Serum Iron Determination in Patients Receiving Therapy with Iron Dextran ("Imferon")

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Imferon (Lakeside Labs., Inc.), an iron dextran complex, is widely used for parenteral therapy with iron. Colorimetry and atomic absorption spectroscopy give different values for serum iron concentration in patients receiving such therapy. Values determined by atomic absorption spectroscopy were usually much higher than those obtained by either a manual or an automated colorimetric procedure, and also were higher than the total serum iron-binding capacity of the same sera. This difference is attributed to circulating iron being present both as iron dextran as as transferrin-bound iron. We conclude that atomic absorption spectroscopy should be used to determine serum iron in patients receiving Imferon.

Additional Keyphrases: colorimetry (bathophenanthroline) vs. atomic absorption spectroscopy • total iron-binding capacity • anemia • transferrin-bound iron

"Imferon" (Lakeside), an iron dextran complex, was introduced in 1954 as the first parenteral iron preparation that could be given intramuscularly (1). Subsequently, intramuscular or, under certain circumstances, intravenous administration of iron dextran has become an established technique in treatment of iron-deficiency anemia. The intravenous route allows introduction of 2 to 3 g of iron into the blood stream (2). Iron dextran is cleared slowly after such infusion, with a half-time of 2.5 to 3 days (3). Body-

References
surface scintillation counting after administration of radiolabeled iron dextran showed radioactivity in the body for longer than three weeks (4).

Serum iron and total iron-binding capacity (TIBC) of serum can be determined either by colorimetry or by atomic absorption spectroscopy. When both procedures were applied in our laboratory, patients receiving iron dextran therapy presented a special problem. During routine analysis for serum iron and TIBC by atomic absorption spectroscopy, we noticed that some patients, mostly those in the hemodialysis unit, had abnormally high values for both. In these patients the serum iron concentration often exceeded the TIBC, indicating that the excess iron was present in some form other than iron–transferrin complex. When iron was determined in the same samples with the aca, the results fell within the physiologic range. Upon inquiry, all such patients were found to have received Imferon intravenously three to 12 days before the blood was sampled. To explain these discrepant results we did the following studies.

Materials and Methods

Reagents

All reagents were prepared from AR grade chemicals (Fisher Scientific Co.) with de-ionized water. A colorimetric procedure (American Monitor, Indianapolis, Ind. 46240) was used with reagents supplied by the manufacturer. Imferon containing 50 mg of elemental iron per milliliter was obtained from the distributor (Lakeside Laboratories, Inc., Milwaukee, Wis. 53201; manufactured by Fisons Limited, Holmes Chapel, England).

Equipment

A Coleman Jr. II spectrophotometer (Coleman Instruments, Maywood, Ill. 60153) was used to prepare a calibration curve for determination of iron concentration by the colorimetric procedure. Wavelength calibration was ascertained with a dicyanum chloride filter at 585 ± 1 nm and linearity was checked with a ammonium cobaltous sulfate solution, 45 g/liter of dilute sulfuric acid (Spectro Chek; Oxford Laboratories, Foster City, Calif. 94404).

Atomic absorption spectroscopic assays were done with a Model 403 spectrophotometer equipped with a three-slot burner head and used with an air/acylene flame (Instrument Division, Perkin-Elmer Corp., Norwalk, Conn. 06856). Analyses were made at a wavelength of 248.3 nm with a single-element hollow-cathode lamp supplied by the manufacturer and a spectral slit width of 0.2 nm.

An Automatic Clinical Analyzer (aca; E. I. du Pont de Nemours and Co., Inc., Instrument Products Division, Wilmington, Del. 19898) was used for the automated measurement of serum iron and TIBC, based on colorimetric procedures. Wavelength calibration was checked according to the manufacturer’s instructions, i.e., filter balancing followed by linearity certification with cobalt (II) sulfate in sulfuric acid, 10 ml/liter (cat. No. 9038000, lot No. 3480).

Corning disposable glassware was used wherever possible in the procedures. Corning disposable pipettes were used to transfer serum samples. All other glassware was treated with dilute nitric acid (200 ml/liter) followed by five successive rinses with de-ionized water, and air dried.

Procedures

The range of linearity for the aca and atomic absorption spectrophotometer was determined by using aqueous ferric chloride standards (Fisher) and assayed serum controls (General Diagnostics). A linear range of 0–10.00 mg/liter was established for both instruments. Manual assays of serum iron and TIBC were done by a colorimetric procedure, modified from the original method by the manufacturer, obtained commercially in kit form from American Monitor (5). Atomic absorption spectroscopic analysis of specimens was routinely done after protein precipitation with trichloroacetic acid (200 g/liter) to avoid potential interference from hemoglobin iron (6).

Serum iron and TIBC were also determined with an aca that had been calibrated with aqueous iron standards (Technical Bulletin, aca, Iron; Du Pont) (7). This procedure is an end-point reaction, done at an acid pH at which the iron–transferrin complex dissociates. The free iron, reduced to the ferrous state (Fe2+) by hydroxylamine (NH2OH), forms a complex with bathophenanthroline, the color of which, measured at 540 nm, is directly proportional to the iron concentration in the sample. Sodium dodecyl sulfate is incorporated into the reaction mixture to prevent precipitation of protein.

Results

Analytical Recovery Studies

A pool of patients’ sera was prepared, and increasing amounts of ferric chloride were added to different aliquots of it. The iron concentration of each aliquot and that of the original pool was determined by atomic absorption spectroscopy, with the aca, and by the manual method.

The serum pool iron content was found to be 800, 530, and 610 μg/liter, respectively, by the three methods. Recovery of added iron ranged from 72 to 114% by the three methods. At each concentration of serum iron, each of the three methods gave about the same percentage recovery; for example, when the iron concentration was adjusted to 2 mg/liter plus its original serum pool value, respective recoveries were 114, 102, and 100%. Resulting curves for all three methods showed a good linear relationship and close parallelism to each other up to 10 mg/liter, when the measured concentration was plotted vs. added iron (Figure 1).

Next, we added various known amounts of iron dextran instead of ferric chloride to aliquots of the same serum pool and repeated the above recovery studies (Table 1). Analytical recovery of the added iron by atomic absorption spectroscopy ranged from 80 to 37%, gradually decreasing with increasing iron dextran concentration. In contrast, we ob-

![Fig. 1](image-url)
Table 1. Analytical Recovery of Imferon-Iron Added to Serum

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<th>Iron added (mg/liter)</th>
<th>Iron recovered (mg/liter)</th>
<th>Recovery, %</th>
<th>AAS*</th>
<th>Iron recovered (mg/liter)</th>
<th>Recovery, %</th>
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*Atomic absorption spectroscopy. Specimens were deproteinized with trichloroacetic acid (200 g/liter) before analysis.

Fig. 2. Comparative analytical recovery of Imferon-iron in a serum pool by atomic absorption spectroscopy (O), Automatic Clinical Analyzer (●), and a manual method (Δ). Each point is the average of five determinations.

We routinely used protein-free filtrates for determination of iron by atomic absorption spectroscopy. Recovery of added iron dextran by this procedure varied but never exceeded 60%. When samples were simply diluted with distilled water and their iron concentrations determined by atomic absorption spectroscopy, recovery approached 100% (Table 2). When the iron in five patients’ sera which contained injected Imferon were determined by atomic absorption spectroscopy after dilution with water, much higher iron concentrations were obtained than by using protein-free filtrates (Table 3). Clearly, a portion of the iron dextran complex in serum, whether injected or added in vitro, is removed along with serum proteins by trichloroacetic acid treatment and thus escapes detection by atomic absorption spectroscopy.

Discussion

Imferon is used extensively for treatment of iron-deficiency anemia by intravenous injection. Reported studies concerning the structure of the preparation, which contains 50 mg of elemental iron and 20 mg of dextran per milliliter (8), indicate that the iron is complexed at the terminal glucose units of the dextran chains, which have been converted into metasaccharinic acid. In solution, the dextran chains can be visualized as radiating randomly from the ferric oxyhydroxide (FeO(OH)) "core" and are attached to it via the terminal metasaccharinic acid units (9).

Our results show that intravenous injection of this iron dextran complex will give rise to a form of circulating serum iron that is detectable by atomic absorption spectroscopy but not by the bathophenanthroline procedure on which both the aca and colorimetric manual methods are based. This is apparently because the iron in the iron dextran complex is in the “core” of it. The iron dextran attachment is probably firm enough to resist dissociation with acid and subsequent complex formation between the iron and bathophenanthroline reagent (10).
Our results also indicate that the circulating iron dextran complex is only partly removed from serum by trichloroacetic acid treatment (Table 3) and that its presence interferes with determination of transferrin-bound iron in protein-free filtrates by atomic absorption spectroscopy. Therefore, water-diluted serum samples should be used to measure total circulating iron. Furthermore, a small proportion of iron dextran (up to 3%, as seen in Table 1) present in a given serum sample may react unaccountably in the colorimetric procedures, becoming significant in patients with exceedingly high concentrations of circulating iron dextran (Table 3). Thus, the use of the aca or manual colorimetric methods involving the bathophenanthroline procedure becomes unreliable for serum iron determination for patients receiving Imferon therapy. Atomic absorption spectroscopy measures total circulating iron, both transferrin- and dextran-bound; colorimetric methods measure a little of the dextran-bound iron, enough to prevent accurate measurement of transferrin-bound iron in some patients. Determination of transferrin-bound iron in these patients is a difficult problem for which we have no solution at the present.

When one wishes to monitor total circulating iron, the best laboratory procedure would be to determine serum iron by atomic absorption spectroscopy on water-diluted serum, as demonstrated in this paper. But in doing so one should be aware of potential error caused by hemoglobin in the serum. Webster (11) has described a modified bathophenanthroline procedure that supposedly measures total circulating iron, i.e., both transferrin- and dextran-bound iron. It may be of interest to compare results by this method with those by atomic absorption spectroscopy, which also measures both.

There are several published studies of rates of clearance of iron dextran from plasma (3, 4, 12–14). These studies and our own experience show that patients who have received iron dextran within the preceding three weeks will show an abnormally high serum iron content. Such patients should be identified and a proper procedure such as the one described in this paper should be used for determining the total circulating iron concentration.

References