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ORIGINAL ARTICLE

Diagnosing empty iron stores in women: unbound iron binding capacity (UIBC) versus soluble transferrin receptor (sTFR)

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ABSTRACT

Unbound iron binding capacity (UIBC) is more accurate than total iron binding capacity (TIBC) and percent transferrin saturation in diagnosing empty iron stores. It is unknown whether UIBC is more or less accurate than soluble transferrin receptor (sTFR). We obtained public-use data from the U.S. National Health and Nutrition Examination Survey (NHANES) 2005-2006 to compare the accuray of UIBC and sTFR in diagnosing empty iron stores in 2337 women aged 12–49 years. We grouped the women according to CRP less than 5 mg/L and pregnancy (four groups) and used three definitions of empty iron stores: Serum ferritin less than 10, 15, and 20 μg/L. Receiver operating characteristic (ROC) curve analysis was used to estimate the diagnostic accuracy. UIBC showed a better diagnostic accuracy than sTFR in all groups and definitions of empty iron stores, except in nonpregnant women with CRP at least 5 mg/L when empty iron stores were defined as ferritin less than 10 and 15 μg/L. Two differences reached statistical significance: In nonpregnant women without inflammation the area under the ROC curve for UIBC was 0.830 compared to 0.793 for sTFR ($p = .007$) when empty iron stores were defined as ferritin less than 20 μg/L. The corresponding figures for pregnant women without inflammation were 0.843 for UIBC and 0.739 for sTFR ($p = .003$). In conclusion, UIBC is a more accurate test than sTFR in diagnosing empty iron stores in women without inflammation.

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Introduction

Iron deficiency is extremely common in women of childbearing age, because the iron loss due to menstruation and pregnancy is not sufficiently compensated with an increased iron intake [1]. Before treatment is started, most physicians want to confirm that the iron stores are exhausted. Then serum ferritin is the test of choice, at least in patients without inflammatory conditions [2]. However, the measurements of iron and transferrin in serum are still in use [3–5], and combined into percent transferrin saturation: 100 × (iron/TIBC), where TIBC is 'total iron binding capacity', i.e. two times the transferrin concentration (in μmol/L) since each transferrin molecule is able to bind two iron atoms. Percent transferrin saturation decreases when iron stores are exhausted. Another way of combining measurements of iron and transferrin, named 'unbound iron binding capacity' (UIBC), is TIBC minus iron concentration. UIBC may also be directly measured in automated instruments. UIBC increases when iron stores are exhausted. We have previously shown that UIBC has a better diagnostic accuracy than transferrin saturation in diagnosing iron deficiency [6,7]. Still another test of iron deficiency is the serum concentration of soluble transferrin receptor (sTFR), which increases when iron deficiency limits hemoglobin production and may be of some help if the patient has an inflammatory condition [3,8]. To the best of our knowledge, the diagnostic accuracies of UIBC and sTFR have never been compared in the same population. To that end, we obtained public-use data from women participants in the US National Health and Nutrition Examination Survey (NHANES) and estimated the diagnostic accuracies of UIBC and sTFR using various serum ferritin concentrations as definitions of empty iron stores.

Methods

Population

We used data from U.S. Department of Health & Human Services, Centers for Disease Control and Prevention, the NHANES 2005–2006 survey [9] on the variables age, sex, the serum concentrations of ferritin, iron, TIBC, sTFR, and C-reactive protein (CRP), in addition to the hemoglobin concentration (Hb), mean erythrocyte volume (MCV), mean erythrocyte hemoglobin (MCH), and erythrocyte volume distribution width (RDW) in blood. The variables were published in separate files. We used the respondent sequence number to compile an analysis file with relevant data for each respondent. The NHANES 2005–2006 sample

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represents 'the total noninstitutionalized civilian population residing in the 50 states and District of Columbia' [10]. However, in 1999–2006 NHANES oversampled certain subpopulations, such as Mexican-American and black persons, individuals with low income, and adolescents aged 12–19. Sampling details are given in [10]. The NHANES 2005–2006 study was the most recent one with data on both iron, TIBC and sTFR. The files contained data from 5268 women, of whom 2689 were registered with ferritin, 2875 with iron and TIBC, 2685 with sTFR, 4191 with CRP, and 4314 with hemoglobin. A complete dataset of these variables were registered with 2559 women, 222 of whom were in the age group 1–5, and 2337 in the age group 12–49, the NHANES population of interest for iron-status indicators since 2003 [11]. Only data from the 2337 women in age the group 12–49 were considered for this work. As we used ferritin to define iron deficiency and ferritin rises during inflammation, we grouped the population into women with CRP $<$ 5 mg/L and those with CRP $\geq$ 5 mg/L [12]. We also grouped according to pregnancy, because the high estrogen production in pregnancy increases transferrin production independently of the iron status [13]. Only women with a positive laboratory pregnancy test or who self-reported pregnancy at examination were considered to be pregnant.

Laboratory methods

According to NHANES, UIBC was measured with a Beckman Synchron LX20 analyser and used to calculate TIBC. A known amount of ferrous iron was incubated with serum to saturate the available binding sites on transferrin, whereupon unbound excess iron was complexed with Ferene to form a blue complex, which was measured photometrically. Iron concentration in serum was measured with a FerroZine-method on the same instrument. The laboratory calculated TIBC = UIBC + iron, which was given in the result file. We had to recalculate UIBC = TIBC - iron, because only TIBC was given in the files. Ferritin and sTFR were measured with Roche Tina-quant reagents on a Roche Diagnostics Hitachi 912 analyzer. CRP was measured with a nephelometric method on a BN2 instrument from Dade Behring (now Siemens), and hemoglobin, MCV, MCH, and RDW on Beckman Coulter MAXM. Details on the analytical methods are given in separate documents [14]. However, the traceability to standards is not given. The ferritin assay was standardized against NIBSC standard 80/578 [15], which is the 2nd international standard for ferritin. According to NHANES, the total analytical coefficient of variation was 5.4% at a ferritin concentration of 77.6 mg/L [14].

Statistical methods

We estimated the diagnostic accuracy as the area under the receiver operating characteristic (ROC) curve [16], using three levels of serum ferritin as the definition of empty iron stores, ferritin $<$ 10 µg/L, $<$ 15 µg/L [17], and $<$ 20 µg/L. To study whether age would affect the diagnostic accuracy of UIBC, we used logistic regression with UIBC, age and an interaction term between UIBC and age as independent variables and the respective definitions of empty iron stores as dichotomous dependent variables. The same was done for sTFR. We also used logistic regression to study the joint diagnostic accuracy of UIBC and sTFR, and to estimate the likelihood ratio of empty iron stores as functions of UIBC [18]. Cut-off values for UIBC were calculated as the values that gave a likelihood ratio of 1, an appropriate value when the physician is most in doubt (when the pretest probability is equal to the treatment threshold [19]). To estimate the median UIBC difference between pregnant women and nonpregnant, due to pregnancy itself (adjusted for covariates), we modelled median UIBC as a function of ferritin, crp, age, and pregnancy, using quantile regression with a multivariable fractional polynomial algorithm that allows for a nonlinear association between UIBC and the three covariates. The same was done for sTFR. The Stata software, version 16 (StataCorp, College Station, TX 77845, USA) was used for all statistical analyses. p-Values less than .05 were considered statistically significant.

Results

Characteristics of the study population grouped according to CRP $<$ 5 mg/L and pregnancy are given in Table 1. Unadjusted median UIBC was 64.3 µmol/L in pregnant women versus 51.7 µmol/L in nonpregnant, a difference of 12.6 µmol/L, but adjusted for ferritin, CRP, and age, median UIBC was only 3.0 µmol/L (5.8%) higher in pregnant women ($p < .001$). Unadjusted median sTFR was 3.2 mg/L in pregnant women and 3.4 mg/L in nonpregnant, i.e. 0.2 mg/L lower in pregnant women. However, adjusted for ferritin, CRP, and age, median sTFR was 0.8 mg/L (23.5%) lower in pregnant women ($p < .001$).

In the regression models used to study whether age would affect the diagnostic accuracy of UIBC and sTFR, the interaction term between age and UIBC or sTFR did not reach statistical significance, except for sTFR when empty iron stores was defined as ferritin $<$ 10 µg/L (results not shown), so grouping by age was not done.

The areas under the ROC curves for UIBC and sTFR in the various groups are given in Table 2. For the largest group (no 1), 1560 nonpregnant women with CRP $<$ 5 mg/L, the areas under the ROC curves for iron, TIBC, transferrin saturation, MCV, MCH, and RDW are listed in Table 3, along with the corresponding figures for UIBC and sTFR.

In most groups and for most definitions of empty iron stores, UIBC had a higher area under the ROC curve than sTFR. The differences between UIBC and sTFR reached statistical significance when empty iron stores were defined as ferritin $<$ 20 µg/L in group 1 ($p = .007$) and in group 2 ($p = .003$). Only in group 3, when empty iron stores were defined as ferritin $<$10 µg/L and $<$15 µg/L, did sTFR show a better diagnostic accuracy than UIBC. Those differences were not statistically significant.

In group 1, the logistic regression model of ferritin $<$ 20 µg/L where sTFR was included in addition to UIBC, the

Table 1. Study population grouped according to CRP < 5 mg/L and pregnancy.

	CRP < 5 mg/L		CRP ≥ 5 mg/L	
Characteristic	Not pregnant	Pregnant	Not pregnant	Pregnant
Group no	1	2	3	4
N	1560	169	426	182
Age, years	19 (12–49)	26 (15–44)	30 (12–49)	26 (17–36)
CRP, mg/L	0.7 (0.1–4.9)	2.9 (0.1–4.9)	8.7 (5–139)	9.7 (5–70)
Ferritin, µg/L	33 (2–611)	22 (3–286)	44 (3–499)	22 (3–195)
Hemoglobin, g/dL	13.5 (8.1–16.7)	12.5 (8.6–16.2)	13.3 (6.2–17.0)	12.5 (8.6–14.7)
UIBC, µmol/L	51.0 (3.6–97.2)	60.1 (29.4–125)	54.5 (24.4–101)	66.7 (23.3–114)
sTFR, mg/L	3.4 (1.1–18.6)	3.1 (1.4–11.7)	3.7 (1.3–28.6)	3.4 (1.3–8.6)

The number of women in each group (*N*) are given, along with median and range (in parenthesis) of age, CRP, ferritin, hemoglobin, UIBC and sTFR.

Table 2. Area under the receiver operating characteristic (ROC) curve (95% confidence interval) for UIBC and sTFR in the four groups of women described in Table 1.

		Ferritin cut-off value for empty iron stores		
Group	Test	< 10 µg/L	< 15 µg/L	< 20 µg/L
1: CRP < 5 mg/L, not pregnant	UIBC	0.918 (0.899–0.936)	0.868 (0.844–0.892)	0.830 (0.806–0.854)
	sTFR	0.893 (0.868–0.919)	0.845 (0.819–0.872)	0.793 (0.767–0.819)
2: CRP < 5 mg/L, pregnant	UIBC	0.860 (0.804–0.917)	0.851 (0.793–0.909)	0.843 (0.786–0.901)
	sTFR	0.856 (0.790–0.922)	0.796 (0.723–0.870)	0.739 (0.664–0.814)
3: CRP ≥ 5 mg/L, not pregnant	UIBC	0.904 (0.837–0.971)	0.866 (0.810–0.922)	0.802 (0.747–0.857)
	sTFR	0.921 (0.873–0.970)	0.885 (0.827–0.942)	0.785 (0.723–0.846)
4: CRP ≥ 5 mg/L, pregnant	UIBC	0.843 (0.785–0.901)	0.824 (0.763–0.885)	0.789 (0.723–0.855)
	sTFR	0.771 (0.685–0.857)	0.788 (0.720–0.856)	0.747 (0.677–0.817)

Table 3. Area under the receiver operating characteristic (ROC) curve (95% confidence interval) for various test in diagnosing empty iron stores in 1560 nonpregnant women with CRP < 5 mg/L (group no 1 in Table 1).

	Ferritin cut-off value for empty iron stores		
Test	< 10 µg/L	< 15 µg/L	< 20 µg/L
UIBC	0.918 (0.899–0.936)	0.868 (0.844–0.892)	0.830 (0.806–0.854)
sTFR	0.893 (0.868–0.919)	0.845 (0.819–0.872)	0.793 (0.767–0.819)
Iron	0.823 (0.784–0.862)	0.764 (0.729–0.799)	0.724 (0.694–0.754)
TIBC	0.869 (0.842–0.896)	0.831 (0.805–0.857)	0.795 (0.770–0.820)
Transferrin saturation	0.872 (0.841–0.902)	0.814 (0.784–0.845)	0.775 (0.748–0.802)
MCV	0.788 (0.748–0.828)	0.725 (0.689–0.761)	0.694 (0.664–0.725)
MCH	0.809 (0.772–0.846)	0.752 (0.719–0.786)	0.711 (0.681–0.741)
RDW	0.869 (0.838–0.899)	0.821 (0.793–0.850)	0.773 (0.746–0.800)

coefficient of sTFR was statistically significant ($p < .001$) and the area under the ROC curve increased from 0.830 for UIBC alone to 0.852 for the combination.

In group 1, the function for the likelihood ratio (LR) of UIBC when empty iron stores were defined as ferritin < 20 µg/L was $LR = \exp(0.1261 \times UIBC - 6.898)$, which gave a cut-off value of 55 µmol/L for LR = 1. The corresponding figures for group 2 were $LR = \exp(0.08629 \times UIBC - 5.329)$, which gave a cut-off value of 62 µmol/L for LR = 1.

Discussion

The results show that UIBC was a more accurate test in diagnosing empty iron stores than sTFR when ferritin was used to define empty iron stores in women without inflammation (Table 2). In the largest group, nonpregnant women without inflammation, UIBC also outperformed iron, TIBC, and transferrin saturation, as well as erythrocyte indices (Table 3).

In nonpregnant women with CRP ≥ 5 mg/L UIBC was less accurate than sTFR when the more stringent definitions of empty iron stores were used. In pregnant women UIBC seemed to be more accurate than sTFR even in women with CRP ≥ 5 mg/L. As seen in Table 2, the diagnostic accuracy of sTFR was more reduced in pregnancy than the diagnostic accuracy of UIBC, and sTFR was relatively more affected than UIBC by pregnancy itself. Anyway, UIBC levels were higher in pregnancy (Table 1), explaining that the cut-off value corresponding to a likelihood ratio of 1 was estimated to be higher in pregnant women. As iron status parameters vary throughout pregnancy [20] and we had no data on gestational age, the results in pregnant women must be interpreted with some caution.

Measuring sTFR in addition to UIBC may not be worthwhile, because the increase in diagnostic accuracy for group 1 was modest compared to UIBC alone.

The credibility of these results depend on the accuracy of ferritin as a gold standard of empty iron stores, an accuracy that may be questioned. The ultimate gold standard of empty iron stores is said to be the absence of stainable reticular iron in technically satisfactory bone marrow smears [21], but bone marrow iron is not a perfect test either

[22–24] and obviously not obtainable in a population survey. So we were left with ferritin for defining empty iron stores. Ferritin is probably the most accurate test compared to bone marrow iron [2]. To study the problem of increased ferritin in individuals with inflammation, we grouped the study population according to CRP < 5 mg/L [12]. As the optimal ferritin cut-off value for empty iron stores is unknown, we used the three different cut-off values of < 10 µg/L, < 15 µg/L, and < 20 µg/L. At these levels ferritin is a very specific test of empty iron stores, although a less sensitive one [2]. The two most stringent ferritin cut-off values are too stringent in many patients with inflammation [8]; however, patients have varying degrees of inflammation, so choosing the optimal ferritin cut-off value for the individual patient is difficult. In the study population, a ferritin cut-off limit of 20 µg/L may be more appropriate to indicate empty iron stores in women with CRP ≥ 5 mg/L, and perhaps even in women with CRP < 5 mg/L, because the mean population hemoglobin begins to fall at a ferritin level of 20 µg/L [25].

Another issue of some concern is that different ferritin assays may not be calibrated to give equal results [26]. For instance, a Tina-quant assay result of 13 µg/L was equivalent to 9–12 µg/L with three other assays [15]. This matter does not disturb the ranking of the diagnostic accuracies, however, as all tests were assessed on equal grounds.

Still another concern is the study population, which by design, was not a representative sample of the U.S. female population at 12–49 years of age [10]. Compared to a relatively healthy, nonpregnant Norwegian female population aged 20–55 years, the part of the nonpregnant study population with CRP < 5 mg/L aged 20–49 years showed about the same prevalence of iron deficiency, as 17.4% had ferritin < 15 µg/L (results not previously shown) versus 21.7% in the Norwegian population [23]. In the Norwegian study, ferritin was measured with Abbott AxSym, which might have measured somewhat lower than Roche Tina-quant [15], so the prevalence of iron deficiency might be even more equal in the two populations. Also, the median hemoglobin concentrations were about the same, 13.5 g/dL in the comparable group of the study population (results not previously shown) versus 13.3 g/dL in the Norwegian population [23]. Nevertheless, the study population was not a clinical one, so the diagnostic accuracy of UIBC and sTFR may not be the same in women attending a health care facility.

If the physician wants a test other than ferritin in diagnosing empty iron stores in women, UIBC would be a good choice, at least in patients without inflammation. Diagnosing empty iron stores when the patient has inflammation is complicated, and sTFR might offer some help in that situation [3]. Otherwise, UIBC has some advantages: It can be easily measured, and its diagnostic accuracy outperforms that of sTFR, TIBC, and percent transferrin saturation. The optimal cut-off value depends, as always, on the cost of true and false positive and negative test results, as well as on the pretest probability of disease [16]; however, it also depends on the population and the definition of empty iron stores.

Disclosure statement

No potential conflict of interest was reported by the authors.

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