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Reference Distributions for Serum Iron and Transferrin Saturation: A Comparison of a Large Cohort to the World's Literature

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The appropriate clinical use of serum iron and transferrin saturation (TSAT) requires satisfactory reference intervals from birth to old age, and for males and females. This study identified 54 publications from 1974 to 2001 that met the criteria used in three prior meta-analyses, and these were analyzed statistically. A summary of our review is presented along with our reference population data on these measurements. This analysis places previous publications in perspective and suggests possible reasons for the observed differences. Previous studies of the individual analytes, serum iron, transferrin, and TSAT values agree with the reference ranges presented in this study, although the entire experience over time and between sexes has not been available before. Our 95% reference ranges are somewhat broader than those of the smaller studies, but they agree well with those of the larger ones. J. Clin. Lab. Anal. 16:246–252, 2002. © 2002 Wiley-Liss, Inc.

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INTRODUCTION

Assessing the status of a person's iron reserves for diagnosis, prognosis, and management of disease has previously been limited by uncertainty concerning the appropriate reference ranges for a given patient. Difficulties are especially evident when one attempts to interpret test results from individuals harboring inflammation. This cytokine-driven process manifests with elevations in levels of the positive acute-phase proteins and decreased levels of the negative acute phase proteins—one of which (transferrin) is the principal transport moiety for iron. The acute phase response, from whatever cause, down-regulates the hepatic synthesis of transferrin, making smaller amounts available for the critical movement of iron from the gut to points of utilization, principally the bone marrow. The usual result is that iron levels are more volatile than transferrin levels and fall more rapidly in individuals with inflammation, while changes in transferrin levels are delayed by a day or two.

This study, like its predecessors (1–3), identified publications (from 1974 to 2001 in the current study) that presented data for serum iron and TSAT, and reviewed the previously presented data for transferrin. All results were standardized against the National Institute for Standards and Technology (formerly National Bureau of Standards) serum reference material (SRM) 937 for iron, and certified reference material (CRM) 470/reference preparation for protein in human serum (RPPHS) (4) for transferrin. No reference preparation exists for total iron binding capacity (TIBC), which is often used to calculate TSAT. All publications provided data on serum iron or TSAT that meet minimal acceptable criteria (5–58). The term "transferrin saturation," used in previous publications, more accurately reflects the relation of iron to TIBC, since direct immunochemical methods for transferrin were usually not used. The large National Health and Nutrition Examination Survey (NHANES), however, provided laboratory details of their major studies (59–61).

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The aim of this work is to review the literature and extract a number of studies that include sufficient information to allow us to properly evaluate each cohort. The different reference ranges and clinical cut-points appear to have resulted from 1) the occurrence of methodological shifts when the required assays were performed, and 2) the complex nature of iron dynamics being overlooked. The conclusion of this analysis also reduces the disharmony in previous studies stemming from the use of small cohorts, not considering the age and sex of patients, the presence or absence of an acute phase response, the fasting state of subjects, and the effects that ubiquitous medications have on the final results.

MATERIALS AND METHODS

Identification of Published Reference Data

The methods used to search for relevant publications were the same as used in the previous studies (1–3). Methodological papers or those that only examined patients with specific diseases were excluded from statistical analysis. Articles were considered acceptable if they provided sufficient numerical information required for statistical analysis. Unlike our previous studies, we did not exclude papers that lacked detailed information on the assay method used, assay characteristics, or reference material for serum iron, since the tests and calibrants for iron have been reasonably stable for years. Performance in U.S. laboratories measuring serum iron ($n = 3{,}369$), as assessed in the CAP Chemistry Survey A (1999), showed good performance overall (overall coefficient of variation (CV) $\leq 6.1\%$). The same holds true for papers presenting data on TSAT wherein transferrin measurement methods, TSAT calculation formulae, or materials were not described. We assumed that the term TIBC is equivalent to transferrin, as others have noted. Most works did not provide gender-specific data. Of the 54 papers reviewed that listed iron values, 17 studied subjects who had been fasted, 12 described subjects who had not been fasted prior to phlebotomy, and 25 gave no indication of the fasting state.

Estimating the Central Estimate and Reference Range From Published Studies

The mean, median, or geometric mean was used as the estimate of the center of the distribution, as in the previous studies. If these statistics were not directly available, they were computed using observed or smoothed centiles (e.g., the 2.5th and 97.5th centiles). If none of these were available or extractable from digitized figures, the study was excluded from all analyses. The observed 95% ranges were used for the reference interval analysis.

Conversion of Reported Results to a Single Reference Material or Measured Unit

Reference to an approved reference material for iron or transferrin was rarely given. It was assumed that the kit manufacturers were using National Institute for Standards and Technology reference material SRM 937 for iron. The papers reviewed gave measured values in a variety of units, most of which could be reduced to a common unit, μmol/L. Conversion from μg/dL to μmol/L was accomplished by multiplying by a factor of 0.179.

RESULTS

Number of Studies Available for Analysis

The collected literature for transferrin values has been previously reviewed (2), as have reference ranges for serum transferrin in a large cohort (62). Because of the critical importance of identifying proposed referent individuals with inflammation, the individual articles were categorized by their attention to evidence of the acute phase response. Of the 54 papers identified for analysis of serum iron or TSAT values, only five used the acute phase response as an exclusion criterion, and the decision level was not provided. Twelve papers acknowledged the importance of the acute phase response and the confounding potential of nonsteroidal antiinflammatory drugs, yet tests to exclude the condition were mentioned only tangentially. Low levels of serum iron or high values of TSAT were the actual focus of these publications. Thirty-seven papers gave no indication that the authors tested for, or recognized the critical importance of, the acute phase response in iron dynamics. As a result, many of the publications included an unknown proportion of referent individuals with unrecognized inflammation, and are therefore likely to have lower iron and TSAT values overall compared to those studies in which such individuals were excluded.

A total of 45 publications were identified that contained information on serum iron in apparently healthy individuals (6,8–24,26–37,39,41–46,48–56). Five did not provide basic information or sufficient numerical data to permit statistical analysis (9,28,36,38,51). Seven did not provide the ages of their subjects (11,12,16,17,24,39,50), and one study gave values in non-convertible units (13). The remaining 33 studies (6, 8, 10, 14, 15, 18–23, 26, 27, 29–35, 37, 41–46, 48, 49, 52–55) were considered acceptable for our analysis of serum iron measurements. Of these, one article had

a typographical error and the unit of measurement was assumed to be μmol/L (37), and one omitted the unit of measurement entirely, so it was assumed to be μmol/L (44).

A total of 32 publications were identified that contained information on TSAT levels in apparently healthy individuals (5,7–10,15,16,18,19,21,22,24,25,29–31,33,38,40–42,45–48,50,51,54–58). Two studies did not provide information on the ages of the study subjects (38,56). The remaining 29 studies were considered acceptable. In addition, one recent paper (58) showed a figure with smoothed centiles, but without statistical data or observed values. Because of the cohort size and timeliness of the work, the figure could not be included in Fig. 1B, but was digitized and incorporated into Fig. 2B.

Of the 54 papers identified, 17 stated that the subjects had fasted prior to phlebotomy, 12 stated that they had not fasted, and 25 made no mention of fasting status.

Comparison of the Central Estimates

Figure 1 shows the central estimates from the published studies of serum iron (A) and TSAT (B). The reported mean (or median) age of the study population is shown on the logarithmic horizontal axis vs. the reported central estimates on the logarithmic vertical axis. Each observation is shown as a circle, with the smallest circles representing estimates based on over 100 observations, medium circles representing 50–99 observations, and the largest circles representing 10–49 observations. If a published observation was based on less than 10 observations, it was combined with an adjacent age group from the same publication, whenever possible. For purposes of comparison, the solid and dashed lines represent the median levels for the two analytes found for males and females, respectively, in our companion study (63).

Figure 1A shows the reported serum iron median values from the 33 publications considered acceptable for analysis of serum iron values. Our median levels for serum iron are lower than the published estimates over the entire age range. At least some of this difference is due to our cohort being composed of subjects who had not fasted prior to phlebotomy (thereby decreasing the values). Most observations at less than 1 year of age were from two studies (8,54).

Figure 1B shows the reported TSAT measurements from the 31 publications that were considered acceptable. Our median TSAT levels for adults are somewhat lower than the published estimates, for the same reason mentioned above. Only the studies that examined the NHANES population provided values for children (15,16,64) and teenagers (65).

Differences in Reference Ranges Reported for Iron and Transferrin Saturation

A review of the publications revealed that analysis of distributions could include consideration of fasting vs. nonfasting data. While it is known that iron absorption is reduced by a meal, the effect on serum levels of iron has not been carefully studied. A few studies show rather marked differences between fasting and postprandial levels by as much as a twofold increase in the fasting state (66,67). When authors stated categorically whether subjects had been fasted or not, measurements were 8% higher for the fasted populations.

In view of the fact that we were not able to evaluate either the authors' methodology or the reference material employed in a manner similar to our previous meta-analysis of transferrin (2), an uncertainty in reported TSAT values remains. Unavoidably, differences among methods, which were not described in the publications, may contribute to the scatter shown in Fig. 1B. Several workers have reviewed the comparability and accuracy of immunochemically measured transferrin vs. TIBC, and have indicated that the former is more precise yet on average compares well with theory (68), with some exceptions (69–71). Twenty-five of the 32 papers reporting TSAT values were published before the introduction of CRM-470, and only two papers stated that the new reference material had been used to standardize the transferrin assay, although manufacturers of transferrin immunoassay kits did shift to the new reference material during this time. Reference materials for transferrin available prior to 1995 were on average 12% higher than the new international reference material for transferrin. With the introduction of CRM 470, median transferrin values fell. The result of this change, however, was that TSAT values rose significantly, as shown by our data (Fig. 1B). The two studies that used CRM 470 values for transferrin reported values that agree with our data. An evaluation of NHANES methods (72) for serum iron faced a similar situation. Although the studies were performed within a single institution, various phases of the study used different methodologies, which resulted in systematic differences in measurements.

Comparison of the Reference Ranges

The reference ranges reported in the studies used in the aforementioned analyses were generally based on an adequate number of observations. Three were based on over 7,000 observations. Cursory verification of the health status of each individual was possible as part of the NHANES national study. Our observed reference ranges were each based on over 25,000 observations. Primary verification of health status was not performed

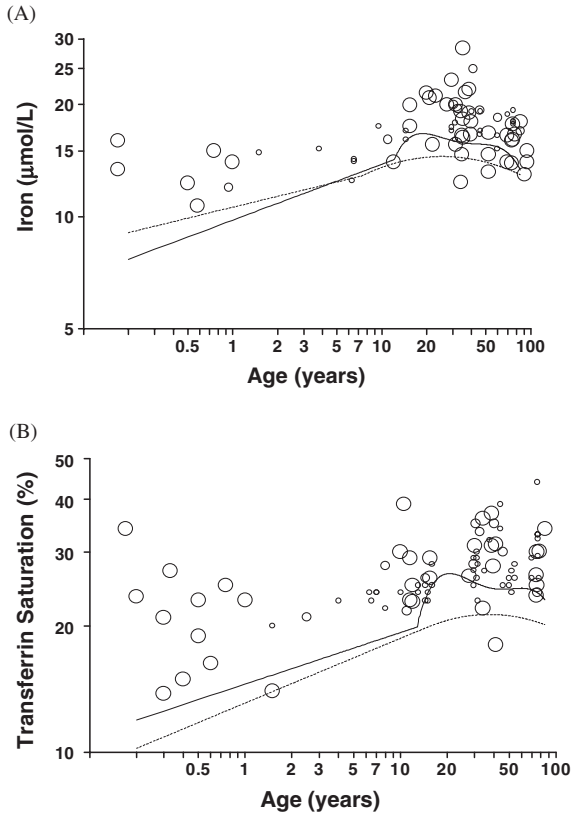

Fig. 1. A summary of published median reference values for iron and TSAT. The published median (or geometric mean) levels for (A) iron and (B) TSAT are displayed on the logarithmic vertical axis vs. the mean (or median) age on the logarithmic horizontal axis. The three symbol sizes represent the number of observations in each group (small, medium, and large circles represent >100, 50–99, and 10–49, respectively). The solid (males) and dashed (females) lines are the regressed median levels from our large cohort study (63).

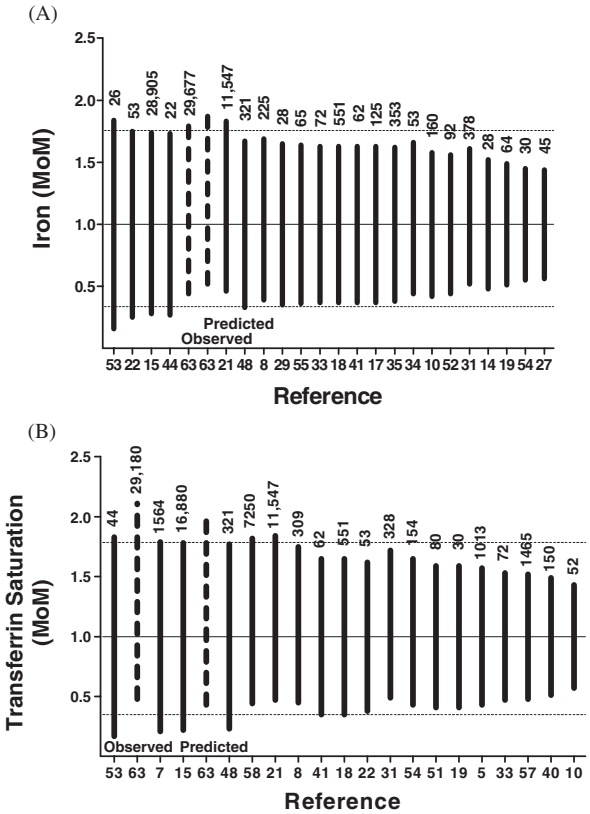

Fig. 2. Reference intervals from published studies. The bars represent the interval between the 2.5th and 97.5th centiles for each study, with the number of subjects above the bar. The horizontal axis shows the reference number for each study. The selected centiles are presented as multiples of the median (MoM), compensating for age and gender differences between studies. The figure shows the analyses for (A) iron and (B) TSAT measurements on identical logarithmic vertical axes. The horizontal dotted lines display the weighted consensus value at the 2.5th and 97.5th centiles for previously published studies. The broken bars represent the observed and predicted reference ranges from our large cohort study (63). The skewed position of the dotted lines in both figures is the result of our population data having been log-transformed while all others were not.

by us, but the cases were reviewed by the referring physicians. Figure 2A shows the 22 previous studies for serum iron for which it was possible to calculate a 2.5th–97.5th centile range (after each study's reference limits were divided by their own population medians for conversion to multiples of the median as a means of normalizing the ranges). The reference numbers are shown on the horizontal axis, sorted by decreasing 95% reference range width. The weighted average of the ranges reported in the literature (excluding those from our study) is shown by two thin horizontal dotted lines. The 95% reference ranges for our study (both observed and predicted from the population parameters) are represented as thick dotted lines. Figure 2B shows a similar analysis for the 19 studies that reported TSAT reference ranges.

When the population distributions were examined for iron and TSAT (63), the log transformation clearly fit the data better than no transformation (data not shown). For this reason, our 95% reference intervals are asymmetric and the tails of the distributions are slightly higher on a linear scale (Fig. 2). However, the width of the interval is similar to those reported by others. Although there are a few low estimates that vary from the consensus, the majority of the published studies are consistent.

Ethnic Group-Specific Reference Ranges

A total of 22 studies included in this analysis reported the race or ethnic heritage of the study subjects. The majority of these studies reported measurements in a Caucasian (or, by inference, predominantly Caucasian)

population. Four papers (14,36,40,52) examined values in Japanese populations, two of which presented sufficient data to show that there were no differences among the Caucasian groups (for TSAT (40) or for serum iron (52)). Five additional studies reported values from Caucasian but ethnically restricted national populations (23,41,43,53,56). In two studies (41,53) there were sufficient data to show that there were no differences between serum iron and TSAT in a Caucasian population.

DISCUSSION

This is the fourth in a series of meta-analyses for serum proteins and related analytes. The results demonstrate that our approach to analyzing a large population (63) produces data comparable to those of previous smaller, more constrained studies, which obtained more detailed individual case data. The companion study reports reference data for serum iron and TSAT based on a large dataset with less reliance on individual health status. This meta-analysis shows that our reference data are somewhat broader and higher than those of small studies, but are consistent with the NHANES results, which are of comparable size (15,16,64,65). Methodological changes over time likely contribute to the degree of scatter seen in Fig. 1. The effect of significant inflammation has been largely removed from our data, but remains as a concern in most published series. It can be seen in Fig. 2 that our cohort has somewhat higher reference values (MoM) for serum iron and TSAT than other studies that did not exclude cases based on laboratory evidence of an acute phase response. In addition, our data has been log-transformed, whereas other studies were not. Furthermore, this study, along with previously published works, contains data from cases wherein the effects of contraceptive medication, female hormone replacement, and pregnancy will be felt, but cannot be readily detected, and will unavoidably (and appropriately) be included as a variation of normal. In addition, the fact that our cohort was from individuals not known to be fasting predictably reduces our serum iron values, as seen in Fig. 1A. Furthermore, our conversion to CRM 470 prior to the start of this analysis results in a decrease in our transferrin values by $\sim$12% (not shown). Moreover, our calculation of TSAT employed the molar ratio (73) while older publications did not, resulting in lower TSAT values in the present study (Fig. 1B).

All parameters of iron metabolism are affected by two easily measured analytes: the amount of the transport protein transferrin, and the amount of iron it transports. All other values devolve from these two. Procedures to quantify these moieties are readily available, perform well, and are inexpensive. With these two values the basic process of iron dynamics can be analyzed and their relationships will add dimension to the understanding of iron dynamics in each case.

This set of reference data for both sexes and throughout life facilitates the incorporation of serum iron values into diagnostic and prognostic patient evaluations in a manner that was not previously possible. Further, it provides a practical basis for avoiding misinterpretation of low serum iron values as a result of inflammation (74). Low levels caused by inflammation will not respond to increased iron intake, whereas iron deficiency as the result of dietary deficiency and normal transferrin levels will respond to iron supplements. These reference ranges provide a starting point for creating a logical assessment of iron metabolic status even in the face of an active cytokine drive. Recommended protocols for examining selected local cohorts (75,76) are available and can now be used more easily to study smaller study groups.

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