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RESEARCH ARTICLE

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Data-driven physiologic thresholds for iron deficiency associated with hematologic decline

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Abstract

Iron-deficiency contributes to a ~50% of anemia prevalence worldwide, but reference intervals for iron status tests are not optimized for anemia diagnosis. To address this limitation, we identified the serum ferritin (SF) thresholds associated with hematologic decline in iron-deficient patients, and the SF thresholds from which an SF increase was associated with hematologic improvement. Paired red blood cell and SF measurements were analysed from two adult cohorts at Massachusetts General Hospital (MGH), from 2008-2011 (N = 48 409), and 2016-2018 (N = 10 042). Inter-patient measurements in the first cohort were used to define optimal SF thresholds based on the physiologic relationship between SF and red cell measurements. Intra-patient measurements (1-26 weeks apart) in the second cohort were used to identify SF thresholds from which an SF increase was associated, with an increase in red cell measurements. The identified optimal SF thresholds varied with age, sex and red cell measure. Thresholds associated with a ~5% decline in red cell index were typically in the range 10-25 ng/mL. Thresholds for younger women (18-45 year) were ~5 ng/mL lower than for older women (60-95 years), and ~10 ng/mL lower than for men. Thresholds from which a subsequent increase in SF was associated with a concomitant increase in red cell measure showed similar patterns: younger women had lower thresholds (~15 ng/mL) than older women (~25 ng/mL), or men (~35 ng/mL). These results suggest that diagnostic accuracy may be improved by setting different SF thresholds for younger women, older women, and men. This study illustrates how clinical databases may provide physiologic evidence for improved diagnostic thresholds.

1 | INTRODUCTION

Adult iron deficiency (ID) is a significant public health burden globally, with approximately ~17% of women and ~13% of men being iron deficient to the point of anemia.[1,2] Because definitive assessment of iron status requires invasive testing,[3] diagnosis of ID is typically performed through measurement of one or more non-invasive iron-status indicators, such as serum iron,[4] serum ferritin (SF)[5] or soluble transferrin receptor,[6,7] which are then compared to a standard reference interval for healthy individuals.[4,8] Serum ferritin is the most commonly used indicator, with typical reference intervals having a lower limit in the range 10-30 ng/mL,[4,8-10] though there is considerable variation between medical centers. Reference intervals typically vary with sex, with lower limits for females ~10-20 ng/mL below those for males,[9-11] but practice varies.[12] As is common practice for reference intervals, the SF reference interval typically reflects the 95% confidence interval for SF in an "ostensibly healthy" population.[13,14] However, given the prevalence of iron deficiency anemia (IDA) among ostensibly healthy people,[8] these reference intervals will have poor sensitivity and specificity. Specifically, that is for determining when iron levels are low enough to affect a patient's hematologic status or for deciding which patients' hematologic status is likely to improve

with iron supplementation, leading some to suggest reconsideration of how SF cut-offs are used to diagnose ID.[15]

Following diagnosis, treatment of IDA is typically performed through a combination of dietary intervention and therapeutic iron supplementation. Treated patients will typically experience an improvement in red blood cell indices, followed by SF increases once iron stores are replete.[8,10] As SF levels increase, red cell indices continue to improve until a plateau is reached, following which further increases in SF do not lead to further improvements in red cell indices.[16] Given this plateau behaviour, it is important to understand whether current clinically implemented SF reference intervals accurately represent the point at which the SF level begins to be associated with a decline in red cell indices. Furthermore, because the menstrual status of females alters the relationship between iron status and hematologic states, these thresholds are expected to differ with both sex and age. Answering these questions retrospectively is possible using large clinical databases that have recently become more routinely available, and which provide opportunities to investigate the functional physiologic relationship between SF and hematologic status and how that relationship varies with age and sex.

2 | METHODS

2.1 | Sample collection

This study was performed retrospectively, through analysis of medical records from two distinct patient cohorts at Massachusetts General Hospital (MGH). Collection of all data was performed under a research protocol approved by the Partners Healthcare Institutional Review Board. Serum ferritin and complete blood counts were measured in the MGH core clinical laboratory under standard hospital protocols. Serum ferritin levels were measured on a Roche COBAS instrument (Roche Diagnostics, Indianapolis, Indiana). Complete blood counts (CBCs) were measured on a Siemens Advia 2120 (cohort 1) and a Sysmex XE-5000 (cohort 2). Complete blood counts included measures of hematocrit (HCT), hemoglobin (HGB), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), red blood cell count (RBC), red cell distribution width (RDW), and, hemoglobin distribution width (HDW - available in first cohort only). We focus on HCT, HGB, MCV and MCH, due to the prevalence of their use in the diagnosis of anemia[17] and management of iron-deficiency. Analysis of the remaining red cell indices is given in the Appendix S1.

The first patient cohort was designed for inter-patient analysis and consisted of all MGH patients who had SF and CBC measurements during a single medical visit (defined as being within 12 hours of each other), between 2008-2011 inclusive. Patients under 18 years old or over 95 years old were excluded, and only the first measurement set was included for each patient. Total cohort size after exclusion was 48 409.

The second patient cohort was designed for intra-patient analysis, and consisted of all MGH patients who had SF and CBC measurements taken during a single hospital visit on at least two distinct occasions, at least one week apart, within a 6-month period, between 2015-2018 inclusive. As with the first cohort, patients under 18 years old and over 95 years old were excluded, and only the first two valid sets of measurements were included for each patient. All patients in the first cohort were excluded from the second cohort. Total cohort size after exclusion was 10 042.

2.2 | Inter-patient analysis

To account for potential confounders, patients were grouped together based on sex and age (younger: 18-45 years old, middle-aged: 45-60, older: 60-95). These groupings were chosen such that in most cases younger women would be pre-menopausal, and older women would be post-menopausal, based on World Health Organisation findings for Caucasian women.[18] The term "older" is used to contrast against the predominantly pre-menopausal group (18-45 years old), and is not meant to reflect a categorisation of "elderly".

Inter-patient analysis was performed by fitting a smoothed curve through the set of red cell index-SF values for each patient group. To reduce the influence of outliers, red cell index values that lay outside the interquartile range for their SF level were excluded from the fitting process. By using the interquartile data, we focus on the physiologic SF-red cell index relationship for a typical patient. And, by smoothing we highlight changes in that relationship that are more likely to reflect altered physiology, and less likely to have been caused by analytic variation or unusual biological variation. Given that the lower limits of most SF reference intervals are in the range 10-30 ng/mL,[9] splines were fit over the range 1-80 ng/mL, using the *csaps* function in MATLAB (The MathWorks, Natick, MA).

Red cell measure-specific cut-offs for iron-deficiency for each age-sex group were defined as the SF level at which the functional relationship illustrated a greater than 5% or greater than 10% decline (for HCT, HGB, MCH, MCHC, MCV, RBC). Or, an increase (for HDW, RDW) in the red cell measure, relative to the population mean in the stable, medium-ferritin region (50-150 ng/mL). For many of these red cell measures, a 5% change roughly corresponds to the reference change value - the smallest change that exceeds what might typically be expected given both analytic variation and normal healthy biological fluctuations.[19] For both the 5% and 10% decline levels, bootstrapping with 10 000 samples was used to calculate a 95% confidence interval for each threshold.

To address potential confounding effects of the retrospective nature of this study, the bias of a single-hospital cohort, and the use of SF as opposed to other iron status indicators, we performed a parallel analysis using the US National Health and Nutrition Examination Survey (NHANES) dataset, from 2001-2010.[20] The NHANES data collection has been described elsewhere, but in brief is a series of prospective health studies using samples representative of the entire US population.[21] Red cell measurements were paired with serum iron,[4] another commonly used iron-status indicator. Following exclusion of children and subjects with missing data, 32 975 paired serum iron and red cell measurements were analysed to identify appropriate

diagnostic thresholds, using the same procedure as outlined above for SF. Note that for the NHANES cohort, HDW was not included, as it was not part of the NHANES collection protocol.

2.3 | Intra-patient analysis

Intra-patient analysis was performed using the same age and sex subgroups as the inter-patient analysis. Responses to iron status improvements were investigated by analysing changes in red cell indices for patients whose serum SF increased by at least 5 ng/mL within a 1-6 month period. An SF increase of 5 ng/mL is greater than what is expected from analytic variation and biological fluctuation.[22]

Patients were sub-divided into groups based on their initial SF level. Groups were defined to explore the same thresholds identified in the inter-patient analysis. Female patients were partitioned based on three SF thresholds: <15, 15-25, and 25-40 ng/mL. Similar classifications were applied to men, using SF < 25, 25-35, and 35-50 ng/mL. For comparison, SF reference intervals for women typically have a lower limit between 10-20 ng/mL, and for men between 20-30 ng/mL.[4,8-11] Mean red cell index changes associated with SF increases were compared to zero, and between groups using two-sided Student's t tests, with a significance threshold of $P = .05$. All statistical tests were performed using MATLAB.

2.4 | Data sharing statement

Raw data for all figures is available upon request from the corresponding authors. Individual patient data is not available for sharing according to IRB approval for this study.

3 | RESULTS

Results are presented from analysis of HGB, HCT, MCH and MCV. Results for HDW, MCHC, RBC and RDW are presented in the Appendix S1.

TABLE 1 Characteristics of the study cohorts

	Total	Younger men	Older men	Younger women	Older women
Inter-patient cohort					
N	48 409	4836	9520	11 278	10 002
Age (years)	53.4 (19.1)	33.9 (8.0)	72.6 (8.6)	34 (7.5)	72.9 (9.2)
Serum ferritin (ng/mL)	80 (30-223)	139 (59-303)	166 (66-419)	33 (15-70)	98 (39-241)
HCT (%)	35.6 (6.1)	38.6 (6.7)	34.9 (6.3)	36.0 (5.0)	33.9 (5.5)
HGB (g/dL)	11.9 (2.2)	13.2 (2.5)	11.6 (2.4)	12.0 (1.9)	11.1 (2.0)
MCH (pg)	28.6 (3.1)	28.8 (2.9)	29.4 (2.8)	28.0 (3.2)	28.7 (2.8)
MCV (fL)	87.3 (8.0)	86.3 (7.2)	89.8 (7.5)	85.7 (7.8)	88.8 (7.8)
Intra-patient cohort					
N	10 042	726	1636	3210	2078
Age (years)	46.2 (22.7)	31.3 (8.5)	71.3 (7.9)	33.2 (7.9)	71.1 (7.9)
Initial serum ferritin (ng/mL)	35.3 (41.5)	46.1 (40.1)	46.7 (45.6)	23.4 (31.4)	38.5 (43.2)
Initial HCT (%)	34.5 (6.1)	38.5 (7.3)	34.0 (6.5)	34.4 (5.3)	32.8 (6.0)
Initial HGB (g/dL)	11.0 (2.3)	12.5 (2.8)	10.8 (2.3)	10.9 (2.0)	10.3 (2.1)
Initial MCH (pg)	26.7 (4.1)	27.3 (3.8)	27.8 (4.0)	26.0 (4.2)	26.9 (4.0)
Initial MCV (fL)	84 (9.8)	84.3 (8.5)	87.3 (9.3)	82.0 (9.6)	85.6 (9.6)
Measurement gap (days)	85 (45)	84 (45)	82 (45)	88 (44)	84 (45)
NHANES 2001-2010					
N	32 975	6218	4135	6990	4168
Age (years)	40.9 (21.8)	30.2 (8.8)	71.2 (7.5)	30.2 (8.5)	71.2 (7.7)
Serum iron (μg/dL)	85.5 (36.6)	96.8 (37.5)	88.6 (34.0)	78.2 (38.9)	77.9 (28.4)
HCT (%)	41.7 (4.4)	45.4 (3.0)	43.0 (4.3)	38.7 (3.4)	39.7 (3.7)
HGB (g/dL)	14.2 (1.5)	15.5 (1.0)	14.6 (1.5)	13.2 (1.2)	13.5 (1.3)
MCH (pg)	30.3 (2.3)	30.4 (1.9)	31.1 (2.2)	30.0 (2.5)	30.7 (2.2)
MCV (fL)	88.9 (5.7)	89.0 (4.7)	91.6 (5.5)	88.0 (6.1)	90.4 (5.4)

Note: Data are presented as Mean (SD), except inter-patient cohort serum ferritin, which is presented as median (25-75th percentile), due to the extreme right-side tail of ferritin measurements.

Abbreviations: HCT, hematocrit; HGB, hemoglobin; MCH, mean cell hemoglobin; MCV, mean corpuscular volume; N, sample size.

Demographic summaries of the patient cohorts and subgroups are given in Table 1.

3.1 | Identifying threshold ferritin levels associated with decreases in red cell indices

To identify SF thresholds systematically, we determined the interpolated relationships between SF and the red cell indices for each patient sub-group (Figure 1A). The physiologic relationship differs significantly between the four population groups, with many declining by 5% and 10% at different SF levels, across a variety of red cell measures. Younger women consistently show a decline in a red cell index at a lower threshold SF than all other groups, for all measurements considered, as shown in Figure 1B. In contrast, older women generally have cut-offs more in line with those of men than of younger women. Older men have higher cut-offs than younger men for MCV and MCH, but the thresholds for HCT and HGB seem comparable. Consistent across all groups, MCH and HGB decline at a higher level of SF than MCV and HCT. This pattern may suggest that hemoglobin production is more sensitive to low-iron than production of cells. This hypothesis is further supported by the fact that RBC count did not respond as sensitively to SF or serum iron reductions as red cell hemoglobin measures (see Appendix S1).

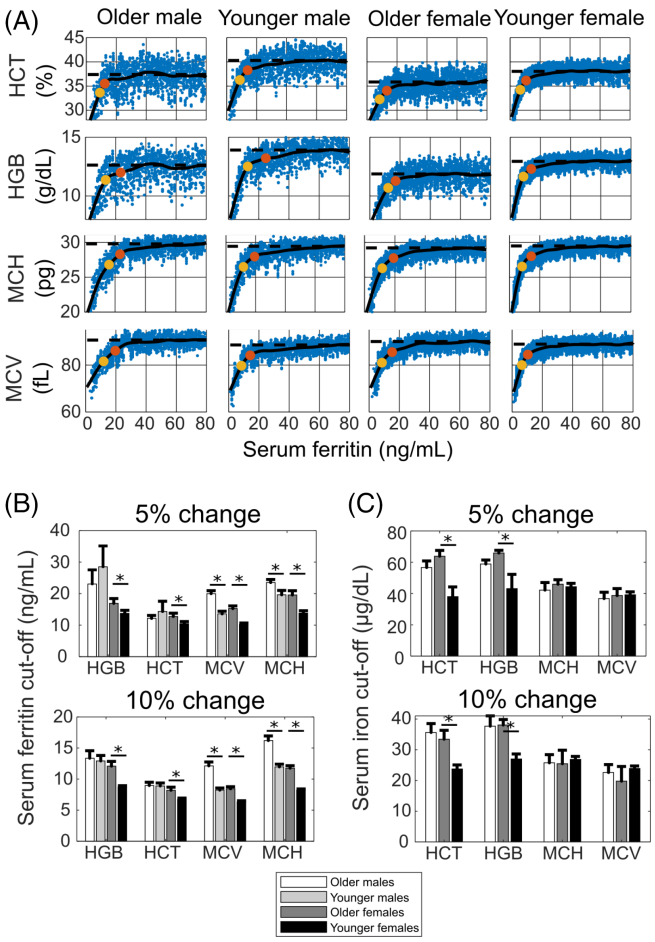

FIGURE 1 Functional relationships between ferritin and four red cell measures, stratified by age and sex. The ferritin-red cell index relationship for each population sub-group is given (A), with data (blue dots) and spline-fit (black line). Circles show decreases of 5% (red circle), and 10% (yellow circle) from the sub-population mean of the red cell index, with the mean determined from all patients with SF in the range 50-150 ng/mL. (B) compares the ferritin values at which these changes occur. For each cut-off, a 95% confidence interval is included, with * denoting when the corresponding intervals for the younger and older group (for each sex) do not overlap. Cut-offs are also given for serum iron, using the NHANES 2001-2010 population dataset (C). Within this cohort, younger males have been excluded, due to lack of available low serum iron data. For both serum ferritin and serum iron, across all four measures the cut-offs for younger women are significantly lower than those of older women. All serum ferritin values are given in ng/mL, all serum iron values are given in μg/dL [Color figure can be viewed at wileyonlinelibrary.com]

This first data set was analysed retrospectively from patients whose CBCs and SFs were collected as part of clinical care, and it is possible that unknown confounding factors biased this set of patients. A similar analysis was performed for the NHANES 2001-2010 dataset, using serum iron as the marker of iron status, with cut-offs summarised in Figure 1C (associated curves are given in the Appendix S1). We find the same relative patterns in this cohort using serum iron as in the MGH cohort using SF. Younger women exhibited consistently lower iron status marker thresholds than older women, with older women having thresholds more similar to those of older men. Younger men were excluded from analysis in the NHANES cohort, due to the low frequency of low serum iron in this sub-group (only 54 young men had serum iron below 30 μg/dL, and only eight had serum iron below 20 μg/dL, with corresponding numbers for young women of 473 and 150 respectively). This small sub-cohort would have an unstable interpolant and subsequently unreliable estimates of serum iron thresholds.

3.2 | Identifying thresholds associated with a concurrent increase in red cell indices and ferritin

The data in Figures 1, Table 1, and the Appendix S1 suggest that the hematologic response to ID may differ significantly between adults based on age and sex, particularly between younger and older women. A related clinical question is whether these patient groups would experience hematological improvement if their iron status improved. To investigate this question, we consider the intra-patient responses of each population group to an increased SF.

In Figure 2, Table 2, and the Appendix S1 we present the changes in red cell indices for all patients who experienced an SF increase of at least 5 ng/mL within a 1-6 month period. Patients are further subdivided according to their initial SF level. As the figure and table show, all groups with SF < 15 ng/mL (women), and SF < 25 ng/mL (men) demonstrated hematologic response to SF increase, showing large improvements in all red cell indices (with the exception of RDW, with discussion in the Appendix S1). In the mildly low SF range (15-25 ng/mL for women; 25-35 ng/mL for men), younger women exhibited only

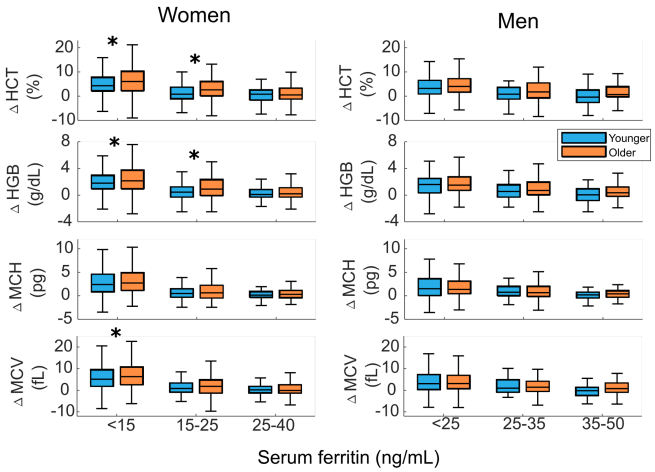

FIGURE 2 Effect of serum ferritin increases on red cell measures, for men and women, stratified by initial ferritin level, and age. The response of the extremely low ferritin (<15 for women, <25 for men) and mildly low ferritin (15-25 for women, 25-35 for men) groups is consistently higher for elderly women than for young women, while for men the response is more similar between the two age groups (for numerical data see Tables 1 and 2). No significant differences in response to ferritin increase are seen in the non iron-deficient group (ferritin 25-40 for women, 35-50 for men). * is used to denote when the young and older group have a statistically significant difference in the given index change ($P < .05$) [Color figure can be viewed at wileyonlinelibrary.com]

a small improvement in red cell indices associated with SF increase, while older women exhibited significantly ($P < .05$) larger improvements in HCT and HGB. Furthermore, older women exhibited small but statistically significant improvements in MCV, HGB, and HCT when initial SF was in the range 25-40 ng/mL, while younger women did not. Conversely, while average improvements following SF increase were statistically significant for both younger and older men with initial SF < 35 ng/mL, there were no statistically significant differences between the two groups. Most red cell indices measures did not improve for men with initial SF in the range 35-50 ng/mL.

4 | DISCUSSION

In this study we have investigated the functional physiologic relationship between SF and red cell indices and find that HCT, HGB, HDW, MCV, MCH, MCHC, and RDW all show changes (increases for HDW and RDW, decreases for all others) that correlate with SF level, once SF has fallen below a threshold value. The precise threshold varies with sex and age and depends on the red cell index, with younger women having the lowest thresholds compared to older women and men of all ages. We also investigated thresholds for SF at which a subsequent increase in SF is associated with an increase in red cell measures. These thresholds also varied with both age and sex, with younger women again having the lowest thresholds. These results suggest that for the purpose of diagnosing iron deficiency, younger women should have lower SF thresholds than older women and men of all ages.

TABLE 2 Mean effect of serum ferritin increases on CBC measures, stratified by initial ferritin level, sex and age

	Women					
	SF < 15		15 < = SF < 25		25 < = SF < 40	
	Younger	Older	Younger	Older	Younger	Older
N	528	223	149	119	118	114
HCT (%)	4.3[b]	6.1	0.8[b]	2.6	0.8[a]	0.1
HGB (g/dL)	1.8[b]	2.15	0.5[b]	0.9	0.1[a]	0.1
MCH (pg)	2.4	2.7	0.5	0.7	0.1	0.5
MCV (fL)	5.1[b]	6.3	0.8	1.8	0.2[a]	0.6
	Men					
	SF < 25		25 < = SF < 35		35 < = SF < 50	
	Younger	Older	Younger	Older	Younger	Older
N	97	251	30	88	42	65
HCT (%)	3.8	4.2	0.8	1.8	−0.2[a]	0.6
HGB (g/dL)	1.6	1.6	0.6	0.7	0.1[a]	0.35
MCH(pg)	1.6	1.4	0.8	0.7	0.2[a]	0.4[a]
MCV (fL)	3.2	3.3	1	1.5	−0.2[a]	0.75[a]

Abbreviations: HCT, hematocrit; HGB, hemoglobin; MCH, mean cell hemoglobin; MCV, mean corpuscular volume; N, sample size.

[a]Denotes values that were not statistically significant from 0 (*P*-value <.05).

[b]Denotes when response of the younger group differed significantly from the corresponding response in the older group (*P*-value <.05). All serum ferritin values are given in ng/mL.

Meta-analysis of iron-deficiency studies shows that lower limits of reference intervals vary from 10-100 ng/mL in scientific studies,[10,11] with the most commonly used cut-offs being in the 10-30 ng/mL range.[9] The results of this study are broadly consistent, with the SF-red cell index curves all exhibiting small declines that start when SF is between 50-100 ng/mL and become more pronounced below 50 ng/mL (Figure 1, Table 2).

We have provided a functional definition of ID as an SF level low enough to be associated with a clinically meaningful change in red cell indices. The precise thresholds identified will depend on the definition of clinically meaningful change. However, the results here suggest that across red cell indices, SF thresholds are consistently lower for younger women than all other groups. Many studies implement and propose different SF cut-offs for men and for women,[8-10,23] but few if any studies propose separating SF intervals for younger and older females or on the basis of menstrual status.

It is well known that menstruation affects iron and hematologic status in women,[24] with women who experience heavy menstrual bleeding having higher risk of developing IDA.[25,26] This increased risk is reflected in the overall prevalence of ID in general population,[8,27] which is highest for menstruating women, particularly in developed nations. Furthermore, the prevalence of ID in women declines once they reach the age range at which menopause most commonly occurs (45-60 years old).[28] For women above 50 years old the prevalence of ID is still significantly higher than that for similarly aged men. However, the difference in prevalence is much lower than between men and women who are 20-49 years old.[27] A simple hypothesis would be that once menstruation stops, women retain the same hematologic sensitivity to low SF, and prevalence of ID declines due to the reduction in iron loss. However, the results in this study suggest that as women age their sensitivity to iron-deficiency also increases, in the sense that their hematologic status begins to change at a higher level of SF. The mechanisms for this difference are unclear. One possible explanation is that there are changes in blood production after menopause, as the hematologic system adapts to a more iron-rich environment. Another factor possibly relevant to the SF differences might be any age-dependent changes in inflammation, though this mechanism would not explain the similar pattern identified for serum iron in the NHANES cohort. Regardless, elucidating the basis for this age- and gender-based change in the functional relationship between iron and hematologic status requires further study and analysis.

Our approach of functionally defining thresholds for diagnosis can be generalized to other situations where a specific biomarker or test is used to assess risk for a specific diagnostic outcome. This approach is particularly well-suited for the use of SF to diagnose iron deficiency anemia, because a low SF is rarely used to diagnose conditions other than iron deficiency anemia.[29] For tests or biomarkers where multiple pathologic states are considered, functional thresholds may have to be defined for each outcome. This approach also requires large databases of patient clinical records which are becoming more routinely available.

The results in this study should be interpreted in context of a few key limitations. Most significantly, while the study cohort is large, it reflects practice at a single hospital and is analysed retrospectively. The data is biased towards an unhealthy population and is not demographically representative of the broader US or world population. This limitation was understood from the initial study conception, and is considered a necessary trade-off to attain a dataset of large enough size to investigate SF response differences between population subgroups. Furthermore, the differences in iron deficiency sensitivity between younger and older women are of a large enough magnitude that it seems reasonable to expect that these relative differences are likely to be reflected in the broader population, but follow-up study is required for greater certainty. Analysis of the NHANES dataset, which yielded qualitatively similar results prospectively in a more healthy population using a different biomarker of iron status, provides independent support for the conclusion that there are age and gender differences in the functional physiologic relationship between patient iron and hematologic status. The use of serum iron instead of SF in the NHANES dataset helps partially control for the bias and limitations of SF, for instance because serum iron is not as confounded by inflammation as SF. However, neither SF nor serum iron are direct measures of total body iron stores, instead being correlated indicators of iron status. Analysis of this phenomenon in a healthy population study, using a wider variety of iron status markers is warranted.

This study used SF as the primary iron-status indicator, due to its clinical prevalence.[4] However, SF is an acute-phase reactant, and is independently affected by inflammation,[11] making it inappropriate in certain clinical settings. The translation of these results to other iron-status markers, as was preliminarily shown in Figure 1C, is a clear avenue for future work.

Finally, the intra-patient analysis in this study considered groups who experienced SF increases within a given timeframe. This approach is different from investigating the influence of direct and controlled iron supplementation on patient outcomes in two major respects. Firstly, this study did not monitor whether treatment was applied to any of the patients. If a patient experiences a significant increase in iron status markers in a short time period, treatment is likely, but not guaranteed. The type of treatment also may vary (eg, intravenous/oral iron supplementation, blood transfusion). Secondly, patients who did not show significant improvements in iron status were excluded from this analysis. The relevance of this finding to thresholds for a particular treatment for iron deficiency cannot be determined without additional information on how frequently that treatment is given to patients with low ferritin, and how frequently and quickly the ferritin increases in response to that treatment. There is a further small confounding bias in the intra-patient cohort, as compared to the inter-patient cohort, as can be seen in the differences in SF levels in Table 2. This difference is due to the biasing effect of requiring a second set of SF-red cell measurements within 6 months, testing which would be undertaken only if there was some clinical expectation that measurements had changed. These limitations could be addressed through the design of a prospective, interventional study of iron supplementation.

We have presented a framework for functionally defining iron deficiency in adults, and we have used this framework to show that the most appropriate SF cut-offs for diagnosis of iron deficiency may

vary by age and sex within adults, and with reference to the hematologic parameter of interest. Importantly, post-menopausal women were shown to experience decline in hematologic parameters at significantly higher levels of SF than younger women. This finding suggests that the diagnostic thresholds for iron-deficiency in pre- and post-menopausal women should be different, with the thresholds for older women being more similar to those of adult men of all ages.

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CONFLICT OF INTEREST

All authors and declare no conflict of interest.

AUTHOR CONTRIBUTIONS

B.H.F., A.L., J.M., R.R., and J.M.H. all contributed to the overarching study design. B.H.F. performed data collection and analysis. A.L., R.R., and J.M.H. provided a technical critique of the data and analysis. J.M., and J.M.H. provided a clinical critique of the data and analysis. B.F. wrote the manuscript. All other authors edited the manuscript.

ETHICS STATEMENT

The study protocol was approved by the local institutional review board (IRB) at Massachusetts General Hospital. We used blood samples that had been collected solely for non-research purposes (such as medical treatment and diagnosis).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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