to assess eligibility to donate using routine procedures at each center. Deferred donors were not enrolled in the study, but a subject could be Hb and/or Hct deferred at any follow-up visit.

Laboratory testing

Biochemical measures—An ethylenediaminetetraacetate (EDTA) plasma sample from each donor was frozen at −20°C and tested to determine ferritin and sTfR(plasma concentrations are approximately 5% lower than those measured in serum).[19] Ferritin was measured using a ferritin assay (ADVIA Centaur, Siemens Healthcare Diagnostics), an immunoassay using direct chemiluminometric technology and a constant amount of two anti-ferritin (Siemens Healthcare Diagnostics). A particle-enhanced immunoturbidimetric assay was used to detect sTfR, in which latex-bound anti-sTfR react with the antigen in the sample to form an antigen/antibody complex that is measured turbidimetrically (Tina-quant sTfR assay, Roche Diagnostics, Indianapolis, IN). Both tests were performed by ARUP Laboratories (Salt Lake City, UT). Biochemical testing was done on all samples at the enrollment and final visits (n = 1659 enrollment/n = 1002 final). This yielded 1002 subjects for paired analysis of biochemical measures.

RBC indices—RBC index testing was done on 1624 of 1659 enrollment visits and 850 of 1002 final visits, yielding paired results (a measurement at both enrollment and final visits) for 788 donors. A blood sample was collected in an EDTA tube either from the predonation diversion pouch or from a postdonation collection (in 12% of enrollment samples and 3% of final samples postdonation Hb values were converted to an estimated predonation Hb by a validated method using the formula

$$\text{Pre vHb (g/dL)} = \text{Post vHb} + 0.8423 - (0.002035 \times \text{Weight [lbs]}).$$[8]

Specimens were kept at room temperature and sent via courier to a local laboratory where testing was completed within 24 hours of collection. ADVIA 2120 or ADVIA 120 hematology systems were used.[20] This flow cytometry– based system uses light scatter, differential white blood cell (WBC) lysis, and myeloperoxidase and oxazine 750 staining to provide a complete blood cell count, a WBC differential, and a reticulocyte count. A cyanide-free method is used to measure Hb colorimetrically. This analysis included only the following variables from the reported RBC values: HYPOm, mean corpuscular Hb concentration of mature cells (CHCMm), percentage of hypochromic reticulocyte RBCs (HYPOr), venous Hb, CHr, and mean corpuscular volume (MCV).

The manufacturer recommends sample testing within 6 hours of draw to avoid a potential effect on some variables as a result of cell swelling that may affect measurement of Hb concentration, that is, HYPOm, HYPOr, CHCM, and MCV, but not Hb content, that is, CHr.[21–23] Only one site was able to test within 6 hours. We found no loss of sensitivity/specificity between 24-hour and shorter (within 6 hr) analysis times: The center with the shortest time to test, a median of 248 minutes, had receiver operating characteristic (ROC) area under the curve (AUC) results for %HYPOm = 0.72 and CHr = 0.69, whereas the three other centers with tests run 1107 to 1341 minutes after collection had higher AUCs for HYPOm (0.75–0.84) and AUC values for CHr, which "bracketed" that of the center with shorter analysis time, 0.65 to 0.77.

The results of the center with the shortest time to test were not statistically different from the other three centers on any of the ADVIA measures (data not shown).

Determination of iron status

A subject was classified as having absent iron stores (AIS) if his or her plasma ferritin at the enrollment visit was less than 12 mg/L. IDE was defined as being present if the log of the ratio of sTfR to ferritin (log[sTfR/ferritin]) was 2.07 or greater. This value corresponded to the 97.5th percentile of the distribution of the log(sTfR/ferritin) in the entire RISE cohort of FT/RA males at enrollment.[8,19]

Statistical analysis

Median values and percentile distributions were calculated for all measures of interest at enrollment and at the final visit. ROC analysis[9] was performed to assess the diagnostic efficiency (AUC is a measure that combines sensitivity and specificity)of the biochemical and the selected RBC indices in detecting two outcomes, AIS and IDE. Sensitivity was defined as the proportion of donors with values defined as abnormal on the measure of interest among donors with AIS and/or IDE. Specificity was calculated as the proportion of donors with normal values on the measures of interest among donors without AIS and/or IDE. Paired sign tests were used to assess if there were longitudinal changes (from enrollment to final) in RBC indices and biochemical measures by cohort. Longitudinal multivariate analysis was used to assess within and between correlation among RBC indices and biochemical measures. All analyses were done using computer software (SAS 9.2, 2008; SAS Institute, Inc., Cary, NC).

RESULTS

RBC indices and biochemical measurements of iron at enrollment and final visits

Tables 1A and 1B show the medians and ranges (2.5th to 97.5th percentile) for the biochemical and RBC measures overall and stratified by cohort at enrollment (Table 1A) and final visits (Table 1B). The percentage of donors with AIS and IDE overall and by cohort is also presented. As previously published, evidence of iron depletion (as measured by AIS and IDE) increases during the study period in FT/RA donors and persists in frequent repeat donors.[19] Consistent with these previous findings, increasing levels of hypochromic RBCs (HYPOm and HYPOr) were observed from enrollment to final visits, especially in female donors (e.g., in FT/RA females HYPOm increased more than threefold, a median of 0.3% to a median of 1.1% at final visit; $p < 0.0001$). Evidence of iron-restricted erythropoiesis was also seen in cellular Hb content, indicated by decreased CHr, CHCMm, Hb, and MCV.

Figure 1 presents the median, 2.5th, 25th, 75th, and 97.5th percentiles as box plots for ferritin, log(sTfR/ ferritin), HYPOm, and CHr among donors who had both an enrollment

and a final visit. Paired analysis (i.e., difference between enrollment and final visit) shows significant intradonor changes among FT/RA donors in iron depletion as measured by ferritin ($p < 0.0001$) and the log(sTfR/ ferritin) ratio ($p < 0.0001$). Parallel changes are seen with both HYPOm and CHr. Intradonor changes in biochemical and RBC indices were less pronounced in the frequent donor cohort with only CHr continuing to show a significant decrease over time. Iron status and RBC indices were correlated between subjects (for example, interdonor correlation between HYPOm and ferritin was $r = -0.58$) and within subjects (for example, intradonor correlation between HYPOm and ferritin was $r = -0.24$).

Relationship between laboratory measures and iron status

ROC analysis of the RBC indices in detecting IDE (Table 2A) and AIS (Table 2B) was performed separately for the enrollment and final visits and the biochemical variables were evaluated by correlation to IDE. Among the RBC variables, HYPOm had the highest AUC for detection of IDE followed by CHCMm and HYPOr (Table 2A, Fig. 2A). The least discriminative measures for detecting AIS in the enrollment subset were MCV and CHr, with the others, including venous Hb in between (Table 2B, Fig. 2B). The RBC index variables have greater efficiency (higher AUCs) in detecting AIS than IDE (all differences significant, $p \leq 0.007$). ROC analysis using the final visit data set showed decreased AUCs for HYPOm, CHCMm, and HYPOr compared to those derived from the enrollment visit and stable or slightly increased AUC values for venous Hb, CHr, and MCV (Tables 2A and 2B).

Sensitivity and specificity of measures for detecting IDE and AIS at specific cutoffs at enrollment

The ROC analysis is further summarized by the sensitivity and specificity of studied measures in detecting IDE and AIS in Tables 3A and 3B. Cutoff values were determined by using normal reference ranges (e.g., CHr < 28 pg) or conventionally accepted values (e.g., venous Hb ≤12.5 g/dL) or by optimizing sensitivity and specificity using ROC curve analysis (e.g., CHr 32.6 pg). Most of the RBC index measures were in the 70% sensitivity/ 70% specificity range except CHr. MCV and venous Hb had poor sensitivity but good specificity at conventional thresholds. Using CHr and HYPOm in conjunction did not improve sensitivity and specificity. The currently accepted eligibility cutoff of 12.5 g/dL Hb in the United States for both sexes yielded a highly specific but insensitive indicator of IDE and AIS. Even if a suggested higher Hb eligibility threshold of 13.5 g/dL for males were used, it would detect only 23% of IDE in male donors.

The correlation of ferritin with IDE was −0.96, and the correlation of sTfR with IDE was 0.54. Multivariate regression analysis showed that log(sTfR/ferritin) of 2.07 equated to a ferritin level of 26.7 μg/L. With this value as the cutoff, ferritin had 95.1% sensitivity and 89.6% specificity to detect IDE. This “optimized” value is consistent with those in other studies.[9,10]

DISCUSSION

The primary objective of this study was to identify the optimal laboratory measures to identify IDE and AIS in active whole blood and double RBC donors. We also examined the ability of Hb to identify iron depletion in presenting donors. The ideal test should have reasonable diagnostic accuracy and be easy to obtain and interpret. The best RBC index was %HYPOm for both AIS and IDE. Hb was much less informative. Of the biochemical tests we found ferritin (at 26.7 μg/L in plasma samples) to be the most useful indication of IDE. sTfR added relatively little to ferritin in predicting IDE. Assessing iron status of blood donors is being considered for operational implementation because of the potential impact on donor health.[24] Adverse effects reported to occur with iron deficiency, even in the

absence of anemia, include fatigue,[25,26] decreased exercise capacity,[27] reduced cognitive function in children and adolescents,[28] and restless leg syndrome.[29,30] Increasing concerns related to these health effects in donors prompted the Australian Red Cross to recommend yearly ferritin testing in frequent donors[16] and the AABB to recommend steps to monitor and prevent iron deficiency in donors.[31] Identification of iron deficiency would potentially facilitate strategies, such as reducing donation frequency or taking oral iron supplements, to improve iron stores. This would be expected to decrease side effects referable to iron deficiency and also Hb deferrals.

RBC variables have been shown to be useful in the detection of iron deficiency in several different iron-deficient populations, including end-stage renal disease patients on recombinant human erythropoietin (rHuEPO) therapy, in pregnant women, and in children.[23,32,33] This technology utilizes incident light scatter to measure cell size and Hb concentration to quantify Hb content in both reticulocytes and mature RBCs. The RBC indices have been found to be even more sensitive indicators of functional iron deficiency than biochemical iron tests in renal failure patients receiving rHuEPO.[34] This may be especially true in renal disease patients and other hospitalized patient populations where serum ferritin is an unreliable indicator of iron stores because it is an acute-phase response protein. In a study of female students with iron deficiency anemia, Kotisaari and colleagues[21] found excellent correlation between sTfR and HYPOm (AUC = 0.98), as well as correction of the abnormal RBC variable in all iron-deficient subjects after oral elemental iron replacement.

Our study suggests that measurement of the proportion of hypochromic RBCs (HYPOm or HYPOr) and CHCMm provide marginally better results than CHr and these assays are best at identifying more severe iron depletion (AIS). Using the lower limit normal reference value of 28 pg for CHr resulted in even worse sensitivity (7%), and the test was no better than using an MCV of 80 fL (Table 3). In a recent single-center study in blood donors, CHr using a cutoff value of 28 pg was reported to have excellent specificity with better sensitivity of 27.3% (males) to 39.1% (females) for detection of AIS.[35] The reason for lower diagnostic efficiency of CHr in our study is uncertain. Although some studies report equal or better results using CHr,[35–37] HYPOm is superior in others, including several hemodialysis and EPO treatment trials[38,39] and studies of iron-deficient young women and hospitalized patients with milder degrees of anemia.[22] Similar to our results, a study performed in iron and recombinant EPO-treated chronic hemodialysis patients also found that a higher cutoff for CHr, 32 pg, improved the sensitivity and specificity of this test in the diagnosis of iron deficiency.[38] As might be expected because of immediate uptake of iron into developing reticulocytes, HYPOr and CHr show more rapid correction than HYPOm after iron replacement in anemic young women.[39] We hypothesize that perhaps the reticulocyte Hb compartment is preserved because regular, committed blood donors are able to compensate for iron lost from blood donation by absorbing more dietary iron and/or by taking iron supplements. Veteran donors, many of whom have been deferred for Hb, may consciously (as they are frequently instructed by the blood center) or unconsciously consume more iron-rich foods and/or iron supplements leading up to their scheduled donation, thus replenishing the reticulocyte Hb compartment and blunting CHr as a measure of IDE. Nevertheless, CHr ranked low as a predictor of AIS and IDE in our study (Table 2).

Two previous groups have employed the ADVIA analyzer in cross-sectional studies to evaluate iron status in blood donors. Radtke and colleagues[7] found slightly lower sensitivity of CHr and HYPOm individually (57%) and combined (69%) in the identification of IDE with better specificity (approx. 90%) than we found in our study. In their analysis, a ferritin level of 20 μg/L or less proved to have the ideal combination of sensitivity and specificity at 88.3 and 92.3%, respectively. In a smaller group of donors Nadarajan and coworkers[40]

found 81 and 89% sensitivity and specificity for RBC-Y (a HYPOm equivalent variable) and 69 and 93% for CHr at 28 pg cutoff, along with 100% sensitivity and 90% specificity for serum ferritin at 15 μg/L. However, there is an inherent bias favoring ferritin comparing this measure vis-à-vis ADVIA variables in a ROC analysis utilizing the sTfR/ferritin ratio as the "gold standard," so we have not analyzed our data in this fashion. Radtke and colleagues and Nadarajan and colleagues used 95th percentile log (sTfR/ferritin) in both males and females, which resulted in a higher ratio of 2.5 to 2.6 to define IDE. This definition is less sensitive than the criterion used in RISE, resulting in their underestimating the prevalence of IDE (8.2% prevalence reported in Radtke and 10% in Nadarajan compared to 42% overall at enrollment in RISE). However, the greater donation frequency in recruited RISE donors is also a factor contributing to the disparate prevalence. Use of nonanemic females with subclinical iron deficiency as "normal" in studies of iron-deficient populations masks the true frequency of this condition.[4,41]

From the AUC analysis (Tables 2A and 2B) and sensitivity and specificity data presented in Table 3, ADVIA RBC measures clearly outperform Hb in the detection of IDE. Hb is an inexact measure of iron depletion because anemia is a late manifestation of iron deficiency. Most donors who are deferred for the current Hb level of 12.5 g/dL or less cutoff are iron deficient but this threshold allows a considerable number of iron-deficient individuals to continue to donate. This is especially true in male donors because men are frankly anemic if their Hb falls under 13.0 g/dL.[42] Using the HYPOm or CHr cutoff values in a particular donor would detect IDE in approximately 70% of "true-positive" individuals while falsely classifying approximately 32% (HYPOm) and approximately 47% (CHr). A plasma ferritin value of 26.7 μg/L (comparable serum level of approx. 30 μg/L) provides the easiest approach with the best discriminatory value overall. Use of ancillary testing in this manner could be used in conjunction with the high specificity and low sensitivity of the 12.5 g/dL Hb standard.

A limitation to our study relates to the logistics of sample processing and testing. We were unable to reliably test samples on the ADVIA instrument within 6 hours of draw as recommended by the manufacturer. From a technical standpoint, swelling of RBCs as they sit over longer intervals may alter certain variables, especially the HYPOm and HYPOr. Although this could have artifactually increased sensitivity and reduced specificity, we saw no evidence of increased sensitivity based on AUC values of donors tested within 6 hours and donors tested after overnight hold but within 24 hours. Another limitation of the study is that although log(sTfR/ferritin) has been shown to reliably estimate body iron content, iron deficiency was not assessed by direct examination of iron in marrow macrophages or response to iron administration.

Since there is a greater proportion and severity of AIS and IDE at the final visit, the reason for the decline in discriminative value of some RBC index measures (e.g., HYPOm) as indicators is unclear. While the enrollment visit sample size (1659) is much larger than the final visit sample size (1002), we hypothesize this decline may be as a result of the study population becoming more homogenous at final visit (no FT donors at final visit), and as shown, within-subject correlation is not as good as between-subject correlation. This presents a limitation in the assessment of iron status based on longitudinal RBC index changes of an individual donor. The enrollment visit venous Hb also appears to be less discriminative compared to final visit venous Hb probably because of less range in Hb values at enrollment, since the donor was required to pass the fingerstick Hb screen (≥12.5 g/dL for entry into study).

It is apparent from Table 3 that regardless of the Hb threshold used in blood donor screening there is considerable uncertainty in the iron status of the donor (iron replete, IDE, or AIS). In

light of the high prevalence of iron deficiency, increasing the acceptable Hb level to qualify to donate blood will have a relatively modest impact on this problem. In deciding whether it is worthwhile to change the Hb standard(s) for blood donors it is important both to assess the adverse effects conferred by iron deficiency (including its severity) and to determine the impact on blood availability to meet the needs for transfusion.

Tests to screen for a condition causing great harm to a subject should have a high degree of sensitivity whereas a test with higher specificity may be suitable if the condition has milder adverse consequences, particularly if there are other undesirable consequences of high sensitivity testing, such as reducing blood availability or adverse consequences of false-positive diagnoses of iron deficiency in donors. At the present time the true impact of iron deficiency on the health of blood donors remains a matter of active scientific inquiry. Therefore, the most appropriate choice of a Hb threshold may be related to the original intended purpose, that is, to prevent anemia, rather than iron deficiency. Ancillary tests (especially ferritin levels) can be employed on at-risk donors to more accurately diagnose iron deficiency and to prevent anemia resulting in Hb deferral. The RISE study has identified frequency of blood donation as the most important determinant of donor iron depletion.[8] This study supports using the ferritin assay to optimally assess iron status and should lead to operationally feasible testing strategies to reduce the high prevalence of iron deficiency in blood donors.

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ABBREVIATIONS

AIS	absent iron stores
AUC	area under the ROC curve
CHCMm	mean corpuscular Hb concentration of mature cells
CHr	hemoglobin content of reticulocytes
FT	first time
HYPOm	percentage of hypochromic mature RBCs
HYPOr	percentage of hypochromic reticulocyte RBCs
IDE	iron-deficient erythropoiesis
log(sTfR/F)	logarithm of the ratio of sTfR to ferritin
RA	reactivated
ROC	receiver operating curve
sTfR	soluble transferrin receptor

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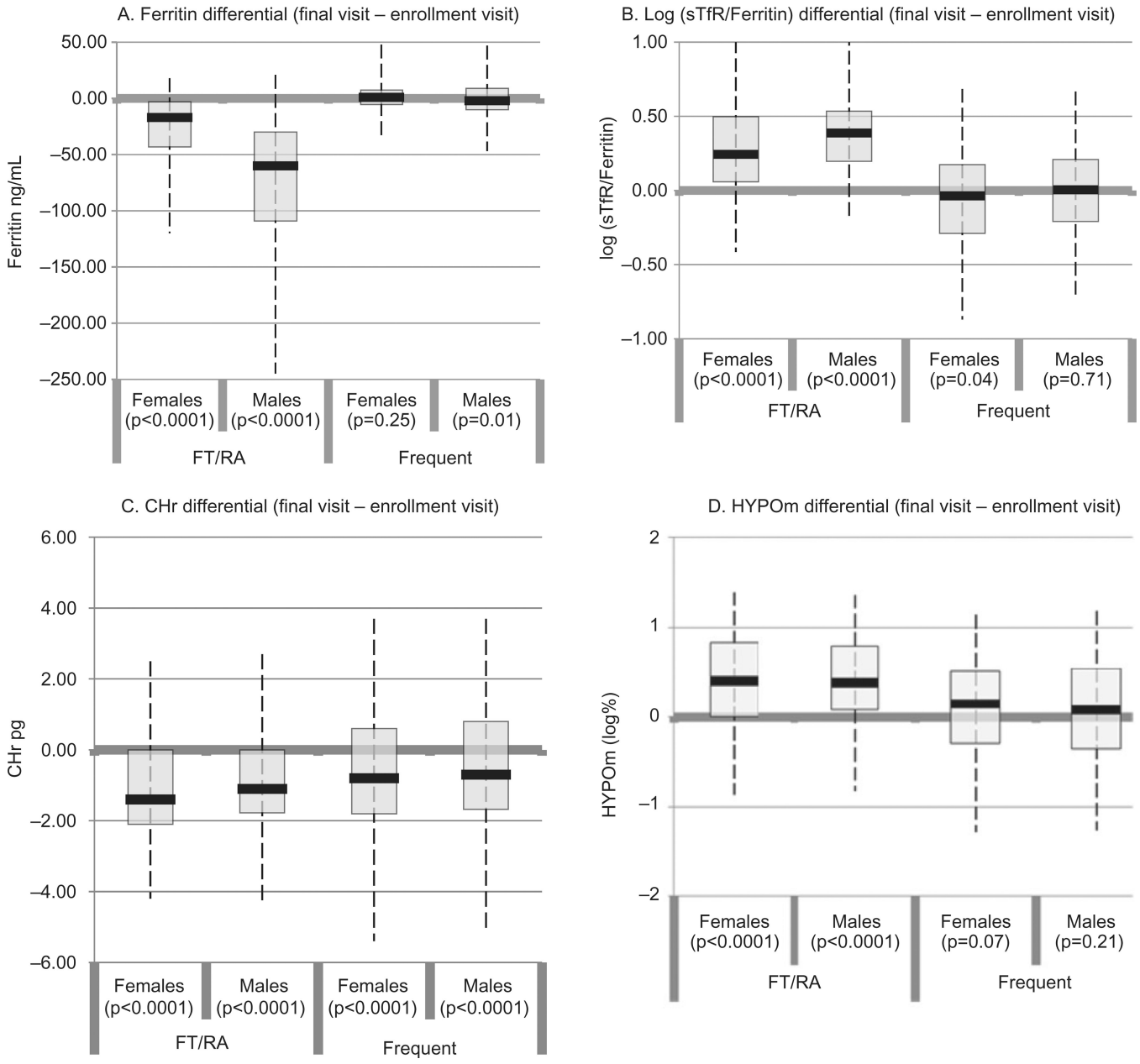

Fig. 1.

Changes in iron variables by cohort over the course of the study. A total of 1002 subjects had biochemical measures and 788 subjects had RBC indices performed at both the enrollment and final visits. (A) Ferritin differential (final visit – enrollment visit); (B) log(sTfR/ferritin) differential (final visit – enrollment visit); (C) CHr differential (final visit – enrollment visit); (D) HYPOm differential (final visit – enrollment visit).

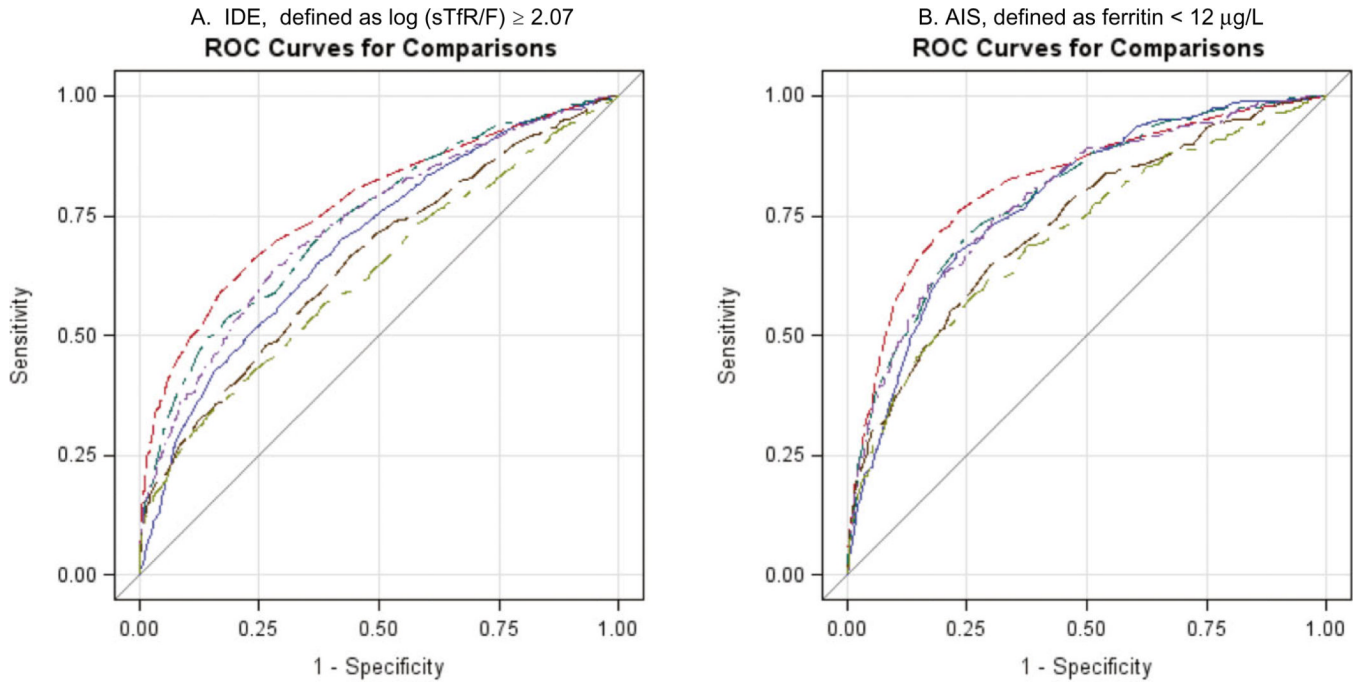

Fig. 2.

ROC curves for biochemical measures and hematology indices at enrollment visit. (A) IDE, defined as log(sTfR/F) of 2.07 or greater. (———) Hb (0.6951); (– – –) HYPOm (0.7692); (— - —) CHCMm (0.7369); (—— ——) CHr (0.6549); (– - – - –) HYPOr (0.7281); (—— ——) MCV (6257). (B) AIS, defined as ferritin less than 12 µg/L. (———) Hb (0.7820); (– – –) HYPOm (0.8177); (— - —) CHCMm (0.7920); (—— ——) CHr (0.7302); (– - – - –) HYPOr (0.7871); (—— ——) MCV (0.7079).

TABLE 1

A. Percentage of subjects classified as having IDE* or AIS† and the medians and ranges (2.5th percentile-97.5th percentile) for the RBC and biochemical variables of interest overall and by cohort at the enrollment study visit‡

		FT/RA		Frequent	
	Overall (n = 1659)§	Females (n = 341)	Males (n = 290)	Females (n = 513)	Males (n = 515)
IDE	42.2	25.8	2.1	68.2	49.7
AIS	15.6	6.2	0	29.2	16.9
Ferritin (ng/mL)	29.0 (6.0–240)	38.0 (9.0–192)	107.5 (30.0–422)	17.0 (5.0–65.0)	25.0 (6.0–111.0)
sTfR (mg/L)	3.0 (1.8–6.2)	2.7 (1.7–5.1)	2.6 (1.6–4.4)	3.2 (1.9–6.7)	3.1 (1.8–7.3)
Log(R/F)	2.0 (1.0–2.9)	1.8 (1.1–2.6)	1.4 (0.7–2.0)	2.2 (1.6–3.0)	2.1 (1.4–3.0)
HYPOm (%)	0.5 (0.0–9.3)	0.3 (0.0–6.8)	0.3 (0.0–1.5)	0.8 (0.1–12.2)	0.5 (0.1–12.8)
CHCMm (g/dL)	34.8 (31.8–37.7)	35.0 (31.8–37.5)	35.7 (33.1–38.2)	34.1 (31.4–36.8)	34.9 (31.4–38.0)
HYPOr (%)	10.2 (1.5–54.5)	9.4 (1.3–51.6)	6.5 (1.0–27.2)	14.5 (1.8–56.1)	9.6 (1.7–62.1)
Hb (g/dL) //	14.2 (12.1–16.7)	13.7 (12.0–15.3)	15.3 (13.4–17.2)	13.5 (12.0–15.3)	14.9 (12.5–16.9)
CHr (pg)	32.4 (27.6–35.7)	32.5 (28.6–35.8)	32.9 (29.9–35.8)	32.0 (27.6–35.6)	32.4 (26.7–35.5)
MCV (fL)	88.6 (78.6–97.5)	89.8 (81.3–97.6)	88.2 (82.4–96.8)	88.6 (78.3–97.6)	87.9 (76.3–96.9)

B. Percentage of subjects classified as having IDE* or AIS† and the medians and ranges (2.5th percentile-97.5th percentile) for the RBC and biochemical variables of interest overall and by cohort at the final study visit‡

		FT/RA		Frequent	
	Overall (n = 1002)§	Females (n = 154)	Males (n = 125)	Females (n = 356)	Males (n = 367)
IDE	50.7	53.9	17.6	65.2	46.6
AIS	21.7	22.7	6.4	28.4	19.9
Ferritin (ng/mL)	23 (5–110)	22.0 (5–110)	45.0 (8–204)	17.5 (4–75)	25.0 (4–99)
sTfR (mg/L)	2.9 (1.6–6.4)	2.7 (1.7–5.6)	2.7 (1.6–4.3)	3.0 (1.6–6.4)	2.9 (1.6–7.0)
Log(R/F)	2.1 (1.3–3.0)	2.1 (1.4–3.0)	1.7 (1.2–2.6)	2.2 (1.5–3.1)	2.0 (1.3–3.2)
HYPOm (%)	0.8 (0–17.2)	1.1 (0–13.4)	0.7 (0–10.8)	1.1 (0–17.2)	0.6 (0–19.4)
CHCMm (g/dL)	34 (30.6–38)	33.6 (31.0–37.4)	34.2 (31.3–40.0)	33.6 (30.6–37.6)	34.3 (30.5–38.1)
HYPOr (%)	16.6 (1.1–74.9)	19.0 (0.9–63.6)	15.2 (0.5–66.8)	19.5 (1.1–72.2)	13.8 (1.2–77.5)
Hb (g/dL) //	13.9 (11.6–16.2)	13.3 (11.5–15.3)	14.8 (12.6–16.7)	13.3 (11.2–15.4)	14.7 (12.0–16.4)
CHr (pg)	31.9 (26.8–35.1)	31.7 (28.7–34.9)	32.0 (27.6–35.3)	31.6 (27.1–34.6)	32.2 (25.5–35.3)

B. Percentage of subjects classified as having IDE* or AIS† and the medians and ranges (2.5th percentile-97.5th percentile) for the RBC and biochemical variables of interest overall and by cohort at the final study visit‡

		FT/RA		Frequent	
	Overall (n = 1002)§	Females (n = 154)	Males (n = 125)	Females (n = 356)	Males (n = 367)
MCV (fL)	87.6 (74.5–96.4)	88.5 (80.7–97.0)	87.7 (81.2–95.2)	87.6 (74.4–96.6)	87.3 (71.9–96.6)

*IDE = log(sTfR/F) ≥ 2.07.

†AIS = ferritin < 12.

‡Data are reported as percent or median (range).

§35 subjects are missing RBC indices.

‖Postdonation values converted to predonation values for 12% of samples.

*IDE = log (sTfR/F) ≥ 2.07.

†AIS = ferritin < 12.

‡Data are reported as percent or median (range).

§152 subjects are missing RBC indices.

‖Postdonation values converted to predonation values for 3% of samples.

TABLE 2

A. AUC for different measures as indexes of IDE, defined as log(sTfR/F) of 2.07 or greater at enrollment and final visits

Measure	AUC (95% CI)	
	At enrollment visits*	At final visits
HYPOm	0.77 (0.74–0.79)	0.66 (0.63–0.70)
CHCMm	0.74 (0.71–0.76)	0.64 (0.60–0.68)
HYPOr	0.73 (0.70–0.75)	0.64 (0.60–0.67)
Hb	0.69 (0.67–0.72)	0.73 (0.69–0.76)
CHr	0.66 (0.63–0.68)	0.65 (0.61–0.69)
MCV	0.62 (0.60–0.65)	0.65 (0.62–0.69)

B. AUC for different measures as indexes of AIS, defined as a ferritin level of less than 12 μg/L

Measure	AUC (95% CI)	
	At enrollment visits*	At final visits
HYPOm	0.82 (0.79–0.85)	0.75 (0.70–0.79)
CHCMm	0.79 (0.76–0.82)	0.72 (0.68–0.77)
HYPOr	0.79 (0.76–0.82)	0.73 (0.68–0.77)
Hb	0.78 (0.75–0.81)	0.81 (0.77–0.85)
CHr	0.73 (0.70–0.77)	0.75 (0.70–0.79)
MCV	0.71 (0.67–0.74)	0.72 (0.68–0.76)

*All differences are significant, $p \leq 0.007$, between RBC index AUCs of IDE compared to AIS at both enrollment and final visits.

TABLE 3

A. Sensitivity and specificity of RBC variables for detecting IDE at enrollment, defined as log(sTfR/ferritin) of 2.07 or greater

Measure	Cutoff value	Sensitivity (%)			Specificity (%)		
HYPOm	>0.55%	72			68		
CHCMm	<34.8 g/dL	71			62		
HYPOr	>10.8%	68			68		
		All	Males	Females	All	Males	Females
Venous Hb*	≤12.5 g/dL	10	5	14	97	100	93
	≤13.0 g/dL	26	11	35	93	99	84
	≤13.5 g/dL	44	23	57	82	96	61
CHr	<32.6 pg	69			53		
	<28 pg	7			100		
MCV	<80.0 fL	9			99		
HYPOm and/or CHr	>0.55% or <28 pg	70			71		
	>0.55% or <32.6 pg	82			47		

B. Sensitivity and specificity of RBC variables for detecting AIS at enrollment, defined as a ferritin level of less than 12 μg/L

Measure	Cutoff value	Sensitivity (%)			Specificity (%)		
HYPOm	>0.55%	85			57		
CHCMm	<34.8 g/dL	84			54		
HYPOr	>10.8%	81			59		
		All	Males	Females	All	Males	Females
Venous Hb*	≤12.5 g/dL	19	16	21	96	100	91
	≤13.0 g/dL	39	29	45	89	99	79
	≤13.5 g/dL	66	53	71	78	95	57
CHr	<32.6 pg	81			49		
	<28 pg	14			99		
MCV	<80.0 fL	15			98		
HYPOm or CHr	>0.55% or <28 pg	84			61		

B. Sensitivity and specificity of RBC variables for detecting AIS at enrollment, defined as a ferritin level of less than 12 μg/L

Measure	Cutoff value	Sensitivity (%)	Specificity (%)
	>0.55% or <32.6 pg	91	39

* All donors had fingerstick measurement of Hb of 12.5 or greater or Hct of 38% or greater.

* All donors had fingerstick measurement of Hb of 12.5 or greater or Hct of 38% or greater.