Chemical and Immunochemical Measurement of Total Iron-Binding Capacity Compared

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Radiometric, colorimetric, and two immunochemical methods for measuring total iron-binding capacity are compared. We evaluated the procedures on the basis of precision, applicability to a pediatric population, and accuracy as assessed by analytical recovery of purified transferrin. The immunonephelometric assay for transferrin provides significant advantages over the other methods examined.

Additional Keyphrases: intermethod comparison · pediatric chemistry · immunochemistry · transferrin

Data comparing immunochemical, radioisotopic, and colorimetric procedures for the measurement of total iron-binding capacity (TIBC)3 and transferrin appear in recent literature (1–4). Resin adsorbents give higher results for TIBC than other adsorbents such as hemoglobin-coated charcoal or light magnesium carbonate powder (1, 2). In a comparison of the radioisotopic and colorimetric methods, no difference was noted if the same adsorbent was used (1, 2). Burrows (3) reported that the radial immunodiffusion measurement of transferrin, the iron-transport protein in serum, yields significantly lower results than do the radioisotopic or colorimetric methods for measurement of transferrin. In contrast, a recent report by Tsung et al. (4) showed that radial immunodiffusion measurement of transferrin gives equivalent results when compared with the colorimetric methods of Ramsey (5). Results obtained with the radioisotopic procedure with use of a resin adsorbent were found to be higher than those by colorimetric and immunochemical methods (4).

We report here a comparison of results by a colorimetric, a radioisotopic, and two distinctly different immunochemical procedures, radial immunodiffusion and immunonephelometry. The bases for comparison were sample size, ease of technical execution, accuracy, precision, and cost.

Materials and Methods

Reagents

Polyethylene glycol (M, 6900 to 7500). Forty grams of polyethylene glycol (PEG) (Fisher Scientific) and 9 g of NaCl were diluted to 1 liter with de-ionized water.

Antihuman transferrin. Antiserum was obtained from Behring Diagnostics, Somerville, N.J. 08876; Meloy Laboratories, Springfield, Va. 22151; and Technicon Instruments Corp., Tarrytown, N.Y. 10591.

Working antiserum solution. Antiserum was diluted 40-fold with the PEG/saline. After the solutions had stood for 30 min they were filtered through a 0.45-μm Millex filter (Millipore Corp., Bedford, Mass. 01730).

Procedure

Nephelometric assay for transferrin measurement. Patients' sera, controls, and standards were diluted 100-fold in physiological saline. The assays were done in cylindrical tubes (10 X 75 mm Kimble test tubes). An antibody blank consisting of 1 ml of diluted antibody and 20 μl of isotonic saline and a diluent blank consisting of 1 ml of the PEG/saline and 20 μl of saline were set up for each assay. For each sample, control, and standard, the following tubes were used: a test cuvette containing 20 μl of diluted sample and 1 ml of diluted antibody, and a sample blank containing 1 ml of PEG/saline and 20 μl of diluted sample. The cuvettes were allowed to stand at ambient temperature for 30 min before the light-scattering reading was made. All scattering measurements were automatically corrected for diluent, antibody, and unknown blank scattering. Unknown sera concentrations were read directly from a multipoint standard curve (Figure 1).

Radial immunodiffusion (RID) for transferrin measurement. All measurements were made according to instructions enclosed for each test kit. RID kits for the measurement of human transferrin were obtained from Behring Diagnostics and Meloy Laboratories.

Fe method for TIBC measurement. Kits were purchased from Mallinckrodt Nuclear Radio Pharmaceuticals, Hazelwood, Mo. 63420. All measurements were made according to instructions enclosed in the kits. Free iron was removed by a solid-phase resin adsorbent in this procedure.

Colorimetric TIBC measurement. The method of Ramsey (5) was used, as modified by the Technicon Corp. Ferric
chloride was added to the sample to saturate the serum transferrin. Light magnesium carbonate was used as the adsorbent. After centrifugation, the samples were assayed with an AutoAnalyzer II by the method of Giovaniello et al. (6), as modified by Stokey (7).

Purified transferrin was obtained from Sigma Chemical Co., St. Louis, Mo. 63178. Its purity was checked by immuno-fixation and electrophoresis on agarose. A single protein band was observed by both types of electrophoresis.

Apparatus

Absorbances were measured with an Acta V spectrophotometer (Beckman Instruments, Fullerton, Calif. 91634) and a Model 55 spectrophotometer (Perkin-Elmer Corp., Norwalk, Conn. 06856).

Light-scattering measurements were made with a nephelometer (Hyland Laboratories, Costa Mesa, Calif. 92626).

Results

Under the conditions chosen for the assay, the change in light-scattering with respect to time was measured at 30 min and five concentrations, ranging from 440 to 7000 mg/liter (Figure 2). At all concentrations examined, we observed little or no change in scatter after 25 min.

Within-run precision for assay of TIBC or transferrin was compared for four different procedures (Table 1). All results for transferrin are expressed as TIBC, with 1.24 as the conversion factor. This derived factor agrees well with an experimentally measured factor reported recently (4). A single serum pool was used for each procedure, except for the immunonephelometric method.

The purified human transferrin was used to evaluate analytical recovery of transferrin by the four procedures. For both of the immunological procedures, sera from five casually selected individuals were used. The transferrin concentration in the purified aqueous pool was determined spectrophotometrically at 280 nm (α = 11.2) (7). Conditions such as pH and buffer were as described by Aisen et al. (7). Aliquots of the purified material were added to each patient’s serum to give a range of five concentrations for each serum sample. The transferrin concentrations measured in patients’ sera ranged from 1930 to 3870 mg/liter (Table 2). These values represent the averaged results of five replicate analyses. For the colorimetric procedure and the $^{59}$Fe radiometric procedure, a single serum pool was used. Four concentrations of added transferrin in this pool were measured. All assays were done in triplicate.

### Table 1. Within-Run Precision for TIBC (Transferrin) Methods

<table>
<thead>
<tr>
<th>Method</th>
<th>n</th>
<th>Mean value (mg/liter)</th>
<th>SD</th>
<th>CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>AutoAnalyzer</td>
<td>25</td>
<td>2350</td>
<td>76</td>
<td>3.3</td>
</tr>
<tr>
<td>$^{59}$Fe</td>
<td>22</td>
<td>2560</td>
<td>35</td>
<td>1.4</td>
</tr>
<tr>
<td>Rad. Immunodiff.</td>
<td>26</td>
<td>2590</td>
<td>166</td>
<td>6.4</td>
</tr>
<tr>
<td>Immunonephelometry</td>
<td>24</td>
<td>2860$^a$</td>
<td>82</td>
<td>2.8</td>
</tr>
</tbody>
</table>

$^a$ Different lot no. of reference specimen.

### Table 2. Comparative Analytical Recovery of Added Human Transferrin by Four Methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Range of concn. (mg/liter)</th>
<th>Analytical recovery, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>AutoAnalyzer</td>
<td>3310–5670</td>
<td>96</td>
</tr>
<tr>
<td>$^{59}$Fe</td>
<td>3560–5350</td>
<td>102</td>
</tr>
<tr>
<td>Rad. Immunodiff.</td>
<td>3000–6790</td>
<td>117</td>
</tr>
<tr>
<td>Immunonephelometry</td>
<td>1900–6870</td>
<td>105</td>
</tr>
</tbody>
</table>

$^a$ Lower figure in each range represents initial concentration before addition of purified transferrin.

### Table 3. Comparison of Transferrin Results for Antisera from Different Sources

<table>
<thead>
<tr>
<th>Source</th>
<th>n</th>
<th>Mean value (mg/liter)</th>
<th>Group range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Technicon</td>
<td>18</td>
<td>3310</td>
<td>1660–4950</td>
</tr>
<tr>
<td>Meloy</td>
<td>18</td>
<td>3290</td>
<td>1570–5020</td>
</tr>
<tr>
<td>Behring</td>
<td>18</td>
<td>3280</td>
<td>1660–4860</td>
</tr>
</tbody>
</table>

$^a$ To convert transferrin in mg/liter to TIBC in μg/liter: (transferrin, mg/liter/transferrin, mg/mmol) · (2 · Fe, μg/μmol/1000) = Fe, μg/liter = TIBC, μg/liter, where 2 is the molar ratio of iron to transferrin.
Table 4. Advantages and Disadvantages of Different Methods for TIBC

<table>
<thead>
<tr>
<th>Method</th>
<th>Sample vol., µl</th>
<th>CV, %</th>
<th>Turnaround time, h</th>
<th>Reagent cost</th>
<th>Technical expertise</th>
<th>Equipment cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>AutoAnalyzer</td>
<td>500</td>
<td>3.3</td>
<td>1.5</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>59Fe</td>
<td>1000</td>
<td>1.4</td>
<td>2.5</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>RID</td>
<td>5</td>
<td>6.4</td>
<td>52.0</td>
<td>++</td>
<td>++++</td>
<td>+++</td>
</tr>
<tr>
<td>Immunonephelometry</td>
<td>10</td>
<td>2.8</td>
<td>0.75</td>
<td>++++</td>
<td>++++</td>
<td>+++</td>
</tr>
</tbody>
</table>

* The number of + signs indicates the relative advantage of item considered.

(Tables 2). Results were expressed as range of concentration for each procedure, range of recoveries calculated as percentages of the theoretical, and the average recovery obtained on all samples for each procedure.

We directly correlated data for transferrin concentrations between 500 and 4000 mg/liter for both RID and immunonephelometry (Figure 3). Linear regression data are given for 24 data pairs.

To investigate the effect of antisera source, we assayed a group of 18 samples by the nephelometric method, using antisera from three suppliers, Technicon, Meloy, and Behring. The mean transferrin value measured was 3310, 3290, and 3280 mg/liter.

Serum from 80 apparently healthy pediatric patients, mixed with regard to sex and race, were assayed by the nephelometric procedure. Ages ranged from one to 16 years. Figure 4 shows the range of transferrin values obtained. We calculated percentile ranges according to the standard statistical procedure [8].

Discussion

Previous investigations have shown the main cause of variation in the TIBC as measured by various methods is the choice of adsorbents used to remove free iron [1, 2]. Comparisons of resin and other adsorbents have evoked speculation that the resin is too strong an adsorbent, removing iron bound to transferrin. A comparison of mean recovery data (Table 2) indicates that, for measurement of TIBC, both light magnesium carbonate and the resin adsorbent gave analytical recoveries very close to 100%; thus we saw little if any difference in the results given by these methods. Adsorbents are directly compared here, because it has been shown that the colorimetric and the 59Fe radiometric procedure give identical data if the same adsorbents are used [1, 2].

Average analytical recoveries for both immunochemical methods exceeded 100%. Although the immunonephelometric method was about 5% higher than the 59Fe procedure, the immunodiffusion procedure averaged 17% higher. Differences from the theoretical recovery were greater at higher concentrations of added transferrin. This observation may explain two findings, the first being that both immunochemical methods had higher mean recoveries than the other procedures investigated, because the immunochemical methods are tested at much higher concentrations of added transferrin (Table 2). Secondly, there was a difference in mean recoveries between the immunological procedures (Table 2), although the linear regression comparison (Figure 3) showed results by the two immunological procedures to correlate well between 500 and 4000 mg/liter. This could be because no points were included above 4000 mg/liter, where the greatest discrepancies in recoveries were noted. Our data indicate that immunochemical and chemical methods give similar results.

We saw no significant difference in results, as evaluated by the t-test, in the means of the data sets (P = 0.05) when antisera from different sources were used. The similarity of the data generated with use of these three antisera tends to rule out the source of antisera as a possible cause of the differences in recoveries between the nephelometric and the radial immunodiffusion procedures (Table 3).

The choice of a procedure for measuring TIBC or transferrin in the routine laboratory depends on several factors. The relative advantages of each method tested are outlined in Table 4. Three persons performing the assays judged the two immunochemical procedures to be easier to do than the other methods examined. From the analytical and practical considerations presented, we think that the immunonephe-
lometric method is the best choice for assessing total iron-binding capacity in a pediatric population.

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References