Quantitating Iron in Serum Ferritin by Use of ICP-MS

Ferritin associated with inflammation can be distinguished from ferritin associated with hemochromatosis.

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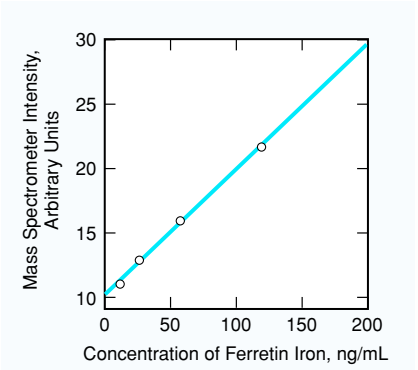
A laboratory method has been devised to enable measurement of the concentration of iron bound in ferritin from small samples of blood (serum). Derived partly from a prior method that depends on large samples of blood, this method involves the use of an inductively-coupled-plasma mass spectrometer (ICP-MS).

Ferritin is a complex of iron with the protein apoferritin. Heretofore, measurements of the concentration of serum ferritin (as distinguished from direct measurements of the concentration of iron in serum ferritin) have been used to assess iron stores in humans. Low levels of serum ferritin could indicate the first stage of iron depletion. High levels of serum ferritin could indicate high levels of iron (for example, in connection with hereditary hemochromatosis — an iron-overload illness that is characterized by progressive organ damage and can be fatal). However, the picture is complicated: A high level of serum ferritin could also indicate stress and/or inflammation instead of (or in addition to) iron overload, and low serum iron concentration could indicate inflammation rather than iron deficiency. Only when concentrations of both serum iron and serum ferritin increase and decrease together can the patient's iron status be assessed accurately. Hence, in enabling accurate measurement of the iron content of serum ferritin, the present method can improve the diagnosis of the patient's iron status.



The **Response of the ICP-MS** is a linear function of the concentration of ferritin iron.

The prior method of measuring the concentration of iron involves the use of an atomic-absorption spectrophotometer with a graphite furnace. The present method incorporates a modified version of the sample-preparation process of the prior method. First, ferritin is isolated; more specifically, it is immobilized by immunoprecipitation with rabbit antihuman polyclonal antibody bound to agarose beads. The ferritin is then separated from other iron-containing proteins and free iron by a series of centrifugation and wash steps. Next, the ferritin is digested with nitric acid to extract its iron content. Finally, a micronebulizer is used to inject the sample of the product of the digestion into the ICP-MS for analysis of its iron content. The sensitivity of the ICP-MS is high enough to enable it to characterize samples smaller than those required in the prior method (samples can be 0.15 to 0.60 mL).

The method has been validated in experiments. In one set of experiments, the linearity of the ICP-MS response (see figure) was established by use of a high-concentration sample diluted to several known lower concentrations. In another set of experiments involving spiked recovery (in which known amounts of iron were added to samples from which natural iron had been stripped), the fraction of added iron recovered was found to lie in the range of 0.96 ± 0.10. In still other experiments, the inter- and intra-assay coefficients of variation for three control samples were found to be 0.12 and 0.09, respectively. These findings suggest that the method can be used to distinguish elevated ferritin due to inflammation and that due to iron overload, using small samples of serum containing ferritin iron at concentrations as low as 10 ng/mL.

This work was done by Scott M. Smith of **Johnson Space Center** *and Patricia L. Gillman of Enterprise Advisory Service, Inc. Further information is contained in a TSP [see page 1].*

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