Performance Characteristics of Three Serum Iron and Total Iron-Binding Capacity Methods in Acute Iron Overdose

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Abstract

Accurate serum iron and total iron binding capacity (TIBC) measurements may be useful in acute iron overdoses. Two alumina column TIBC methods were found to measure increased TIBC when free iron was present. A homogeneous TIBC method gave consistent results until iron concentrations exceeded 500 μg/dL (90 μmol/L), when it began to underestimate the TIBC. Serious iron overdoses require chelation therapy with deferoxamine. Iron recovery was reduced by up to 50% for all 3 methods with clinically achievable concentrations of deferoxamine 8,400 μg/dL (150 μmol/L). TIBC measurements by both alumina column methods were reduced by deferoxamine in the presence of free iron and unaffected when the iron concentration was less than the TIBC. The homogeneous TIBC method yielded falsely elevated results in the presence of free deferoxamine. Procedures that measure TIBC by addition of excess ferric iron followed by alumina adsorption are not suitable for monitoring TIBC in acute iron overdose. The homogeneous TIBC assay can be used in acute iron overdose but underestimates TIBC when iron concentrations exceed 500 μg/dL (90 μmol/L). None of the methods examined are useful for measuring iron or TIBC in the presence of deferoxamine.

Acute iron poisoning continues to be a significant problem, especially in the pediatric population. The American Association of Poison Control Centers reported 29,260 cases of exposure to iron-containing products in 1997, with 79% occurring in children younger than 6 years and 12% in children 6 to 19 years of age.1 Iron exposure accounted for 2.0% of all exposures in children younger than 6 years, a figure that has remained remarkably constant for the past 15 years.2 Oral iron preparations contain ferrous iron (Fe2+), which is absorbed readily in the gastrointestinal tract. Once absorbed, Fe2+ is rapidly converted to ferric iron (Fe3+), which enters the systemic circulation. Transferrin, the major iron-binding protein in serum, binds Fe3+ tightly. It has been hypothesized that once the serum iron concentration exceeds the binding capacity of transferrin, the unbound or “free” iron is responsible for the systemic toxic effects.3-5 This hypothesis has not been validated in the clinical setting, in part because of limitations of some laboratory methods for measuring the serum total iron-binding capacity (TIBC) and also because the rapid distribution of free iron into tissue compartments makes the time of sample collection critical.6-8

Questions about the appropriate diagnosis and management of acute iron overdose still remain. When should chelation therapy be administered? Some have recommended chelation with deferoxamine when free iron is present (ie, the serum iron concentration is greater than the TIBC).3,4,9-12 More recently, others have indicated that the TIBC is not measured consistently in acute overdose situations by certain methods, one using magnesium carbonate and the other using an iron-absorbing resin, and, therefore, should not be relied on.6,7 It remains to be determined whether appropriate measurement of the TIBC can facilitate correct medical decisions about chelation.

Another therapeutic decision is when to terminate chelation therapy. Some have recommended discontinuing
deferoxamine when serum iron levels fall below the TIBC.9,13 Another more recent recommendation is to discontinue deferoxamine when the serum iron is less than 150 μg/dL (27 μmol/L).14 One confounding factor for patient management is that the administration of deferoxamine potentially can interfere with measurements of serum iron and TIBC.15,16

Citing such problems, a recent review article recommended against the use of TIBC measurements in the setting of iron overload.17 However, some widely used clinical laboratory methods for measuring TIBC have not been evaluated for performance in acute iron overdose. We studied the performance characteristics of 3 sets of previously unstudied serum iron and TIBC assays under conditions simulating acute iron overdose and subsequent deferoxamine therapy.

Materials and Methods

Deferoxamine mesylate, ferric chloride hexahydate, bovine serum albumin, and human immunoglobulin G (IgG) were purchased from Sigma Chemical (St Louis, MO). A pool of off-the-clot serum was obtained by combining serum samples from the clinical laboratory and adjusting the pH to 7.4 with sodium hydroxide. Aliquots of the serum pool were spiked with the appropriate amount of a ferric chloride stock solution (50,000 μg/dL of iron [8,950 μmol/L]) to achieve the desired final iron concentration.

Iron and TIBC reagents from Ortho Clinical Diagnostics (Raritan, NJ) were used according to the manufacturer’s instructions on a Vitros 250 analyzer (Ortho Clinical Diagnostics). The iron method used a dry multilayered slide in which Fe₃⁺ is removed from transferrin at pH 4.0. The Fe₃⁺ is reduced to Fe²⁺ by ascorbic acid. The Fe²⁺ binds to a dye, N-(4-(2,4-bis(1,1-dimethylpropyl)phenoxo)butyl)-5-methoxy-6(2,3,6,7-tetrahydro-8-1H.5H-benzaquinolinizin-9-ylazo)-3-pyridine sulfonamide, to form a colored complex that is quantified by reflectometry at 600 nm. TIBC was measured on the Vitros 250 by the addition of excess ferric citrate to simulate samples that might be obtained in an acute iron overdose. The iron and TIBC concentrations were measured in these samples using 3 sets of iron-TIBC methods. The results are shown in Figure 1. Panels A and B demonstrate that the TIBC measured by the Vitros and aca Star methods, both of which use an alumina column to remove nontransferrin-bound iron, is dependent on the serum iron concentration. When the iron concentration exceeds the TIBC of the original serum pool, the measured TIBC begins to increase. The slopes of these lines, which were determined by plotting TIBC vs iron for the 3 highest iron concentrations for each experiment, varied modestly from experiment to experiment when different lots of alumina columns were used, but the TIBC always increased with increasing iron concentrations.

For the Vitros the mean slope was 0.63 (range, 0.52–0.75; n = 5). For the aca Star, the mean slope was 0.83 (range, 0.66–0.99; n = 2). The BMC method shown in panel C demonstrates an inverse relationship between serum iron and TIBC at iron concentrations higher than 500 μg/dL (90 μmol/L). The decrease in TIBC with increasing iron concentrations observed by this method also varied some with different lots of reagents, but the decrease was always less than 10% at serum iron concentrations up to 500 μg/dL (90 μmol/L). When the measured percentage of iron saturation of TIBC was plotted against the theoretical value calculated using the TIBC of the original serum pool for each method, it was noted that the maximum percentage of saturation was less than 150% for the Vitros method and less than 125% for

unbound iron. Iron is measured in the cartridge eluate by the iron method outlined.

Iron and unsaturated iron-binding capacity (UIBC) reagents from Roche/Boehringer Mannheim (Indianapolis, IN; subsequently referred to as the “BMC” method) were used on a Hitachi 747 (Roche/Boehringer Mannheim) analyzer according to the manufacturer’s instructions. The BMC iron method releases Fe³⁺ from transferrin with guanidinium chloride at pH 5.0 followed by reduction to Fe²⁺ with ascorbic acid. The Fe²⁺ then forms a colored complex with Ferrozine, the absorbance of which is monitored at 570 nm. In the BMC UIBC method, an excess of ferrous ammonium sulfate is added in the presence of hydroxylamine at pH 9.1. The available iron-binding sites are saturated, and the excess unbound Fe²⁺ is reacted with Ferrozine. The UIBC is equal to the difference between the amount of iron added and the excess unbound iron measured. The TIBC is equal to the sum of the serum iron and the UIBC.
the aca Star method Figure 2AI and Figure 2BI. The measured TIBC saturation exceeded the theoretical for the BMC method when the percentage of saturation exceeded 200% Figure 2CI. In an actual pediatric iron overdose case in which the total serum iron was 1,940 µg/dL (347 µmol/L), the TIBC measured on a Vitros analyzer was 1,760 µg/dL (315 µmol/L), yielding an apparent saturation of 110%. This result is consistent with the in vitro spiked serum results.

Additional experiments were performed using the alumina columns and iron saturating solution from the Vitros TIBC method in an attempt to determine why TIBC values were increased in the presence of free iron. The iron concentration in the ferric citrate saturating solution was found to be 380 µg/dL (68 µmol/L). Aliquots (200 µL) of this saturating solution were passed through an alumina column, and the iron concentration of each effluent aliquot was measured to determine the capacity of the alumina for Fe^{3+}. The iron concentration was less than the limit of detection (2 µg/dL [0.4 µmol/L]) until 3,000 µL of eluate had been collected, at which point the iron concentration was 310 µg/dL (55 µmol/L).

In a second experiment, a serum pool (TIBC, 254 µg/dL [45 µmol/L]) was spiked with ferric chloride solution to achieve iron concentrations ranging from 300 to 4,100 µg/dL (54–734 µmol/L). The samples were prepared for TIBC determinations by adding 1,000 µL of Vitros iron saturating solution to 500 µL of sample. Aliquots (300 µL) of the alumina column eluate were assayed for iron. In all cases, the concentration of iron in the last aliquot through the column was lower than the first. The difference between the first and last aliquots ranged from 5% to 45% and showed no apparent concentration dependence.

In a third set of experiments, a serum pool (iron, 80 µg/dL [14 µmol/L]; TIBC, 352 µg/dL [63 µmol/L]) was spiked with ferric chloride to a final iron concentration of 546 µg/dL (98 µmol/L). The TIBC was measured using Vitros alumina columns and 3 different saturating solutions (500 µL of sample + 1,000 µL of solution). The TIBCs measured using the Vitros saturating solution, water, and a
2-mmol/L concentration of sodium citrate were all 553 μg/dL (99 μmol/L).

Another set of experiments was performed to determine the effect of specific serum proteins on TIBC measurements by an alumina column method. Solutions containing bovine serum albumin (6% wt/vol in water) or human IgG (3% wt/vol in a 154-mmol/L concentration of sodium chloride) were spiked with ferric chloride to final iron concentrations of 500 and 1,000 μg/dL (90 and 180 μmol/L). A 1-mL aliquot of each solution was passed through a Vitros alumina column, and the iron concentration of the column eluate was measured. A plot of the iron concentration vs TIBC yielded slopes for the albumin and IgG solutions of 0.39 and 0.09, respectively (data not shown).

The effects of deferoxamine on iron and TIBC measurements made using all 3 methods were determined by using a serum pool spiked with aqueous ferric chloride solution. Reductions in the measured iron concentration for a given spiked pool were linear with increasing concentrations of deferoxamine, and all 3 methods showed similar reductions Figure 3A, Figure 3C, and Figure 3E. Deferoxamine had minimal effect on the Vitros and aca Star TIBC measurements for pools with low iron concentrations, but pools with high concentrations of iron showed linear reductions in the measured TIBC with increasing concentrations of deferoxamine Figure 3B and Figure 3D. The TIBC of the serum pool measured by the BMC assay showed a linear increase with increasing concentrations of deferoxamine Figure 3F. Serum pools with the highest iron concentrations showed only modest increases in the TIBC measured by the BMC method with increasing concentrations of deferoxamine.

Discussion

In the management of iron overdose, it has been proposed that deferoxamine therapy is indicated when the concentration of iron exceeds the TIBC. This guideline assumes that bound iron is relatively nontoxic, an assumption that may not be valid. Under normal physiologic conditions, essentially all of the iron-binding capacity of serum is accounted for by transferrin. Unoccupied transferrin sites
Figure 3 Effect on deferoxamine on iron and total iron-binding capacity (TIBC) measurements. The serum pool with no exogenous iron is indicated by x; the pool with 200 μg/dL (36 μmol/L) of added iron, a triangle; the pool with 400 μg/dL (72 μmol/L) of added iron, a circle; the pool with 800 μg/dL (143 μmol/L) of added iron, a diamond; and the pool with 1,600 μg/dL (286 μmol/L) of added iron, a square. The dashed line indicates the initial TIBC values in A, C, and E. A and B, Results for the Vitros method (Ortho Clinical Diagnostics, Raritan, NJ). C and D, Results for the aca Star method (Dade Behring, Deerfield, IL). E and F, Results for the BMC method (Roche/Boehringer Mannheim, Indianapolis, IN).
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The capacity of the alumina in these columns to bind iron is in the iron-adsorbing capacity of the alumina columns. These findings are in contrast to the situation remove unbound iron, in which increasing the amount of TIBC. This effect does not seem to be due to a limita­tion in the iron-adsorbing capacity of the alumina columns. The capacity of the alumina in these columns to bind iron from a protein-free aqueous solution is more than adequate for the quantities of iron applied even in a typical overdose situation. Dilution of the sample or passage through a second alumina column did not reduce the overestimation of the TIBC. These findings are in contrast to the situation observed with methods that use magnesium carbonate to remove unbound iron, in which increasing the amount of magnesium carbonate used can correct the problem.

The ability of the alumina column to retain nontransferrin-bound iron seems to be reduced by the presence of serum proteins, particularly albumin. As iron concentrations exceed the transferrin-associated binding capacity, weak interactions between excess iron and serum proteins may compete with iron-binding sites on alumina particles and prevent removal of non-transferrin-bound iron by the alumina columns. The maximum ratio of measured iron to measured TIBC in spiked serum specimens was less than 1.5, even when the ratio of iron to iron-binding sites on transferrin exceeded 5. Both alumina column methods that were evaluated exhibited similar effects. Although such effects can lead to measured TIBCs that substantially exceed the normal range, there was no instance in which the TIBC exceeded the serum iron concentration. The use of these assays in an overdose situation could lead to a poor correlation between the apparent free iron and the clinical outcome but would not be expected to falsely suggest a nondangerous situation as a result of the TIBC exceeding the serum iron concentration.

When the BMC homogeneous UIBC method was studied, the opposite effect was seen, with TIBC decreasing as serum iron concentrations increased above 500 μg/dL (90 μmol/L). This effect is not readily explained as the result of iron-protein adduct formation, nor does it seem to result from working outside the linear range of the assay, since sample dilution did not alter the results. While the homogeneous TIBC method seems to give consistent TIBC at iron/TIBC ratios from 0.2 to 2, it remains to be proven in the clinical setting whether the assay is of greater value for predicting which patients will require deferoxamine therapy and which will not.

It has been suggested that deferoxamine therapy can be discontinued when the serum iron concentration is less than the TIBC. However, deferoxamine decreases the analytic recovery of serum iron in a concentration-depend­ent fashion. All of the iron methods studied showed comparable decreases in measured iron concentration; increasing concentrations of deferoxamine with the Vitros assay were slightly less affected and with the aca Star assay were slightly more affected than the BMC assay. This slight variability between assays may reflect differences in the assay conditions used to dissociate and reduce Fe³⁺ to Fe²⁺. The affinity of deferoxamine for Fe³⁺ is very high with an affinity constant of 10³¹. The 3 iron methods we studied use a mild reducing agent to reduce Fe³⁺ to Fe²⁺, which is then quantified with a chromogen. These reducing agents apparently do not completely dissociate Fe³⁺ from deferoxamine, leading to an underestimation of the actual iron concentration. An accurate colorimetric measurement of the serum iron concentration in the presence of deferoxamine can be achieved with a relatively high concentration of a stronger reducing agent, such as thioglycolate or ascorbate-citrate.

The effect of deferoxamine on TIBC assays is highly assay dependent. When the iron concentration was less than the TIBC, the addition of deferoxamine produced no signifi­cant change in Vitros or aca Star TIBC values. It has been reported that deferoxamine can remove no more than 10% to 15% of iron from totally saturated transferrin. This would explain the apparent lack of effect. However, when the iron concentration exceeded the transferrin-associated IBC, leading to an increase in the TIBC, deferoxamine decreased the TIBC measured by both alumina column methods in a concentration-dependent fashion, similar to what was seen for serum iron measurements. Presumably, the iron that had been weakly bound to serum proteins formed a complex with deferoxamine that was removed by the alumina column or was not available to the iron-specific chromogens used in either TIBC assay. The net result was that this deferoxamine-bound iron no longer contributed to the TIBC.

Deferoxamine led to an increase in the TIBC values measured by the BMC method. Presumably, some of the added iron becomes bound to deferoxamine and is not
available for chromogenic detection. This results in a decrease in the measured free iron and an increase in the apparent UIBC and, consequently, the TIBC.

Previous studies have found somewhat contradictory effects of deferoxamine on TIBC measurements. In 1 set of experiments, addition of the iron-deferoxamine complex, ferrioxamine, was found to have no effect on TIBC of serum that did not contain free iron using a magnesium carbonate colorimetric method.7 These results are consistent with our results for the alumina column TIBC methods. In another set of experiments, the addition of deferoxamine to serum that did not contain any free iron increased the TIBC when a magnesium carbonate separation step was used in conjunction with an electrochemical method that used alcoholic hydrochloric acid.16 These results parallel what we observed with the homogeneous BMC method, but the mechanism producing the effect is assay-dependent. Deferoxamine has a serum elimination half-life of 50 minutes.22 To avoid potential assay interference, specimens should not be collected for iron or TIBC measurements for 4 to 5 half-lives or at least 4 hours after the last administration of deferoxamine.

In conclusion, previous reports have indicated that TIBC methods that use magnesium carbonate to absorb excess unbound iron may measure an elevated TIBC when the iron concentration exceeds the available transferrin binding sites. This problem could be corrected by increasing the amount of magnesium carbonate used. We have shown that alumina column TIBC methods exhibit a similar effect, but passage through an additional alumina did not correct the effect. Therefore, procedures that measure TIBC by addition of excess iron followed by adsorption with alumina are not suitable for estimating TIBC in acute iron overdose. In contrast, a homogeneous UIBC method seems to provide consistent TIBC results until the iron concentration exceeds 500 µg/dL (90 µmol/L), at which point this method begins to underestimate the TIBC. Because iron concentrations greater than 90 µmol/L (500 µmol/L) are predictive of systemic toxic effects,23 measurement of TIBC is unlikely to affect management in such situations. This more consistent TIBC method might be useful in an acute overdose situation to rule in a significant iron ingestion requiring chelation therapy if the serum iron concentration were less than 90 µmol/L (500 µmol/L) but substantially greater than the TIBC. Similarly, a serum iron concentration substantially less than the TIBC would suggest a low probability of systemic toxic effects, provided the sample was collected at the appropriate time. While this algorithm is reasonable on a theoretical basis, it has not been tested empirically.

Deferoxamine uniformly decreases iron recovery in a concentration-dependent fashion and interferes with TIBC measurements in a method-dependent fashion. Alumina column methods are not affected when transferrin is not completely saturated with iron. When the iron concentration exceeds the available transferrin-binding sites, deferoxamine diminishes the overestimation of the TIBC in a concentration-dependent fashion. Deferoxamine increases the TIBC measured by the homogeneous UIBC method. Specimens should not be collected for iron and TIBC measurements by any of the methods studied until at least 4 hours after the last administration of deferoxamine to minimize interferences.

References


