IDPAS #1093

5

MEASUREMENT OF IRON STATUS

5.1 IRON STATUS

Normal iron status implies both the presence of erythropoiesis which is not limited by iron and a small reserve of 'storage iron' to cope with normal physiological functions. The ability to survive the acute loss of blood (iron) which may result from injury is also an advantage. The limits of normality are difficult to define and some argue that physiological normality is an absence of storage iron (Sullivan, 1992a) but the extremes of iron deficiency anaemia and haemochromatosis are well understood.

Apart from too little or too much iron in the body there is also the possibility of maldistribution. An example is anaemia associated with inflammation or infection, where there is a partial failure of erythropoiesis and of iron release from the phagocytic cells in liver, spleen and bone marrow which results in accumulation of iron as ferritin and haemosiderin in these cells (Figure 5.1). Thus determination of iron status requires an estimate of the amount of haemoglobin iron (usually by measuring the haemoglobin concentration in the blood) and the level of storage iron. Occasionally, further investigations into iron loss, iron absorption and flow rates within the body are also required.

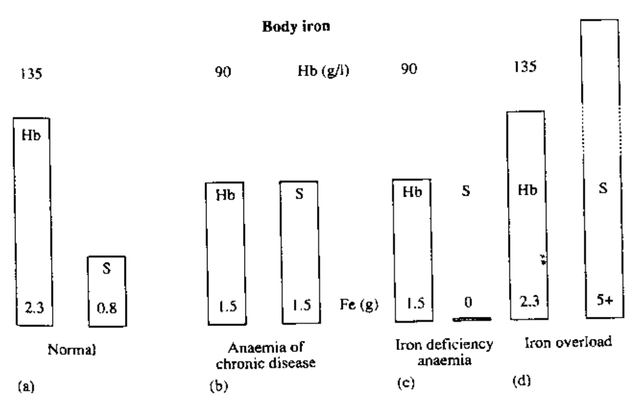

Figure 5.1 Body iron. Relationship between total haemoglobin (Hb) and storage iron (S): (a) normally; (b) in the anaemia of chronic disease; (c) in iron deficiency anaemia; and (d) in iron overload caused by genetic haemochromatosis. (The 'normal' example is for a 70 kg subject with a haemoglobin concentration of 135 g/l.)

5.2 METHODS FOR ASSESSING IRON STATUS

The methods which have been used to assess iron status are summarized in Table 5.1. Some methods are not applicable to normal subjects but have value in the standardization of indirect methods.

5.2.1 Haemoglobin

The measurement of haemoglobin concentration depends on the conversion of haemoglobin to cyanmethaemoglobin and determination of the absorbance at 540 nm. An international standard is widely available for calibration and the measurement is included in the analysis provided by electronic blood cell counters in most haematology laboratories (Dacie and Lewis, 1991). Developmental changes in haemoglobin concentration have been reviewed by Yip (1994). Concentrations are higher in adult men than women (White *et al.*, 1993) but otherwise vary little with age in adult life. Haemoglobin concentrations increase with long-term exposure to high altitude (compensation for reduced oxygen supply) and are higher in cigarette smokers (White *et al.*, 1993; Yip, 1994) in compensation for reduced oxygen capacity due to the formation of carboxyhaemoglobin.

The investigation of anaemia begins with the blood count in order to distinguish between anaemia due to inadequate supply of iron or, more rarely, to B_{12} or folate deficiency. Modern automated cell counters provide a rapid and sophisticated way of detecting the changes in red cells which accompany a reduced supply of iron to the bone marrow (Williams, 1990): a low mean cell volume (MCV), a low mean cell haemoglobin concentration (MCH) and a high red cell distribution width (RDW). Cell analysers can thus indicate the presence of microcytic anaemia which can be due to a reduced supply of iron to the bone marrow or to a deficit in

Table 5.1 Assessment of body iron status

Measurement	Representative reference range (adults)	Diagnostic use
Functional iron		
Haemoglobin conc – Males	130–180 g/l	Assessing the severity of IDA; response to a therapeutic trial of iron confirms IDA. Not applicable to assessment of iron overload.
– Females	120–160 g/l	
Red cell indices – MCV	80–94 fl	
– MCH	27–32 pg	
Tissue iron supply		
Serum iron	10–30 µmol/l	Decreased saturation of transferrin, reduced red cell ferritin and increased zinc protoporphyrin, indicate impaired iron supply to the erythroid marrow. Raised saturation of transferrin used to assess risk of tissue iron-loading (e.g. in haemochromatosis or iron-loading anaemias).
Saturation of transferrin	16–60%	
Red cell zinc protoporphyrin	<80 µmol/mol Hb (<70 µg/dl red cells)	
Red cell ferritin (basic)	3–40 ag/cell	
Serum transferrin receptor	2.8–8.5 mg/l	Particular value in identifying early iron deficiency and, in conjunction with a measure of iron stores, may be of value in distinguishing this from ACD.
Iron stores		
Quantitative phlebotomy	<2 g iron	All measures are positively correlated with iron stores except TIBC which is negatively correlated. Quantitative phlebotomy, liver iron concentration, chelatable iron and MRI are of value only in iron overload. Bone marrow iron may be graded as absent, normal or increased, and is most commonly used to differentiate ACD from IDA.
Tissue biopsy iron		
– liver (chemical assay)	3–33 µmol/g dry wt	
– bone marrow (Prussian blue stain)		
Serum ferritin	15–300 µg/l	Serum ferritin is of value throughout the range of iron stores.
Urine chelatable iron (after 0.5 g I.M. desferrioxamine)	>2 mg/24 hr	
Non-invasive methods (MRI etc.)		
Serum TIBC/transferrin	47–70 µmol/l	In IDA a raised TIBC is characteristic.

Key: IDA = Iron deficiency anaemia
ACD = Anaemia of chronic disorders
TIBC = Total iron-binding capacity.

haemoglobin synthesis such as in thalassaemia. Further tests are usually necessary to distinguish between simple iron deficiency (absence of storage iron) and a supply deficiency that is secondary to another disease process (Figure 5.2).

5.2.2 Quantitative phlebotomy

A direct way of measuring iron stores is by quantitative phlebotomy (removing up to 500 ml/week until anaemia develops). This gives a measure of the amount of iron available for haemoglobin synthesis. If blood is removed at a rate of 500 ml/week, most of the iron used for haemoglobin synthesis (250 mg Fe/week) is obtained from the stores (ferritin and haemosiderin) rather than by absorption (Torrance and Bothwell, 1980). Quantitative phlebotomy has been applied to validate the concept that serum ferritin concentrations in normal subjects reflect the level of available storage iron (Walters *et al.*, 1973). It is also used to determine the initial level of storage iron during treatment of haemochromatosis.

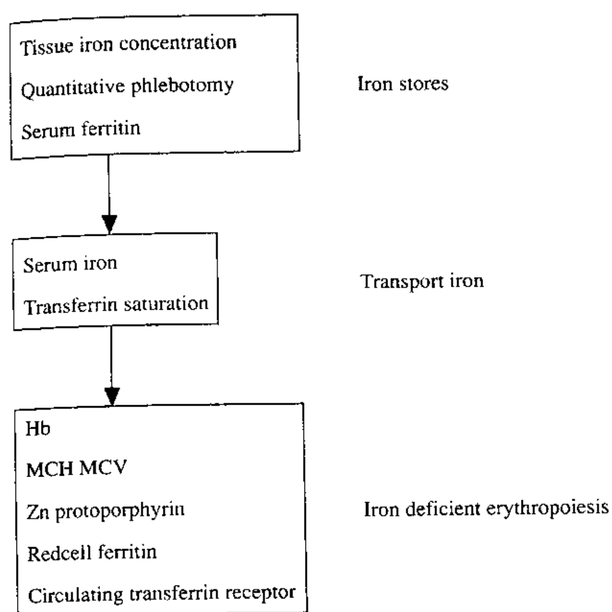

Figure 5.2 Indicators of iron status.

5.2.3 Tissue iron concentrations

The liver and bone marrow are important and relatively accessible storage sites and the amount of iron present in a tissue biopsy can be estimated either visually, using the Prussian blue reaction on tissue sections, or chemically. There is generally a good correlation between iron concentrations in liver and bone marrow (Gale *et al.*, 1963). Methods for chemical and histological assessment of tissue iron concentration have been described in detail by Torrance and Bothwell (1980). Mean liver non-haem iron concentrations lie between 80 and 300 μg/g wet weight (Charlton *et al.*, 1970). Chemical determination of liver iron concentrations is most widely applied for the demonstration of iron overload and allows the important distinction to be made between the relatively minor elevations of liver non-haem iron sometimes found in patients with cirrhosis of the liver, and iron overload associated with inherited haemochromatosis (Bassett *et al.*, 1986). Estimation of iron concentration in bone marrow is, in contrast, usually by the histochemical method and is often used to detect iron deficiency. In particular, assessing marrow iron histologically distinguishes between 'true' iron deficiency and other chronic disorders in which there is impaired release of iron from reticuloendothelial cells.

5.2.4 Serum iron/total iron binding capacity and transferrin saturation

The International Committee for Standardization in Hematology (Expert Panel on Iron) has recommended a reference method which is simple and reliable (ICSH, 1990). The simultaneous precipitation of serum proteins and the release of iron from transferrin in the presence of a reducing agent is followed by centrifugation to remove denatured protein and detection of the ferrous iron in the supernatant with a chromogen. The method avoids non-specific absorbance caused by interference with serum proteins and there is relatively little interference by haem iron or copper. Methods which do not require centrifugation have obvious advantages in terms of automation, and techniques in which iron is released from transferrin without precipitating serum proteins are also widely applied (Persijn *et al.*, 1971). Procedures for measuring serum iron are available for most clinical chemistry autoanalysers, but there are concerns about accuracy (Tietz and Rinker, 1994).

Measurement of the serum iron concentration alone provides little useful clinical information because of the considerable variation from hour to hour and day to day in normal individuals. Transferrin iron is only 0.1% of the total body iron and the transferrin iron pool turns over 10–20 times each day. Changes in supply and demand due to infection, inflammation or injury (e.g. surgery) therefore cause rapid changes in serum iron concentration. Normal ranges for serum iron concentration are given in Table 5.2 and change little with age in adult life. Low concentrations are found in patients with iron deficiency anaemia and high concentrations in patients with iron overload (Bothwell *et al.*, 1979). However, many hospital patients have a low serum iron concentration which is a response to inflammation, infection or surgery, and does not necessarily indicate an absence of storage iron. High concentrations are found in liver disease, hypoplastic anaemias, ineffective erythropoiesis and iron overload.

More information may be obtained by measuring both the serum iron concentration and the total iron binding capacity (TIBC), from which the percentage of transferrin saturation with iron may be calculated. The TIBC is a measurement of transferrin concentration and may be estimated by saturating the transferrin iron binding capacity with excess iron and removing the excess with solid magnesium carbonate, charcoal or an iron exchange resin. This is followed by determination of the iron content of the saturated serum (Bothwell *et al.*, 1979). The unsaturated iron binding capacity (UIBC) may be determined by methods which detect remaining iron able to bind to chromogen, after adding an excess of iron to the serum. The problems inherent in these direct assays have been summarized by Bothwell *et al.* (1979). As for the serum iron determination, protocols for clinical chemistry analysers often include a method for

Table 5.2 Serum iron, total iron binding capacity and transferrin saturation during development

Age	Number	Serum iron (μmol/l) Median	(95% range)	TIBC (μmol/l) Mean ± SD	% Saturation Median	95% range	Reference
0.5 months	40*	22	11–36	34 ± 8	68	30–99	Saarinen and Siimes (1977a)
1 months	40*	22	10–31	36 ± 8	63	35–94	Saarinen and Siimes (1977a)
2 months	40*	16	3–29	44 ± 10	34	21–63	Saarinen and Siimes (1977a)
4 months	40*	15	3–29	54 ± 7	27	7–53	Saarinen and Siimes (1977a)
6 months	40*	14	5–24	58 ± 9	23	10–43	Saarinen and Siimes (1977a)
0.5–2 years	121	12.2	3.6†	–	22	7†	Koerper and Dallman (1977)
2–12 years	164	12.5	3.6†	–	25	7†	Koerper and Dallman (1977)
12–18 years	74	14.3	3.6†	–	27	7†	Koerper and Dallman (1977)
Adult female	517	16.1±7.4‡		68.0 ± 12.6	24.6	± 11.8	Jacobs *et al.* (1969)
Adult male	499	18.0±6.3		63.2 ± 9.1	29.1	± 11.0	Jacobs *et al.* (1969)

* Approximate number, male and female infants who were not iron deficient
† Lower limit of 95% range, male and female
‡ mean ± SD

TIBC and doubts about accuracy apply as to serum iron (Tietz and Rinker, 1994). An alternative approach is to measure transferrin directly by immunological assay. Nephelometric methods are widely used and there is generally a good correlation between the chemical and immunological TIBC (Huebers *et al.*, 1987). However, Bandi *et al.* (1985) reported great variability among a number of immunochemical assays for transferrin.

Normal ranges for both TIBC and transferrin saturation are given in Table 5.2. A transferrin saturation of 16% is usually considered to indicate an inadequate iron supply for erythropoiesis (Bainton and Finch, 1964). Of more significance is a raised TIBC (greater than 70 μmol/l), which is characteristic of a deficiency of storage iron. The measurement of TIBC or transferrin may sometimes lead to an apparent saturation of greater than 100% if there is non-transferrin iron present in the serum. Such non-transferrin iron may be ferritin if there is significant liver damage which releases ferritin into the blood. Some of the ferritin iron may be assayed in the determination of serum iron (ICSH, 1990) and other low molecular weight forms of iron may also be present in iron overloaded patients. These measurements of serum iron, which for many years formed the basis of much clinical investigation of iron metabolism, are now seen to provide an inadequate index of storage iron where they have been largely replaced by the assay of serum ferritin. In screening for idiopathic haemochromatosis, however, it is essential to measure both the serum iron concentration and the TIBC or transferrin concentration.

5.2.5 Erythrocyte protoporphyrin

This assay has been performed for many years as a test for lead poisoning. More recently, there has been considerable interest in its use in evaluating the iron supply to the bone marrow. The 'free' protoporphyrin concentration of red blood cells increases in iron deficiency. A widely used technique directly measures the fluorescence of zinc protoporphyrin (μmol/mol haem) in a haematofluorometer (Labbe and Rettmer, 1989). The small sample size (about 20 μl of venous or skin-puncture blood), simplicity, rapidity and reproducibility within a laboratory are advantages. Furthermore, the test has an interesting retrospective application. Because it takes some weeks for a significant proportion of the circulating red blood cells to be replaced with new cells, it is possible to make a diagnosis of iron deficiency anaemia some time after iron therapy has commenced. Chronic diseases that reduce serum iron concentration, but do not reduce iron stores, also increase protoporphyrin levels (Hastka *et al.*, 1993).

The measurement of erythrocyte protoporphyrin levels as an indicator of iron deficiency has particular advantages in paediatric haematology and in large-scale surveys in which the small sample size and simplicity of the test are important. The normal range in adults is <80 μmol/mol haem. Mean values in normal women are slightly higher than in men (Yip, 1994). Garrett and Worwood (1994) found a mean of 44 (range 30–68) μmol/mol haem in women and 41 (29–64) in men. Mean concentrations vary relatively little with age but are slightly higher for children aged 1–3 years than the mean for adult blood (Deinard *et al.*, 1983; Yip, 1994). In the general clinical laboratory, however, it provides less information about iron storage levels in anaemic patients than the serum ferritin assay.

5.2.6 Serum ferritin

It was only after the development of a sensitive immunoradiometric assay (IRMA) that ferritin was

detected in normal serum or plasma (Addison *et al.*, 1972). Reliable assays, both RIA (labelled ferritin) and IRMA (labelled antibody), have been described in detail (Worwood, 1980a). More recently radioactive labels have been supplanted by enzyme-linked immunoassays (ELISA) with colorimetric and fluorescent substrates or by antibodies with chemiluminescent labels. The solid phase may be a tube, bead, microtitre plate or (magnetic) particle. Numerous variations have been described and serum ferritin included in the latest (batch and random access) automated analysers for immunoassay. A simple enzyme-linked assay with standard reagents has been described and may have application as a reference method (Worwood *et al.*, 1991b) in evaluating automated methods.

The use of a reference ferritin preparation to calibrate the assay is recommended. The second WHO standard for the assay of serum ferritin is reagent 80/578 (obtainable from the National Institute of Biological Standards and Control, PO Box 1193, Potters Bar, Herts EN6 3QH, UK).

Serum ferritin concentrations are normally within the range 15–300 μg/l: values are lower in children than adults, and from puberty to middle age mean concentrations are higher in men than in women (Worwood, 1982). Mean concentrations and ranges throughout life are given in Tables 5.3 and 5.4.

The mother's iron status appears to have relatively little influence on cord serum concentrations, mean values for which are in the range 100–200 μg/l. In a sample of Australian office workers, blood donation and alcohol intake influenced serum ferritin concentrations along with diet in women (Leggett *et al.*, 1990). The significant increase associated with alcohol consumption in both men and women has been confirmed in the *Health Survey for England* (White *et al.*, 1993). In this survey, ferritin levels also increased with increasing body mass index. In older men and women, mean concentrations are similar. In elderly, unselected patients high levels of ferritin are often associated with pathology (Touitou *et al.*, 1985). Good correlations have been found between serum ferritin concentrations and storage iron mobilized by phlebotomy, stainable iron in the bone marrow, and the concentration of both non-haem iron and ferritin in the bone marrow. This suggests a close relationship between the total amount of storage iron and serum ferritin concentrations in normal individuals, which was directly demonstrated by Walters *et al.* (1973). Serum ferritin concentrations are relatively stable in healthy persons (see later). In patients with iron deficiency anaemia, serum ferritin concentrations are less than 12–15 μg/l (depending on the assay) and a reduction in the level of reticuloendothelial stores is the only common cause of a low serum ferritin concentration. This is the key to the use of the serum ferritin assay in clinical practice (Worwood, 1982).

Table 5.3 Serum ferritin concentrations (μg/l) in infants, children and adolescents

Number of children	Age	Population	Selection	Mean	Range	Reference
46	0.5 months	Helsinki	Non-anaemic	238	90–628	Saarinen and Siimes (1978)
46	1 months	Helsinki	Non-anaemic	240	144–399	Saarinen and Siimes (1978)
47	2 months	Helsinki	Non-anaemic	194	87–430	Saarinen and Siimes (1978)
40	4 months	Helsinki	Non-anaemic	91	37–223	Saarinen and Siimes (1978)
514	0.5–15 years	San Francisco[f]	Non-anaemic	30[a]	7–142	Siimes *et al.* (1974)
323	5–11 years	Washington	Low income families	21[a]	10–45[d]	Cook *et al.* (1976)
117	5–9 years	Nutrition Canada Survey	Random	15[b]	2–107[e]	Valberg *et al.* (1976)
335	6–11 years	Denmark	Random, urban	29[a]	12–67[c]	Milman and Ibsen (1984)
126 male 125 female	12–18 years	Washington	Low income families	23[a] 21[a]	10–63[d] 6–48[d]	Cook *et al.* (1976)
98 male 106 female	10–19 years	Nutrition Canada Survey	Random	18[b] 17[b]	3–125[e] 2–116[e]	Valberg *et al.* (1976)
269 male 305 female	12–17 years	Denmark	Random, urban	28[a] 25a	11–68[c] 6–65[c]	Milman and Ibsen (1984)

[a] median
[b] geometric mean
[c] 5–95% interval
[d] 10–90% interval
[e] 95% confidence interval
[f] there were no significant differences in median values for ages 6–11 months, 1–2, 2–3, 4–7, 8–10 and 11–15 years.

Table 5.4 Serum ferritin concentrations (µg/l) in adults

Age (years)	Men			Women		
	No.	Mean	5–95%	No.	Mean	5–95%
18–24	107	80	15–223	96	30	5–73
25–34	211	108	21–291	226	38	5–95
35–44	202	120	21–328	221	38	5–108
45–54	166	139	21–395	177	60	5–217
55–64	140	143	22–349	162	74	12–199
65–74	127	140	12–374	138	91	7–321
75+	80	110	10–309	99	77	6–209
Total	1033	121	16–328	1119	56	5–170

Source: White *et al.*, 1993. Subjects being treated with drugs for iron deficiency (n = 26) were included.
For other surveys of populations in North America and Europe, see Cook *et al.* (1976), Finch *et al.* (1977); Jacobs and Worwood (1975); Millman *et al.* (1986); and Valberg *et al.* (1976).

Iron overload causes high concentrations of serum ferritin, but so may liver disease and some forms of cancer. High concentrations of serum ferritin can only be ascribed to iron overload after careful consideration of the clinical situation.

5.2.7 Red cell ferritin

The ferritin in the circulating erythrocyte is but a residue of that present in its nucleated precursors in the bone marrow. Normal erythroblasts contain ferritin which is immunologically more similar to heart (H subunit) than liver ferritin (L subunit) and mean concentrations are about 10 fg ferritin protein/cell (10^{-15} g/cell) (Hodgetts *et al.*, 1986). Concentrations decline in late erythroblasts, decline further in reticulocytes and only about 10 ag/cell (10^{-18} g/cell) remains in the erythrocyte (measured with antibodies to L ferritin) again with somewhat higher levels detected with antibodies to H type ferritin (Cazzola *et al.*, 1983). Red cell ferritin concentrations have been measured in many disorders of iron metabolism, usually with antibodies to L ferritin. In general, red cell ferritin levels reflect the iron supply to the erythroid marrow and tend to vary inversely with red cell protoporphyrin levels (Cazzola *et al.*, 1983). Thus in patients with rheumatoid arthritis and anaemia, low values of red cell ferritin are found in those with microcytosis and low serum iron concentrations regardless of the serum ferritin levels. Red cell ferritin levels do not therefore necessarily indicate levels of storage iron. High levels of red cell ferritin are also found in thalassaemia, megaloblastic anaemia and myelodysplastic syndromes, presumably indicating a disturbance of erythroid iron metabolism in these conditions.

Because it is necessary to have fresh blood in order to prepare red cells free of white cells (which have much higher ferritin levels), the assay of red cell ferritin has seen little routine application despite possible diagnostic advantages (Cazzola and Ascari, 1986), such as differentiation between hereditary haemochromatosis (higher concentration) and alcoholic liver disease (lower concentration).

5.2.8 Serum transferrin receptor

Soluble transferrin receptors are detectable in the circulation by immunoassay and appear to reflect the number of transferrin receptors on immature red cells and thus the level of bone marrow erythropoiesis (Cazzola and Beguin, 1992). The levels determined depend on the assay. A mean level of 5.6 mg/l has been reported by Flowers *et al.* (1989). The assay is potentially of considerable value as it provides an alternative to the very cumbersome ferrokinetic studies which were previously necessary. In normal subjects the serum transferrin receptor level also provides a sensitive indicator of functional iron deficiency in subjects with absent iron stores but who have not yet developed iron deficiency anaemia (Skikne *et al.*, 1990). The serum transferrin receptor level is not elevated in patients with acute infection, including hepatitis, in chronic liver disease and other patients with the anaemia of chronic disease if there is no co-existing iron deficiency. It is not yet clear what happens to receptor levels in cases where there is iron deficiency (Petterson *et al.* 1994; Zoli *et al.* 1994). In chronic disease the normal levels seem to reflect the lack of any increase in erythropoietic activity in the bone marrow (Ferguson *et al.*, 1992). The ability to distinguish the anaemia of chronic disease from iron deficiency anaemia makes the transferrin receptor assay a potentially valuable addition to

haemoglobin and ferritin in clinical practice and in epidemiological surveys of iron status (Ferguson *et al.*, 1992). However, its usefulness in specific groups, e.g. adolescents, has recently been questioned (Kivivuori *et al.*, 1993).

5.2.9 Non invasive methods for determining tissue iron concentrations

Dual-energy computed tomography, magnetic susceptibility and magnetic resonance imaging are all being exploited but at present are only applicable to the detection of iron overload (Stark, 1991).

5.3 METHODOLOGICAL AND BIOLOGICAL VARIABILITY OF ASSAYS

The blood assays vary greatly in both methodological and biological stability. Haemoglobin concentrations are stable and the simple and well-standardized method of determination (Dacie and Lewis, 1991) ensures relatively low day-to-day variation in individuals (Table 5.5). Automated cell counters analyse at least 10 000 cells and thus reduce errors. The more complicated procedures involved in immunoassays mean higher methodological variation for ferritin assays (coefficient of variation, CV, of at least 5%) and this, coupled with some physiological variation, gives an overall CV for serum ferritin for an individual over a period of weeks of the order of 15%. There is, however, little evidence of any significant diurnal variation in serum ferritin concentration (Dawkins *et al.*, 1979). The serum iron determination is an example of extremes with reasonably low methodological variation coupled with extreme physiological variability giving an overall 'within subject' CV of approximately 30% when venous samples are taken at the same time of day. A diurnal rhythm has been reported with higher values in the morning than in late afternoon, when concentrations may fall to 50% of the morning value (Bothwell *et al.*, 1979). The circadian fluctuation is due largely to variation in the release of iron from the reticuloendothelial system to the plasma. Results from a number of studies of overall variability are given in Table 5.5 but it should be noted that the type of blood sample, length of study period and the statistical analysis vary from study to study. The somewhat higher variability for Hb and ferritin reported by Borel *et al.* (1991) may be due to their use of capillary blood and plasma. Pootrakul *et al.* (1983) have demonstrated that mean plasma ferritin concentration is slightly higher in capillary specimens than venous specimens and that within and between sample variation was approximately three times greater. Variability was less in capillary serum but still greater than venous serum.

These results have clear implications for the use of these assays in population studies (Dallman *et al.*, 1984; Looker *et al.*, 1990; Wiggers *et al.*, 1991) and in the assessment of individual patients (Borel *et al.*, 1991). For accurate diagnosis, either a multiparameter analysis is required or the assay of several samples.

5.4 APPLICATION OF BLOOD ASSAYS FOR DETERMINATION OF IRON STATUS

Two important applications need to be considered:

- population surveys
- evaluation of patients in hospital or clinics.

5.4.1 Use in population surveys

Factors other than variation in iron stores which might influence the analyses should always be considered. In older people there may be a higher incidence of chronic disease and in some parts of

Table 5.5 Overall variability of assays for iron status (within-subject, day-to-day CV for healthy subjects)

Reference	Haemoglobin	Serum ferritin	Serum iron	TIBC	EP
Dawkins *et al.* (1979)	–	15 (MF)	–	–	–
Gallagher *et al.* (1989)	1.6 (F)	15 (F)	–	–	–
Statland and Winkel (1977)	–	–	29 (F)	–	–
Statland *et al.* (1976)	–	–	27 (M)	–	–
Statland *et al.* (1977)	3 (MF)	–	–	–	–
Pilon *et al.* (1981)	–	15 (MF)	29 (MF)	–	–
Romslo and Talstad (1988)*	–	13 (MF)	33 (MF)	11 (MF)	12 (MF)
Borel *et al.* (1991)	4 (MF)	14 (M)	27 (M)	–	–
		26 (F)	28 (F)	–	–

* anaemic patients

the world infection may be prevalent. High zinc protoporphyrin levels or normal transferrin receptor levels in conjunction with normal or high serum ferritin levels make it possible to detect chronic disease in anaemic subjects. Blood donation may reduce iron stores significantly. There is no information on seasonal factors influencing most of these analyses although seasonal changes in red cell parameters have been reported (Kristal-Boneh *et al.*, 1993). Only limited information on changes during the menstrual cycle is available (Kim *et al.*, 1993).

The serum ferritin assay is the only method which can provide a semiquantitative indication of the levels of storage iron but its application is limited by both methodological and biological variation. Starvation, or even fasting for a short period, can cause elevation of the serum ferritin concentration (Lundberg *et al.*, 1984) and vitamin C deficiency may reduce it (Chapman *et al.*, 1982b). Two definitions are commonly used but sometimes confused. **Iron deficiency anaemia** refers to anaemia caused by the lack of iron in the body. **Iron deficient erythropoiesis** refers to an impairment of iron supply to the bone marrow in the absence of storage iron and may occur with haemoglobin levels still in the reference range. Overlap in laboratory values for iron stores between anaemic and non-anaemic individuals means that using haemoglobin measurements alone leads to an overestimate of the prevalence of iron deficiency anaemia. Similarly the use of only one indicator of storage iron levels or tissue iron supply – ferritin, transferrin saturation or erythrocyte protoporphyrin – may overestimate the number of individuals with lesser degrees of iron deficiency. The concepts are illustrated in a paper by Cook *et al.* (1986) in which they show that more precise figures for the prevalence of iron deficient erythropoiesis and iron deficiency anaemia may be obtained by using multiple criteria (abnormal values for any two of serum ferritin, transferrin saturation or erythrocyte protoporphyrin). The 'multiple-test' approach has been further refined with the introduction of soluble transferrin receptor measurements.

Skikne *et al.* (1990) employed quantitative phlebotomy to evaluate the use of serum ferritin concentrations and soluble transferrin receptor concentrations to indicate levels of iron stores. The log of the ratio of these two measurements (receptor/ferritin as μg/μg) shows a linear relationship with iron stores (mg Fe/kg body weight). This combination makes it possible to evaluate iron stores and also the impairment of iron supply for production of functional iron proteins which develops as more and more body iron is lost.

The results of the application of the multiple test and the single test approaches are clearly contrasted in two papers (Cook *et al.*, 1986; Hallberg *et al.*, 1993a). The final confirmation about the correctness of these prevalence figures will require an examination of the response to iron supplementation (Garby *et al.*, 1969).

In order to allow greater consistency in the comparison of iron status data from population surveys, the Task Force has suggested cut-off points for children (Table 5.6) and adults (Table 5.7).

5.4.2 Use in individual patients

In combination with measurement of blood haemoglobin, assays of zinc protoporphyrin (ZPP) and transferrin saturation can indicate iron deficient erythropoiesis. Furthermore a high TIBC provides good evidence that there is no storage iron. However, most adult hospital patients have anaemia which is secondary to infection, inflammation, malignant disease or surgery. If information about the amount of storage iron is required, it is generally necessary to assay serum ferritin or to assess tissue iron levels directly. Iron deficient erythropoiesis in the presence of normal or raised levels of storage iron suggests secondary anaemia, not

Table 5.6 Suggested cut-off points for differing iron status in young children from epidemiological studies (source: Oski *et al.* 1983)

Factor	Iron sufficient	Iron depleted non-anaemic	Iron deficient erythropoiesis	Iron deficient anaemia
Hb g/l	≥ 110	≥ 110	≥ 110	< 110
Ferritin μg/l*	≥ 12	< 12	< 12	< 12
Transferrin saturation %	≥ 10	≥ 10	< 10	< 10
EPP μmol/mol haem	< 100	< 100	≥ 100	≥ 100

* Others have adopted more stringent criteria, e.g. ferritin of 7 μg/l has been suggested by Siimes *et al.* (1974) and various surveys, including the DH nationally representative sample of children have adopted this figure.

Table 5.7 Suggested cut-off points for defining iron status in adults from epidemiological studies

Factor	Iron overload	Iron sufficient	Iron depleted non-anaemia	Iron deficient erythropoiesis	Iron deficient anaemia
Hb[a] g/l	–	≥ 130 men ≥ 120 women	≥ 130 men ≥ 120 women	≥ 130 men ≥ 120 women	< 130 men < 120 women
Ferritin[b] μg/l	> 300 men > 200 women	≥ 13	< 13	< 13	< 13
Transferrin saturation %	> 60% men > 50% women	> 16%	≥ 16%	< 16%	< 16
EPP[c] μmol/mol haem	–	< 80	< 80	≥ 80	≥ 80
Transferrin receptor mg/l	–	< 8.5	< 8.5	> 8.5	>8.5

[a] WHO (1972) recommended cut-off points for IDA are below 120 g/l for women and below 130 g/l for men, but the Health Survey of England (1991) uses 110 g/l as the lower cut-off point for men and women.
[b] The Adult Survey uses 13 μg/l ferritin (Ft) as the lowest cut-off point but defines less than 25 μg/l as low iron stores.
[c] EPP is usually measured as ZPP. If washed cells are used, the cut-off value should be 40 μmol/mol haem.

primary iron deficiency. Thus the serum ferritin assay should provide an excellent discriminator between iron deficiency anaemia and the anaemia of chronic disease. In practice, interpretation of serum ferritin is more complicated.

In the anaemia of chronic disease, the most important factor controlling serum ferritin concentrations is the level of storage iron. Serum ferritin levels are higher than those found in patients with similar levels of storage iron but without infection and inflammation (Worwood, 1980b). There is experimental evidence from studies of rat liver that the rapid drop in serum iron concentration which follows the induction of inflammation may be due to an increase in apoferritin synthesis which inhibits the release of iron to the plasma (Konijn and Hershko, 1977). Interleukin-l (IL-l) is the primary mediator of the acute-phase response which in iron metabolism is indicated by the drop in plasma iron concentration (Dinarello, 1984). There is direct evidence from studies of cultured human hepatoma cells that IL-1β (which also causes changes in protein synthesis that mimic the acute phase response in cultured hepatoma cells) directly enhances the rate of ferritin synthesis by control of translation (Rogers *et al.*, 1990).

Many clinical studies have demonstrated that patients with the anaemia of chronic disease, and no stainable iron in the bone marrow, may have serum ferritin concentrations considerably in excess of 15 μg/l and there has been much debate (Witte, 1991) about the practical application of the serum ferritin assay in this situation. Values < 15 μg/l indicate the absence of storage iron and values > 100 μg/l indicate the presence of storage iron. It is the 'grey' area from 15 to 100 μg/l which is difficult to interpret. It would seem logical to combine the assay of serum ferritin with a measure of disease severity such as the ESR or C-reactive protein. Witte *et al.* (1986) have described such an approach and claimed to be able to confirm or exclude iron deficiency (absence of storage iron in the bone marrow) in almost all patients with secondary anaemia. However these conclusions have been challenged (Coenen *et al.*, 1991). As described earlier, measurements of soluble transferrin receptor concentration may provide a valuable diagnostic aid for this problem area.

In assessing the adequacy of iron stores for replenishing haemoglobin the degree of anaemia must also be considered. Thus a patient with haemoglobin concentration of 100 g/l may benefit from iron therapy if the serum ferritin concentration is below 100 μg/l (Cavill *et al.*, 1986).

The other major influence confounding the use of the serum ferritin assay to determine iron stores is liver disease. The liver contains much of the storage iron in the body and any process that damages liver cells will release ferritin. It is also possible that liver damage may interfere with clearance of ferritin from the circulation. It was suggested by Prieto *et al.* (1975) that the ratio of serum ferritin to aspartate aminotransferase activity might provide a good index of liver iron concentration. Glycosylated ferritin concentrations might relate directly to storage iron concentrations, while non-glycosylated levels would relate to the degree of liver damage (Worwood, 1979). However, neither the ferritin : aspartate aminotransferase ratio (Valberg *et al.*, 1978; Batey *et al.*, 1978) nor the measurement of glycosylated ferritin levels (Worwood *et al.*, 1980; Chapman *et al.*, 1982a) has proved to be any more reliable than the simple assay of serum ferritin as an index of liver iron concentration. In patients with liver damage a low value always indicates absent iron stores; normal values indicate absent or normal levels but rule out iron overload; whereas high values may indicate either normal or high iron stores and further investigation may be necessary to distinguish these.

5.5 SPECIFIC APPLICATIONS

The general application of the ferritin assay for the determination of iron storage levels has been described. There are a number of common diagnostic applications where a more specific application of the various indicators of iron metabolism is justified.

5.5.1 Iron deficiency in infancy and childhood

The serum ferritin concentration is a less useful guide to iron deficiency in children than in adults partly because of the low concentrations generally found in children over 6 months of age. ZPP provides a useful indicator of iron-deficient erythropoiesis, although high values may indicate lead poisoning rather than iron deficiency. The small sample volume for ZPP determination is also an advantage in paediatric practice. Hershko *et al.* (1981) studied children in villages from the Golan Heights in Israel and concluded that erythrocyte protoporphyrin (EP) was a more reliable index of iron deficiency than serum ferritin and serum iron. They suggested that a significant incidence of chronic disease affected both ferritin and iron values. However, Zanella *et al.* (1989) did not find that EP was a better predictor of iron deficiency than ferritin.

5.5.2 Treatment of iron deficiency anaemia

Oral iron therapy at conventional doses (60 mg Fe, three times daily) has little immediate effect on serum ferritin levels which rise slowly as the haemoglobin concentration increases. With double doses there is a rapid rise of serum ferritin to within the normal range (within a few days) which probably does not represent the increase in storage iron (Wheby, 1980). Intravenous iron causes a rapid rise in ferritin concentrations, which may be above the normal range, and then gradually drop back to the normal range.

5.5.3 Screening blood donors for iron deficiency

Regular blood donation reduces storage iron levels and this has been demonstrated in a number of surveys of blood donors (Skikne *et al.*, 1984; Milman and Kirchoff, 1991). A well-known difficulty is that the conventional 'copper sulphate' screening test for anaemia (Mollison *et al.*, 1988) is somewhat inaccurate and donors may be deferred unnecessarily. It has been suggested that secondary screening using ZPP would provide an immediate confirmation of iron deficiency (Raftos *et al.*, 1983; Schifman and Rivers, 1987). Despite the availability of the serum ferritin assay during the last 20 years, there has been little attention to the fundamental relationship between storage iron levels and the ability to give blood. Screening blood donors by routinely assaying serum ferritin may make it possible to predict the development of iron deficiency anaemia, identify donors with high iron stores who may give blood more frequently than usually permitted, and also identify donors homozygous for genetic haemochromatosis who are beginning to develop iron overload (Worwood *et al.*, 1991a; Worwood and Darke 1993).