CPD Article

Accuracy of Various Iron Parameters in the Prediction of Iron Deficiency in an Acute Care Hospital

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Abstract

Introduction: Iron parameters like serum ferritin and iron saturation are routinely used in diagnosing iron deficiency. However, these tests are influenced by many factors. We aimed to review the accuracy of iron parameters among inpatients in an acute care hospital. Materials and Methods: From October 1997 to April 2002, bone marrow aspirate samples from patients on whom concurrent iron studies had been done were analysed. Accuracy of the various iron parameters was analysed using receiver operating characteristic curves. Results: Among 92 bone marrow aspirate samples, 58, 86 and 83 had a concurrent serum ferritin, serum iron and percentage iron saturation done respectively. Serum ferritin is the best marker for predicting the presence of iron deficiency. This is followed by percentage iron saturation and lastly by serum iron. At the most optimal, a serum ferritin of <60 ng/mL has a positive likelihood ratio of 24.35, sensitivity of 69.6%, specificity of 97.1% and positive predictive value of 94.1%. An iron saturation of <7% has a positive likelihood ratio of 21.62, sensitivity of 44.1%, specificity of 98.0% and positive predictive value of 93.8%. Seven out of 50 samples, which had concurrent measurements of iron saturation and serum ferritin, were discordant in indicating the presence of iron deficiency. In 6 cases, the iron saturation was the spurious result. Conclusion: A serum ferritin of <60 ng/mL or a percentage iron saturation of <7% is highly predictive of iron deficiency among inpatients in an acute care hospital.

Ann Acad Med Singapore 2005;34:437-40

Key words: Ferritin, ROC curve, Sensitivity and specificity, Transferrin

Introduction

The diagnosis of iron deficiency anaemia has traditionally relied on the measurement of iron parameters like serum iron, total iron binding capacity, iron saturation and serum ferritin. The reference ranges provided by most laboratories are derived from apparently normal individuals and represent the values of 95% of the normal population.

The inpatient population represents a heterogeneous group of individuals with various disease conditions; many of these patients suffer from inflammatory disorders. Inflammatory states and other diseases like myocardial infarct and malignancies are known to cause variations in the iron parameters used for the diagnosis of iron deficiency. Specifically, the above diseases cause a reduction in serum iron, and an increase in serum ferritin. Accordingly, iron (or transferrin) saturation levels are also affected unpredictably although the literature usually quotes 15% as

the level below which iron deficiency can be predictably found.[1] Reports investigating the accuracy of the various iron parameters in predicting iron deficiency in various subgroups of patients showed that serum ferritin is most useful in many conditions (liver cirrhosis, rheumatoid arthritis and other inflammatory rheumatic diseases, hospitalised patients).[2-5] Transferrin saturation (or iron saturation) was found to be most useful in HIV patients in another report,[6] and both were found to be inaccurate in a report on haemodialysis patients.[7] For those studies which found serum ferritin to be a useful predictor of iron deficiency, a variety of serum ferritin levels were recommended, ranging from 30 ng/mL to 75 ng/mL, for optimal sensitivities and specificities.

The interpretation of iron parameters can be confusing if different parameters indicate differently the presence or absence of iron deficiency. While a "trial of iron therapy"

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may seem innocuous, studies have suggested that iron overload can have growth-promoting effects on microorganisms and tumour cells and can concurrently inhibit T cell-mediated immunity and the intracellular killing capacity of neutrophils.[8,9]

We therefore embarked on a study to review the predictive value of iron parameters in a single acute care hospital in predicting the presence of iron deficiency among inpatients. We also analysed the situations where results of different iron parameters indicate discordantly the presence or absence of iron deficiency.

Materials and Methods

From October 1997 to April 2002, bone marrow aspirate samples from patients on whom concurrent full blood count and iron studies have been done were analysed. Samples were included for analysis only if the bone marrow aspirate and iron studies were done within 1 week of each other. These samples came from patients who had a variety of clinical diseases and many had evidence of ongoing inflammation.

All bone marrow aspirate samples were stained for iron using the conventional Perl's reaction with Prussian blue.[10] The amount of iron stores were graded by a single assessor (PK) unaware of the results of the other iron studies. The amount of iron stores were graded on a scale of 0 (absent) to 5+ (marked increase), with 1+ to 3+ being normal iron stores and ± being reduced iron stores. With each iron stain, a positive control was concurrently performed. The bone marrow iron was taken as the reference standard for the diagnosis of iron deficiency and only aspirates which had a score of 0 were considered iron-deficient.

Serum ferritin was measured using a microparticle enzyme immunoassay on the Abbott AxSym chemistry analyzer (Abbott Laboratories, Abbott Park, Illinois, USA). Serum iron was measured using the FerroZine photometric method on Roche/Hitachi analyser (Roche Diagnostics Asia Pacific, Singapore). Transferrin was measured by a latex-enhanced immunonephelometric assay on the BN 100 nephelometer (Dade Behring Asia, Singapore) for the first 66 out of the 83 samples that had a percentage iron saturation result. For these 66 samples, the total iron binding capacity was calculated by multiplying the transferrin levels with a conversion factor of 25.6.[11] For the remaining 17 samples which had a percentage iron saturation result, the total iron-binding capacity was measured by summing the serum iron and the unsaturated iron-binding capacity, which was measured directly by saturating the serum transferrin with a standard amount of iron at an alkaline pH and then measuring the excess unbound iron.

The accuracy of the various iron parameters was analysed using MedCalc™ statistical software programme (MedCalc

Software, Belgium), plotting receiver operating characteristic (ROC) curves for ferritin levels, percentage iron saturation and serum iron levels. Bone marrow iron was used as a dichotomous classification variable, where only a marrow iron score of 0 was considered iron-deficient and any other marrow iron score was considered iron-replete. The diagnostic performances (sensitivity, specificity, positive and negative likelihood ratios) of the various iron parameters at different cut-off points for the diagnosis of iron deficiency were plotted on a ROC curve. From the ROC curves, the levels of the various iron parameters at their optimal likelihood ratios for diagnosing iron deficiency were derived.

Results

Samples

Altogether, 92 bone marrow aspirate samples met the inclusion criteria. Among these 92 samples, 58 had a concurrent serum ferritin level, 86 had a concurrent serum iron level and 83 had a concurrent percentage iron saturation measured. The patients represented in this study included 54 females and 38 males, and the median age of these patients was 63 years (range, 13 to 90).

Among the bone marrow samples analysed, the amount of stainable iron ranged from 0 to 5+. The distribution of amount of stainable iron in these 92 samples is shown in Table 1.

Diagnostic Performances of Iron Parameters

The ROC curves for serum ferritin, percentage iron saturation and serum iron levels, together with the area under the curves (with their 95% confidence intervals), are shown in Figure 1. From the figure, it can be seen that amongst this group of hospital inpatients, serum ferritin was the best marker for predicting the presence of iron deficiency as characterised by the largest area under the curve. This was followed by percentage iron saturation and lastly by serum iron levels.

From the ROC curves, the level of serum ferritin at the optimal positive likelihood ratio for diagnosing iron

Table 1. Iron Stores in Marrow Samples

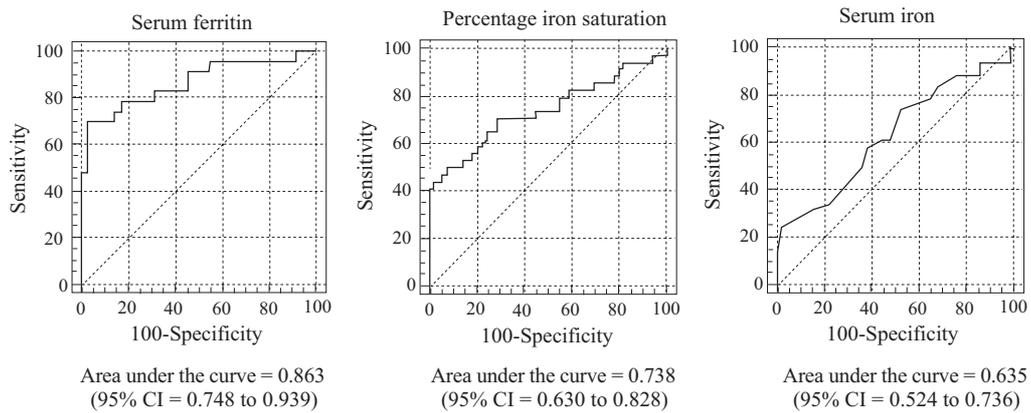| Bone marrow iron	Number
0	40
±	10
1+	9
2+	11
3+	15
4+	6
5+	1
Total	92

Fig. 1. Receiver operating characteristic curves for ferritin, percentage iron saturation and serum iron.

deficiency (likelihood ratio = 24.35) was found to be <60 ng/mL, which is higher than the lower limit of the normal population (15 ng/mL in our laboratory). Similarly, the percentage iron saturation at the optimal positive likelihood ratio for diagnosing iron deficiency (likelihood ratio = 21.62) was found to be <7% and not 15%, as published in textbooks. The diagnostic performances of the various iron parameters at these different levels are shown in Table 2. As a comparison, the diagnostic performances of these iron parameters at levels commonly used for the diagnosis of iron deficiency by the clinical community are also included.

Discordant Samples

Fifty samples had concurrent measurements of iron saturation and serum ferritin levels. Among these samples, there were 7 samples where both results were discordant in indicating the presence or absence of iron deficiency (iron deficiency defined as iron saturation of <7% or serum ferritin levels <60 ng/mL). As shown in Table 3, in all these discordant cases except one, serum ferritin was concordant with bone marrow iron and the iron saturation was the spurious result.

Discussion

Our results show that among patients in an acute care hospital like ours, both serum ferritin and iron saturation

Table 3. Samples where Serum Ferritin and Iron Saturation were Discordant in Indicating Iron Deficiency

	Number	%
Iron saturation indicates iron deficiency, ferritin does not	1	
Ferritin concordant with bone marrow iron	1	100
Iron saturation concordant with bone marrow iron	0	0
Ferritin indicates iron deficiency, iron saturation does not	6	
Ferritin concordant with bone marrow iron	5	83
Iron saturation concordant with bone marrow iron	1	16

levels have a high specificity in the diagnosis of iron deficiency at the respective levels of <60 ng/mL and <7%, levels predicted to have optimal likelihood ratios by the ROC curves. At these levels, there is a >93% positive predictive value with regard to the presence of iron deficiency. However, sensitivity at these levels is low and 30% of iron-deficient patients will not be picked up if we apply the serum ferritin level of 60 ng/mL as a criterion for iron deficiency. Nonetheless, this is an improvement compared to using the normal reference ranges, where more than 60% of iron-deficient patients will not be picked up. Our results are also corroborated by several reports[2-6] which found that using levels of serum ferritin from 30 ng/mL to 75 ng/mL can optimise the sensitivity and specificity of diagnosing iron deficiency.

Table 2. Diagnostic Performance of Iron Parameters at Various Levels

Test value	Number	Positive likelihood ratio	Sensitivity (%)	Specificity (%)	Positive predictive value (%)
Serum ferritin <60 ng/mL	17	24.35	69.6	97.1	94.1
Serum ferritin <15 ng/mL	8	NA	39.1	100	100
Serum ferritin <100 ng/mL	21	4.87	69.6	85.7	76.2
Iron saturation <7%	16	21.62	44.1	98.0	93.8
Iron saturation <15%	36	2.26	64.7	71.4	61.1

NA: not applicable

Results of our ROC curve for iron saturation is rather surprising when compared to those found in the literature,[1,6] which has traditionally recommended an iron saturation level of 15% to 20%, below which iron deficiency can be diagnosed with reasonable accuracy. Our analysis recommended a far lower level of 7%. At a recommended level of 15%, sensitivity and specificity are only 65% and 71% respectively. The reason for this discrepancy is not obvious, although the concomitant clinical disorders of the patients and the medications (iron supplementation) that they were using might have played a role (see below).

Discordant results between different iron parameters are common and make up about 15% of our analysed samples (if the strict criteria as proposed above is applied – iron saturation <7% or serum ferritin <60 ng/mL). Although the absolute number of discordant results is few (n = 7), the discordance in the results is reflective of the high specificity of serum ferritin and the low sensitivity of percentage iron saturation at their respective levels.

One obvious weakness of this study is the non-uniformity of the study population. The marrow samples come from patients with different diagnoses. There is also no information on whether these patients were on iron supplementation at the time of testing. If a significant number of these iron-deficient patients were on iron supplementation (which would have increased the serum iron levels but would have had no effect on bone marrow iron stores), this might have caused the low sensitivity of iron saturation in diagnosing iron deficiency.

Should clinicians practising in an acute care hospital setting now base their diagnoses of iron deficiency on the "more optimal" levels of serum ferritin and percentage iron saturation proposed in this study? The answer really depends on the patient. In the absence of any inflammation or malignancy that can cause an increase in acute phase reactants, there is no reason not to base the diagnosis of iron deficiency on the reference ranges of the iron parameters found in a normal population. In the presence of inflammation or malignancy, the severity of the disorder should also be considered. Being aware of the interaction between inflammation and the various iron parameters, and that the diagnostic performances of the various iron parameters can change in the presence of other concomitant diseases, is an important step in the interpretation and use of iron studies to diagnose iron deficiency.

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