ANNEX 2

Indicators of the iron status of populations: ferritin

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Contents

1. Sources of data

This review summarises the genetics, biochemistry and physiology of serum ferritin, discusses variables affecting the assay of ferritin, and examines how ferritin may be used to assess the iron status of populations. It builds on a review of earlier studies of serum ferritin concentration in health and disease by Worwood (*1*) and on several national and international reviews of using serum ferritin to determine iron status (*2–4*). The Centers for Disease Control and Prevention Nutrition Laboratory has recently prepared a *Reference Manual for Laboratory Considerations – Iron Status Indicators for Population Assessments* (2003), which covers all aspects of the processing of blood samples.

2. Introduction

The iron storage protein ferritin is found in both prokaryotes and eukaryotes. It consists of a protein shell with a molecular mass of about 500 kDa composed of 24 subunits. The protein shell encloses a core of ferric-hydroxy-phosphate which can hold up to 4 000 atoms of iron. Proteins with a similar overall structure are found throughout the plant and animal kingdom as well as in bacteria, although bacterial ferritin appears to have evolved separately as it has no amino acid sequence homology with animal ferritins. Bacterial ferritin from *Escherichia coli* for example, contains haem (about one per two subunits), as well as a core of non-haem iron. Ferritin is ancient in evolutionary terms and also has a long biochemical history. Since it was first isolated (*5*) two main issues have dominated ferritin research: its structure, and the mechanism of iron uptake and release. Recently the molecular biology of ferritin has come to the fore and the molecule has become a model for studies of how synthesis is regulated at the level of genetic translation. A detailed review of the structure and function of ferritin has been published (*6*).

3. Genetics

A range of isoferritins is found in various human tissues. These are composed of combinations of two types of subunit, H and L (*7*). The expressed gene for the H-subunit is on chromosome 11 at 11q13 (*8*) and that for the L-subunit is on chromosome 19 at 19q13-ter. There are however multiple copies of the ferritin genes. Most of the H sequences (about 15 copies) on a number of chromosomes appear to be processed pseudogenes (i.e. without introns) with no evidence for their expression. The same applies to the other 'L' sequences found on chromosomes 19, 21 and X. However an intronless gene on chromosome 5q23.1 codes for mitochondrial ferritin – a newly identified H-type ferritin (*9*). For the expressed L-gene in the rat there are three introns located between exons coding for the four major α–helical regions of the peptide sequences. Human H and L genes have a similar structure although the introns differ in size and sequence. The messenger ribonucleic acid (mRNA) for the human ferritin genes contains about 1.1 kb. The H-subunit is slightly larger than the L-subunit (178 amino acids compared with 174 amino acids) but on electrophoresis in polyacrylamide gels under denaturing conditions the apparent differences in relative molecular mass are rather greater (21 kDa and 19 kDa). Human H and L sequences are only 55% homologous whereas the degree of homology between L-subunits and H-subunits from different species is of the order of 85% (*6*).

4. Structure

A ferritin subunit has five helices and a long inter-helical loop. The loop L and the N-terminal residues are on the outside of the assembled molecule of 24 subunits. The C-terminal residues are within the shell. H and L chains adopt the same conformation within the molecule. A description of the three-dimensional structure of apoferritin will be found in a recent review (*6*).

In human tissues H-rich isoferritins (isoelectric point (pI) 4.5–5.0) are found in heart muscle, red blood cells, lymphocytes, monocytes, HeLa cells and other, but not all, cultured cells (*10*). L-rich isoferritins are more basic (pI 5.0–5.7) than H-rich isoferritins and are found in the liver, spleen and placenta. The pI of ferritin is not significantly affected by its iron content, which varies from tissue to tissue and with the tissue iron content.

Ferritin is purified from tissues by taking advantage of three properties: the ability to withstand a temperature of 75 °C; the high density of the iron-rich molecule, which allows concentration by ultracentrifugation; and crystallisation in the presence of cadmium sulphate. However it should be noted that, whereas ultracentrifugation tends to concentrate molecules rich in H-subunits, crystallisation from cadmium sulphate solution tends to give a lower overall recovery and selects molecules rich in L-subunits (*11*).

5. Haemosiderin

Ferritin is a soluble protein but is degraded to insoluble haemosiderin which accumulates in lysosomes. Both ferritin and haemosiderin provide a store of iron that is available for protein and haem synthesis. Normally much of the stored iron in the body (about 1 g in men and less in pre-menstrual women and children) is present as ferritin, but during iron overload the proportion present as haemosiderin increases. Purified preparations of ferritin always contain a small proportion of molecules in the form of dimers, trimers and other oligomers (*12*). These may be intermediates in the formation of haemosiderin. Andrews et al. (*13*) isolated a soluble ferritin from iron-loaded rat liver lysosomes which contained a peptide of molecular mass 17.3 kDa which may be a precursor of insoluble haemosiderin. Peptides extracted from preparations of haemosiderin have been found to react with antibodies to ferritin (*14*,*15*).

6. Regulation of ferritin synthesis and breakdown

Ferritin synthesis is induced by administering iron. In 1966 Drysdale and Munro (*16*) showed that the initial response of apoferritin synthesis to the administration of iron was by regulating translation rather than transcription. This requires the movement of stored mRNA from the ribonucleoprotein fraction (RNP) to the polysomes (*17*) followed by an increased rate of translation of ferritin subunits. This response is the same for H and L subunits. However, after administering iron there is an eventual increase in the rate of transcription of the L-subunit gene. This causes an increase in the ratio of L- to H-subunits during ferritin synthesis after administering iron (*18*). The translational control mechanism involves the 5' untranslated region of the ferritin mRNA which contains a sequence forming a 'stem-loop' structure. This is called an 'iron response element' (IRE). Similar cytoplasmic proteins (IRP- and -2)

bind to the IRE in the absence of iron but are inactivated (IRP-1) or degraded (IRP-2) when iron supply increases (*19*). Binding to mRNA prevents ferritin synthesis, but in the absence of binding, polysomes form and translation proceeds. The protein IRP-1 is the iron-sulphur protein, aconitase, encoded by a gene on chromosome 9 which functions as a cytosolic aconitase in its iron-replete state. A model involving conformation changes which permit RNA binding has been proposed (*20*). A related mechanism operates in reverse for the transferrin receptor. Here there are stem-loop sequences in the 3' untranslated region, and protein binding prevents degradation of mRNA. Hence iron deficiency enhances transferrin receptor synthesis. Erythroid ALA synthase, aconitase, DMT1 and ferroportin-1 also have IREs.

Although ferritin is generally considered to be an intra-cellular protein and most of the mRNA in the liver is associated with free ribosomes, there is evidence of the synthesis of ferritin on membrane-bound polysomes (21). This finding may be of special relevance to the origin of plasma ferritin (see below).

The way in which ferritin is degraded remains largely a mystery. Studies of rat liver cells (16) indicate that the half-life of a ferritin molecule is about 72 hours, and is extended by iron administration. The relationship between ferritin breakdown and formation of haemosiderin is unclear, as is the fate of the iron core after the degradation of the protein shell.

7. Functions related to iron storage

The major function of ferritin is clearly to provide a store of iron which may be used for haem synthesis when required. Iron uptake *in vitro* requires an oxidizing agent, and iron release requires a reducing agent (reviewed by Harrison and Arosio (*6*). There are differences in the rate of iron uptake between apoferritins with varying proportions of H and L-subunits; H-rich isoferritins having the highest rate of iron uptake *in vitro* (*22*). Such isoferritins are found in cells which either have a high requirement for iron for haem synthesis, such as nucleated red cells and cardiac muscle, or which do not appear to be involved in iron storage, such as lymphocytes. In the tissues where iron is stored, such as the liver and spleen, the ferritin contains mostly L-subunits. Recent studies with recombinant H_{24} and L_{24} molecules have demonstrated that the ferroxidase activity of ferritin is a property of the H-subunit and that L24 molecules have little ability to catalyse iron uptake (*6*). The maturation of monocytes to macrophages *in vitro* is associated with the loss of acidic isoferritins (*23*). Iron storage therefore seems to require ferritin that is rich in L-subunits.

8. Are there tumour specific ferritins?

There has been considerable interest in specific 'carcino-fetal' ferritins , which are molecules peculiar to fetal or malignant cells. This term originated in a paper by Alpert et al. (*24*) to describe the acidic ferritins found in rat fetal liver cells and in some neoplastic tissues. It is now accepted that the variation in isoelectric point of ferritin molecules from various tissues is effectively explained in terms of the two-subunit model of Arosio et al. (*7*). Later, Moroz et al. (*25*) chose human placental ferritin as a possible source of unique, antigenic "onco-fetal" ferritin, and produced a monoclonal antibody (H9) which bound to placental ferritin but not to liver or spleen ferritin. Recently the unique subunit in placental ferritin has been fully characterised (*26*) and named placental immunomodulatory ferritin (PLIF). The PLIF coding region is

composed of ferritin heavy chain (FTH) sequence lacking the 65 C-terminal amino acids, which are substituted with a novel 48 amino acid domain (C48). In contrast to FTH, PLIF mRNA does not include the iron response element in the 5'-untranslated region, suggesting that PLIF synthesis is not regulated by iron. The authors suggest that the p43 subunit of PLF may represent a dimer of PLIF (22 kDa). Furthermore, PLIF transcripts exist at a very low copy number compared with ferritin heavy chain in placental tissues at term delivery (40 weeks). Despite the development of an immunoassay for placental isoferritin (*27*) the assay has not been widely exploited in the diagnosis of cancer and its specificity for malignancy has not been established. Low concentrations of placental ferritin in serum during pregnancy may indicate abnormal gestation (*28*).

9. Ferritin as a regulator of erythropoiesis

A role for ferritin in the regulation of haemopoiesis, apparently unrelated to iron storage, was proposed by Broxmeyer et al. (*29*) who showed that the protein responsible for a 'leukaemia-associated inhibitory activity' (LIA) was an acidic isoferritin. This protein fraction, and an acidic isoferritin preparation from the spleen of a patient with chronic myeloid leukaemia, suppressed colony formation *in vitro* of CFU-GM, BFU-E and CFU-GEMM progenitor cells from the marrows of normal donors, but was ineffective in marrow or blood from patients with acute leukaemia, myelodysplasia and some other haematological disorders.

Ferritin appears to act *in vitro* on progenitors which are in the DNA synthesis (S) phase of the cell cycle (*30*). Sala et al. (*31*) were unable to confirm the original results of Broxmeyer et al. (*29*) and since 1992 there has been little work on this inhibitory activity of ferritin.

10. Plasma (serum) ferritin

It was only after the development of a sensitive immunoradiometric assay (IRMA) that ferritin was detected in the serum or plasma of normal individuals (*32*). Reliable assays, both radioimmunoassay (RIA) using labelled ferritin and IRMA using labelled antibody, have been described in detail (*33*). These assays have since been supplanted by enzyme linked immunoassays (ELISA) using 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 is included in the latest batch and random access, automated analysers for immunoassays.

10.1 Relationship to storage iron levels

Serum ferritin concentrations are normally within the range 15–300 µg/l and are lower in children than adults (Table 1 and 2). Mean values are lower in women before the menopause than in men, reflecting women's lower iron stores caused by the losses during menstruation and childbirth. The changes in serum ferritin concentration during development from birth to old age reflect changes in the amounts of iron stored in tissues (*34*). A mother's iron status appears to have relatively little influence on the concentration in cord serum, and mean values are in the range 100–200 µg/l. There is a good correlation between serum ferritin concentration and storage iron

TABLE 1

The normal range in mean serum ferritin concentration (µg/l) of adults by sex and age group[a]

	Men				Women			
Age range (years)	Sample size	Mean serum ferritin concentration (µg/l)	5th percentile	95th percentile	Sample size	Mean serum ferritin concentration (µg/l)	5th percentile	95th percentile
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

[a] Subjects being treated with drugs for iron deficiency (n = 26) were included.
Adapted from White et al. (*40*), with permission of the publisher.
For other surveys of populations in North America and Europe, see Cook et al. (*42*), Finch et al. (*43*), Jacobs and Worwood (*44*), Milman et al. (*45*), Valberg et al. (*46*), Custer et al. (*47*).

TABLE 2

The mean and normal range in the serum ferritin concentration (µg/l) of infants, children and adolescents

Number of children	Age	Population	Selection	Mean serum ferritin concentration (µg/l)	Range serum ferritin concentration (µg/l)	Reference
46	0.5 months	Helsinki	Non-anaemic	238	90–628	Saarinen and Siimes (*48*)
46	1 month	Helsinki	Non-anaemic	240	144–399	Saarinen and Siimes (*48*)
47	2 months	Helsinki	Non-anaemic	194	87–430	Saarinen and Siimes (*48*)
40	4 months	Helsinki	Non-anaemic	91	37–223	Saarinen and Siimes (*48*)
514	0.5–15 years[a]	San Francisco	Non-anaemic	30[b]	7–142	Siimes et al. (*49*)
323	5–11 years	Washington	Low income families	21[b]	10–45[c]	Cook et al. (*42*)
117	5–9 years	Nutrition Canada Survey	Random	15[d]	2–107[e]	Valberg et al. (*46*)
335	6–11 years	Denmark	Random, urban	29[a]	12–67[f]	Milman and Ibsen (*50*)
126 male 125 female	12–18 years	Washington	Low income families	23[b] 21[b]	10–63[c] 6–485	Cook et al. (*42*)
98 male 106 female	10–19 years	Nutrition Canada Survey	Random	18[d] 17[d]	3–125[e] 2–116[e]	Valberg et al. (*46*)
269 male 305 female	12–17 years	Denmark	Random, urban	28[b] 25[b]	11-68[f] 6–65[f]	Milman and Ibsen (*50*)

[a] 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
[b] Median
[c] 10–90 percentile
[d] Geometric mean
[e] Confidence interval
[f] 5–95% interval

mobilized as a result of phlebotomy. This suggests a close relationship between the total amount of stored iron and the serum ferritin concentration in normal individuals (*35*). Serum ferritin concentration decreases with blood donation (*36–38*) and increases with alcohol intake (*36,39,40*). The significant association with alcohol consumption in both men and women has been confirmed in the Health Survey for England (40). In this survey the ferritin concentration was also higher with increasing body mass index. In women after the menopause the ferritin concentration increases but remains lower than in men (Table 1 and 6). In unselected elderly patients a high concentration of ferritin is often associated with disease (*41*). The serum ferritin concentration is relatively stable in healthy persons (see below). In patients with iron deficiency anaemia, the serum ferritin concentration is typically less than 12–15 µg/l. This threshold has been established in a number of studies by determining the serum ferritin concentrations of patients with iron deficiency anaemia (see below) and a reduction in the level of reticuloendothelial iron 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 (*34*). A high concentration of serum ferritin is found during iron overload, but there are other causes as well.

10.2 Serum ferritin in acute and chronic disease

The acute phase refers to a series of events that occur in response to infection or tissue damage. The local reaction is termed inflammation and the systemic response is referred to as the acute phase response. The acute phase response may be induced by toxic chemicals, physical trauma, infection, inflammation, malignancy, tissue necrosis (e.g. myocardial infarction) and immunisation. The clinical and metabolic features of the acute phase response include fever, leucocytosis, thrombocytosis and metabolic alterations, as well as changes in the concentration of a number of plasma proteins. The changes in several plasma proteins including ferritin during infection, inflammation and trauma are discussed in the review by Northrop-Clewes.

In the anaemia of chronic disease the most important factor controlling serum ferritin concentration is the level of storage iron. However the serum ferritin concentration is higher than in patients with similar levels of storage iron but without infection and inflammation. There is experimental evidence from studies of rat liver cells 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 (*51*). Interleukin-1 (IL-1) is the primary mediator of the acute-phase response which, in iron metabolism, is indicated by a drop in plasma iron concentration (*52*). There is direct evidence from studies of cultured human hepatoma cells that IL-1ß (which also causes changes in protein synthesis which mimic the acute phase response in cultured hepatoma cells) directly enhances the rate of ferritin synthesis by control of translation (*53*).

Few longitudinal studies of serum ferritin have been reported. After experimentally inducing fever in normal volunteers, ferritin concentrations reached a maximum after 3 days and gradually returned to normal values over the next 10 days (*54*). The increases were relatively small, with ferritin concentrations increasing by about 20 µg/l per 24 hour after giving etiocholanolone. After acute infection, there were increases of about 3 fold in serum ferritin concentration, with the maximum concentration reached within 1 week (55). Concentrations then declined slowly over several weeks. After myocardial infarction, ferritin concentrations began to rise after 30

hours, reached a peak after about 4 days, and then remained above initial levels for up to two weeks (*55,56*). The increases in serum ferritin were smaller than those found for acute infection. After surgery there was a rapid decline (in 2 days) in the concentration of haemoglobin, serum iron and transferrin, with the greatest fall shown by serum iron (*57*). The concentration of C-reactive protein (CRP) rose less rapidly from <5 mg/l to over 100 mg/l, reaching a maximum value at 5 days, and then declined to normal values over the subsequent 4 weeks. Most of the decrease was in the first 10 days. Ferritin increased to a maximum concentration at about 5 days, but the mean increase was less than 50%. After 4 weeks values returned to those before surgery. Serum transferrin receptor (sTfR) concentrations showed little change after surgery.

The combined effect of these changes on blood viscosity and erythrocyte aggregation may be detected by directly measuring plasma viscosity or the erythrocyte sedimentation rate (ESR). Changes occur slowly and these measures are of greater use in monitoring chronic disease than in detecting the immediate response to injury.

The Expert Panel on Blood Rheology of the International Committee for Standardization in Haematology (ICSH), has published guidelines on measuring the ESR and blood viscosity (*58*). Suitable methods for determining the CRP concentration include nepholometry and turbidimetry, and they should be able to detect the protein at concentration as low as 5 mg/l. An international reference standard is available (*59*).

Many clinical studies have demonstrated that patients with anaemia of chronic disease and no stainable iron in the bone marrow may have a serum ferritin concentration considerably in excess of 15 µg/l and there has been much debate about the practical application of the serum ferritin assay in this situation (*60*). A ferritin concentration of <15 µg/l indicates the absence of storage iron while concentrations >100 µg/l indicate the presence of storage iron. Concentrations in the range of 15–100 µg/l serum ferritin are 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 the concentration of CRP. Witte et al. (*61*) described such an approach and claimed to be able to confirm or exclude iron deficiency, defined as an absence of stainable iron in the bone marrow, in almost all patients with secondary anaemia. However these findings have not been confirmed (*62*). This lack of success in "correcting" serum ferritin concentrations for the effect of inflammation or infection is probably due to the different responses to acute disease shown by ferritin and CRP. Although other acute phase proteins may show similar responses in time, the small changes in concentration reduce the value of the marker as an indicator of disease. Minor infections in children, without changes in other markers of infection, may cause long-term increases in serum ferritin concentration (*63*). Minimal inflammation, detected using a highly sensitive assay for CRP, led to a low serum iron concentration in infants (*64*). As described earlier, measurements of soluble transferrin receptor concentration may provide a valuable diagnostic aid for this difficult area of nutritional assessment.

In assessing of the adequacy of iron stores to replenish haemoglobin, the degree of anaemia must also be considered. Thus a patient with a haemoglobin concentration of 100 g/l may benefit from iron therapy if the serum ferritin concentration is below 100 µg/l (*65*). This is discussed below in terms of the predictive power of diagnostic tests.

10.3 Serum ferritin and liver disease

The other major influence confounding the use of the serum ferritin concentration to estimate iron stores is liver disease. The liver contains much of the iron stored in the body, and any process that damage 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. (*66*) that the ratio of serum ferritin to aspartate aminotransferase activity might provide a good index of liver iron concentration. Glycosylated ferritin concentrations might be related directly to storage iron concentrations, while the concentration of non-glycosylated ferritin would relate to the degree of liver damage (*67*). However neither the ferritin:aspartate aminotransferase ratio (*68*,*69*) nor the measurement of glycosylated ferritin concentration (33,70) have proved to be any more reliable than the simple measurement of serum ferritin concentration as an index of liver iron concentration. In patients with liver damage a low serum ferritin concentration always indicates absent iron stores, a normal concentration indicates absent or normal iron stores but rules out iron overload, whereas a high concentration may indicate either normal or high iron stores and further investigation may be necessary to distinguish between the two.

10.4 Serum ferritin concentration and malignancy (*34*)

A high concentration of ferritin is seen in most patients with pancreatic carcinoma, lung cancer, hepatoma and neuroblastoma, although in most cases of cancer of the oesophagus, stomach and colon, the serum ferritin concentration is within the normal range. In breast cancer, the concentration is usually raised in patients with metastatic disease, but the assay has not proved to be useful in predicting metastasis. Patients with acute leukaemia generally have a higher serum ferritin concentration than normal but this is not the case for patients with chronic leukaemia. In Hodgkin's disease the concentration of ferritin increases with the stage of disease, but is not related to the histological type of disease.

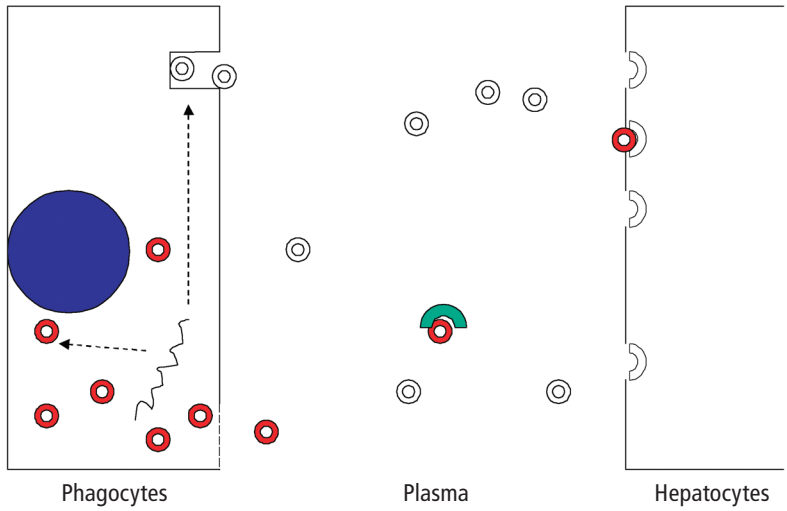
The concept of carcino-fetal ferritin has been introduced above and a logical extension of the concept is to search for changes in the immunological properties of serum ferritin in order to detect malignant disease or monitor the effect of therapy. A number of assays have been described using acidic isoferritins derived from HeLa cells (*71–73*) or heart ferritin (*74*,*75*) and have been applied to serum from patients with cancer. The results have been inconsistent, but later studies using a monoclonal antibody (*76*) confirm some of the studies with polyclonal antisera and indicate that the concentration of H-rich isoferritin in serum is very low compared with L-rich isoferritins, even in patients with cancer. An assay for placental isoferritin has not improved tumour specificity (see above). It is likely that the high concentration of ferritin in the serum in malignancy is due to an increase in the concentration of storage iron, to liver damage, or to inflammation, as well as a consequence of the direct release of ferritin from the tumour. Whatever the cause is, the result is an increase in the concentration of L-rich isoferrritin in the serum rather than accumulation of 'tumour-specific' isoferritins.

10.5 Exceedingly high serum ferritin concentrations

The factors controlling plasma ferritin concentration are: 1) synthesis, 2) release from cells), 3) clearance from the plasma (Figure 1). There are no instances yet known in which a very high ferritin concentration is due to abnormalities in ferritin clearance, but abnormalities occur in both synthesis and release.

In iron overload the serum ferritin concentration is unlikely to exceed 4 000 µg/l in the absence of concomitant liver damage (*33*) but in liver necrosis the ferritin concentration may be in excess of 50 000 µg/l (*66*). The stimulation of synthesis by a combination of iron and cytokines can lead to a ferritin concentration of >20 000 µg/l in adult-onset Still's disease (*77,78*). In the reactive haemophagocytic syndrome there is an inappropriate activation of monocytes leading to haemophagocytosis and cytokine release. A ferritin concentration of up to 400 000 µg/l has been reported in children (*79,80*) and adults (*81*). Patients with acquired immunodeficiency syndrome (AIDS) may also have a reactive haemophagocytosis syndrome and high concentrations may also occur in AIDS sufferers with disseminated histoplasmosis (*82*).

FIGURE 1
A diagrammatic representation of a macrophage (left), plasma in the centre, and on the right, a liver parenchymal cell



Cytosolic ferritin (O) is released directly from damaged cell membranes into plasma or secreted (top) after synthesis on membrane bound polysomes and glycosylation (◎). In the circulation non-glycosylated ferritin may interact with ferritin binding proteins followed by removal of the complex from the circulation. Many cells also carry ferritin receptors, presumably for both secreted ferritin and cytosolic ferritin (see text). Injection of spleen ferritin into the circulation in man is followed by rapid uptake by the liver.
Adapted from Worwood (*83*), with permission of the publisher.

10.6 High serum ferritin concentrations and congenital cataract

An interesting cause of a high ferritin concentration in the absence of iron overload is associated with inherited cataract formation. In has now been demonstrated that mutations in the 'stem loop' structure of the ferritin L subunit may lead to synthesis of the 'L' subunit of ferritin that is no longer regulated by iron concentration (*84*). This causes an increase in the serum ferritin concentrations up to about 1 000 µg/l) in the absence of iron overload.

11. Biochemistry and physiology of plasma ferritin

11.1 Immunological properties and iron content

Plasma ferritin resembles liver or spleen ferritin immunologically and is recognised by polyclonal or monoclonal antibodies raised against these ferritins (see above). In patients with iron overload plasma ferritin has a relatively low iron content in purified preparations of 0.02–0.07 µg iron/µg protein (*85,86*) or a mean of 0.06 µg iron/µg protein when measured by immunoprecipitation (*87*). Purified horse serum ferritin has an iron content of <0.01 µg iron/µg protein (*88*). In the liver and spleen of patients with iron overload the iron content of ferritin is >0.2 µg iron/µg protein. Despite these findings several recent papers have indicated that serum ferritin has a much higher iron content. In 1997 ten Kate et al. (*89*) purified ferritin by immuno-precipitation and measured the iron content by atomic absorption spectrophotometry. They found a mean iron saturation of ferritin of 24% in normal serum giving a concentration of 0.13 µg iron/µg protein. They suggested that the extensive purification used in earlier studies had lead to a loss of iron, although this is unlikely unless reducing agents were present in the buffers used. Herbert et al. (*90*) claimed that the measurement of serum ferritin iron by a similar procedure provided an accurate assessment of the whole range of human body iron status, unconfounded by inflammation. Later Nielsen et al. (91) determined the iron content of serum ferritin derived from patients with iron overload and tissue damage using the method of ten Kate et al. (*89*). The iron saturation was about 5% and they found that the assay for ferritin iron was of little benefit in the diagnosis of iron overload. Yamanishi et al. (*92*) determined ferritin iron concentrations from 0.02 to 0.04 µg iron/µg ferritin protein in serum samples with ferritin concentrations greater than 2 000 µg/l. It should be pointed out that in 1956 Reissmann and Dietrich found that iron-rich ferritin was only detectable in the circulation after liver necrosis (*93*). Another consideration is that, in a normal subject with a serum iron concentration of 20 µmol/l and a serum ferritin concentration of 100 µg/l, the ferritin iron concentration would be only 1% of the transferrin iron concentration, even if the iron content is assumed to be high (0.15 g/µg protein). Clearly specific antibodies and the effective washing of the immunoprecipitate are essential if ferritin iron is to be detected.

11.2 Glycosylation

On isoelectric focusing both native and purified serum ferritin display a wide range of isoferritins covering the pI range found in human tissues (*85,94*) yet on anion exchange chromatography, serum ferritin is apparently a relatively basic isoferritin (*85*). The reason for this discrepancy and the heterogeneity of ferritin on isoelectric focusing appears to be glycosylation. In normal serum about 60% of ferritin binds to concanavalin A (*67*) whereas tissue ferritins do not bind. Incubation with neuraminidase converts the acidic ferritins of serum to the basic isoferritins but the pI of acidic heart ferritin is unaffected (*95*). A carbohydrate containing G subunit has also been identified in purified preparations of serum ferritin in addition to the H and L subunits (*86,96*).

11.3 Origin of serum ferritin and its clearance from the circulation

These findings suggest that some ferritin may enter the circulation by secretion, rather than by release from damaged cells. In hepatocytes there is direct evidence of

regulated secretion of glycosylated ferritin (*97*). Secreted ferritin may originate from phagocytic cells that degrade haemoglobin (Figure 1). When there is tissue damage, direct release of cytosolic ferritin through damaged cell membranes becomes important. In patients with ferritinaemia resulting from necrosis of the liver, the plasma ferritin shows reduced binding to concanavalin A (*67*). Findings in patients with haemophagocytosis or in the early stages of haemochromatosis confirm the importance of phagocytic cells (see below).

Another explanation for the differences between plasma and tissue ferritins may be differences in their clearance from the circulation. Plasma ferritin labelled with [^{131} I] was removed only slowly [T½<24h] from the plasma of normal subjects after intravenous injection (*98*) but spleen ferritin labelled in the same way was cleared very rapidly with a T½ of about 9 minutes (*99*). Such a rapid clearance may be due to interaction with ferritin receptors on hepatocytes (*100*) which appear to have a higher affinity for liver ferritin than for serum ferritin, at least in experiments on rats. Rapid clearance may also be initiated by interaction with ferritin binding proteins in the plasma (*101–104*). Several isoferritins may be released into the plasma but the ones which normally accumulate are L_{24} molecules and glycosylated molecules that are rich in L-subunits and again contain little iron. The L_{24} molecules take up iron slowly *in vitro* and have been termed 'natural apoferritin' (*105*). These molecules may accumulate in the plasma because their clearance by receptors, or their interaction with binding proteins, requires at least some H-subunits. The glycosylated protein may have little opportunity to acquire iron during secretion.

12. Red cell ferritin and its diagnostic use

The ferritin present in circulating erythrocytes is but a tiny residue of the amount in its nucleated precursors in the bone marrow. Normal erythroblasts contain ferritin which is immunologically more similar to heart ferritin than liver ferritin (i.e., ferritin rich in H-subunits) and mean concentrations are about 10 fg ferritin protein/cell (*106*). The concentration declines throughout the process of cell maturation and only about 10 ag/cell (10^{-18} g/cell) remains in the erythrocyte when measured with antibodies to L-ferritin, with a somewhat higher concentration detected using antibodies to H-type ferritin (*107,108*). Red cell ferritin concentration has generally been measured with antibodies to L-ferritin and reflects the iron supply to the erythroid marrow. The concentration tends to vary inversely with the red cell protoporphyrin concentration (*107*). Thus in patients with rheumatoid arthritis and anaemia, a low concentration is found in those with microcytosis, and a low serum iron concentration is observed regardless of the serum ferritin concentration (*109*). The red cell ferritin concentration does not therefore necessarily indicate the concentration of iron in storage. The red cell ferritin concentration may be useful to differentiate between hereditary haemochromatosis and alcoholic liver disease (*110*) and possibly to distinguish heterozygotes for haemochromatosis from normal subjects (*107*). The mean red cell ferritin content in patients with untreated inherited haemochromatosis was found to be about 70 times normal, and fell during phlebotomy. In some patients the concentration was still high after phlebotomy even when the serum ferritin concentration was within the normal range. This was shown to reflect the concentration of iron in liver parenchymal cells, which was still higher than normal (*110*). Furthermore the ratio of red cell ferritin (ag/cell) to serum ferritin (µg/l) was found to be about 0.5 in hereditary haemochromatosis but only 0.03 in patients with alcoholic

cirrhosis, thus clearly separating the two conditions. There may also be advantages of red cell ferritin over the assay of serum ferritin to estimate iron stores in patients with liver damage because the red cell ferritin concentration should not be greatly influenced by the release of ferritin from damaged liver cells. However, a high concentration of red cell ferritin is also found in individuals with thalassaemia (*111*,*112*), megaloblastic anaemia (*113*) or myelodysplastic syndromes (*108*) presumably indicating a disturbance of erythroid iron metabolism in these conditions.

Despite these specific diagnostic advantages (*114*) an assay for red cell ferritin has seen little routine application. This is because it is necessary to have fresh blood in order to separate the red from white cells, which have a much higher ferritin concentration.

13. Ferritin in urine

Although methods to estimate the concentration of ferritin in urine have been described and urine ferritin concentration is correlated with the concentration in serum (*115*,*116*) the technique has received little attention.

14. Assay of serum ferritin

Methods to prepare ferritin, raise antibodies to ferritin, and immunoassays for serum ferritin have been fully described (*33*) along with a reliable enzyme immunoassay (*117*,*118*). The serum ferritin assay is a routine measurement in most diagnostic laboratories and further discussion is not warranted. Several immunoassays for ferritin have been evaluated for the Medical Devices Agency of the UK (*119*,*120*). However some discussion of possible pitfalls and standardization is justified.

14.1 Samples

In many assays both plasma and serum give the same results but in some cases plasma collected in EDTA gives different values to serum. Samples may be stored at -20 °C or -80 °C for several years. Several rounds of freezing and thawing do not lead to changes in serum ferritin concentration, nevertheless freezing and thawing should be kept to a minimum.

14.2 Pitfalls

There are a number of theoretical and practical problems associated with the assay of serum ferritin. In theory, there may be problems because ferritin consists of a family of isoferritins which differ in subunit composition and thus in isoelectric point, and it is possible to generate specific antibodies which recognise particular isoferritins (see above). In practice, this has not been a problem because, in general, the ferritin found circulating in the plasma is similar to the L-rich ferritin found in liver or spleen (see above). A more practical concern is the very wide range in ferritin concentration that can be encountered in serum. In hospital patients the ferritin concentration can range from <1 µg/l in some patients with iron deficiency anaemia to in excess of 100 000 µg/l in patients with necrosis of the liver. The early two-site immunoradiometric assays suffered from a problem called the “high-dose hook” effect. In this situation a very high ferritin concentration could give readings in the lower part of the standard curve. In order to ensure that results were not artefactually low due to

the high dose hook effect, it is necessary to do the assay at two dilutions and to show that the greater dilution reduced the apparent ferritin concentration.

Interference by non-ferritin proteins in serum may occur with any method, but particularly with labelled antibody assays. Serum proteins may inhibit the binding of ferritin to the solid phase when compared with the degree of binding in buffer solution alone. Such an effect may be avoided by diluting the standards in a buffer containing a suitable serum, or by diluting serum samples as much as possible. For example, for two-site immunoradiometric assays, the sample may be diluted 20 times with buffer while the standards are prepared in 5% normal rabbit serum (if antibodies have been raised in rabbits) in buffer. Further dilution of the sera is then carried out with this solution. Some methods give low recoveries of ferritin from plasma collected in EDTA, and the use of the plasma samples should therefore be investigated carefully.

Another problem has occurred because antibodies to some animal proteins are sometimes present in human serum. These can interfere with the assay of serum ferritin, giving a spuriously high ferritin concentration (*121*).

14.3 Standardization

The serum ferritin assay is often used in epidemiological surveys in which the iron status of populations is examined. For such an assay to be comparable between surveys it is important that a common standard is used and that the assays are calibrated against this. The first WHO standard for the assay of serum ferritin was introduced in 1990 (reagent 80/602), and was replaced by the second international standard in 1993 (reagent 80/578). A third international standard, which is a recombinant ferritin, is now in use (reagent 94/572). The introduction of an international standard has led to considerable improvements in the standardization of the assay for ferritin (*11,122*) but differences in reference ranges were still being reported (*123*) and there are problems with comparisons during longitudinal studies (see later). Almost all commercial assays in current use have been calibrated against either the first or second international standard, although these have not been available for some years.

The United Kingdom National External Quality Assessment Scheme (UK NEQAS) has recently investigated the recovery of the third international standard for assay systems used by UK participating laboratories. In most cases recoveries were acceptable and ranged from 90 to 110%. However in two cases recoveries were 69% and 157%. Despite this, the performance of the assays of ferritin in serum samples in the NEQAS haematinics scheme was considered to be acceptable (*124*). The authors concluded that these results call into question the suitability of the third international standard for calibration. However, another concern is that none of the assays appeared to have been calibrated against the current standard which has been used for the last ten years.

Discrepancies remain in the definition of normal ranges of serum ferritin concentration. Some manufacturers define a normal range as the ferritin concentration found in unselected, apparently normal subjects. However, a proportion of the normal population have almost no storage iron without being anaemic, particularly young women, and a smaller proportion will be anaemic. The “normal range” in young females will thus include ferritin concentrations found in iron deficiency. This confusion between “normality” and iron deficiency causes difficulties in the interpretation of ferritin concentrations.

Evaluation of several commercial assay systems suggests that most are free from problems of protein interference or high dose "hook effects" and are well standardized (*119*), but these points should be investigated before adopting a system for routine use.

15. Methodological and biological variability of measures of iron status

Assays of blood for indicators of iron status vary greatly in both methodological and biological stability. Haemoglobin concentrations are stable and a simple and well-standardized method ensures a relatively low day to day variation in individuals (Table 3). Automated cell counters can analyse at least 10 000 cells and thus reduce errors. The more complicated procedures involved in immunoassays lead to a greater variation in ferritin assays, with a coefficient of variation of at least 5%. This variation, coupled with some physiological variation, gives an overall coefficient of variation 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 (*125*). There is no information on seasonal factors influencing most of these analyses, although seasonal change in red cell parameters have been reported (*126*).

The effect of menstruation on indicators of iron status was examined in 1712 women aged 18-44 years during the Second National Health and Nutrition Examination Survey (NHANES II) after adjusting for potential confounders. Adjusted mean values of haemoglobin (Hb), transferrin saturation (TS), and serum ferritin (SF) concentration were lowest for women whose blood was drawn during their menses and highest for women examined in the luteal or late luteal phase of their menstrual cycle (Hb = 130 vs 133 g/l; TS = 21.2% vs 24.8%, $P < 0.01$ for both; and SF = 17.2 vs 24.0 µg/l, $P < 0.05$). The prevalence estimate of impaired iron status was significantly higher for women whose blood was drawn during the menstrual phase than for women whose blood was drawn during the luteal and late luteal phases. The authors concluded that cyclical variations in indicators of iron status are a potential source of error when iron status is assessed in surveys of large populations that include women of reproductive age (*127*).

Results from a number of studies of overall variation are shown in Table 3, but it should be noted that the type of blood sample, the length of study period, and the statistical analysis performed, vary from study to study. The somewhat higher variability in the haemoglobin and ferritin concentration reported by Borel et al. (*128*) may be due to their use of capillary blood and plasma. Pootrakul et al. (*129*) have demonstrated that the mean plasma ferritin concentration is slightly higher in capillary blood specimens than in venous specimens and that the variation within and between samples was approximately three times greater. Variability was less in capillary serum but still greater than venous serum. However the increased variability of capillary samples may be related to the blood sampling technique as Cooper and Zlotkin (*130*) found little difference in variability between venous and capillary samples.

Starvation or even fasting for a short period can cause an increase in the serum ferritin concentration (*131*) while a vitamin C deficiency may reduce the ferritin concentration (*70*). Moderate exercise has little effect on serum ferritin concentration

(*132*) although exhaustive exercise leads to an increase in serum ferritin concentration due to muscle damage and inflammatory reactions (*133,134*).

These variations have clear implications for the use of these assays in population surveys (*3,135*) or in the assessment of patients (*128*). For accurate diagnosis either a multi-parameter analysis is required or the assay of several samples (see below).

TABLE 3
The coefficient of variation (%) reported in assays of iron status (within-subject, day-to-day variation in otherwise healthy subjects)

Haemoglobin	Serum ferritin	Serum iron	Total iron binding capacity	Zinc protoporphyrin	Serum transferrin receptor	Reference
–	15 (MF)	–	–			Dawkins et al. (*125*)
2 (F)	15 (MF)	–	–			Gallagher et al. (*136*)
–	–	29(F)	–			Statland and Winkel (*137*)
–	–	27 (M)	–			Statland et al. (*138*)
3 (MF)	–	–	–			Statland et al. (*139*)
–	15 (MF)	29 (MF)	–			Pilon et al. (*140*)
–	13 (MF)[a]	33 (MF)[a]	11 (MF)[a]			Romslo and Talstad (*141*)
4 (MF)	14 (M) 26 (F)	27 (M) 28 (F)	– –			Borel et al. (*128*)
–	27 (MF)	29 (MF)	7 (MF)		14 (MF)	Maes et al. (*142*)
– –	26 (F)[b] 15 (M)[b] 28 (F)[c] 12 (M)[c]				14 (F) 12 (M) 11(F) 10 (M)	Cooper and Zlotkin (*130*)
–	–	–	–	5 (MF)	–	Hastka et al. (*143*)
3 (F)[d]	11 (F)[d]	26 (F)[d]	4 (F)[d]		13 (F)[d]	Ahluwalia (*144*)

M, males only; F, females only; MF, males and females
[a] Anaemic patients
[b] Venous blood
[c] Capillary blood
[d] 70–79 years old healthy women

16. The predictive value of indicators of iron metabolism

Despite years of investigation there is little reliable comparative information on indicators that will distinguish reliably between the presence and absence of storage iron. Most investigators have used the grade of stainable iron in the bone marrow as a "gold standard". This involves an invasive procedure and so limits greatly the number of patients that can be investigated. It is often difficult to justify bone marrow aspiration to determine the iron status of a patient, and it is even more difficult in the case of normal volunteers. Furthermore, the examination of a stained bone marrow aspirate is not a reproducible procedure as there can be observer error (*145*) while inadequate specimens and a lack of correlation with response to iron therapy have been described (*146*). An alternative is to demonstrate a change in haemoglobin concentration in response to oral iron therapy, and this has been the method of choice in paediatric practice.

16.1 Iron deficiency anaemia in adults

Iron deficiency anaemia (IDA) in adults occurs typically due to a gradual decline in the iron content of the body due to a loss of haemoglobin and a depletion of iron stores. In the absence of malabsorption there is a good response to iron therapy. Menstrual blood loss is the most common cause of IDA in pre-menopausal women, but blood loss from the gastro-intestinal tract or malabsorption of iron are common causes in men and post-menopausal women. People with asymptomatic colonic or gastric carcinoma may present with IDA and it is essential to exclude these conditions as the cause of anaemia in elderly men and women (*147*).

Early studies of patients with iron deficiency anaemia characterised by microcytic anaemia, low serum iron concentration, high total iron binding capacity (TIBC), and either an absence of stainable iron in the bone marrow or who had a subsequent response to therapeutic iron, showed that serum ferritin concentrations were less than 12–16 µg/l (1). Hallberg et al. (*148*) determined the serum ferritin concentration of 203 women aged 38 years who had undergone bone marrow examination. They concluded that a value of <15 µg/l was the best predictor of iron deficiency (confirmed by an absence of stainable iron in the bone marrow) and noted that this threshold was similar to one derived from earlier population surveys and studies of clinical cases (*148*).

Almost all measures currently used to assess iron status show a high sensitivity and specificity in distinguishing between subjects with iron deficiency and those with iron stores and a normal haemoglobin concentration, but only in the absence of any other disease process. Zanella et al. (*149*) examined the sensitivity and predictive value of serum ferritin and zinc protoporphyrin (ZPP) concentrations to identify iron deficiency. Iron deficiency anaemia was defined as a haemoglobin concentration below 13.5 g/dl for men and 11.8 g/dl for women, a transferrin saturation below 16%, and an increase in haemoglobin concentration after oral iron therapy (*149*). The subjects with iron deficiency but without anaemia were blood donors whose haematological profile over time indicated possible iron deficiency and whose haemoglobin concentration improved in response to iron supplementation. The overall sensitivity and specificity of diagnosis were 82% and 95% for serum ferritin and 61% and 95% for ZPP. However while the sensitivity was over 90% for both ferritin and ZPP in cases of severe anaemia, in the absence of anaemia the sensitivity dropped to 70% for ferritin and less than 50% for ZPP. In a systematic review of the diagnostic value of various laboratory tests to diagnose iron deficiency it was concluded that serum ferritin was the most powerful test for simple iron deficiency in both populations and hospital patients. However this analysis did not include the transferrin receptor (*150*).

16.2 Detection of iron deficiency in acute or chronic disease

In practice there are two different questions: first, identifying patients with inflammation, infection, malignancy and renal failure and an absence of storage iron in reticuloendothelial cells; and second, identifying a functional iron deficiency in which there is an inadequate iron supply to the bone marrow in the presence of storage iron in reticuloendothelial cells. This problem is usually encountered among patients with renal failure receiving erythropoietin to correct anaemia.

Table 4 summarises a number of studies in which bone marrow iron has been determined and the sensitivity and specificity of various assays to determine iron status have been compared. Despite varying results between studies, some general points may be made.

TABLE 4

The sensitivity/specificity of methods to diagnose iron deficiency in the presence of chronic disease[a]

	Reference											
	Van Tellingen et al. (*156*)	Lee et al. (*157*)[b]	Mast et al. (*158*)	Kotru et al. (*152*)	Punnonen et al.(*159*)[b]	Baumann Kurer et al. (*160*)	Bultink et al. (*161*)	Means et al. (*162*)	Joosten et al. (*163*)	Balaban et al. (*164*)	Mast et al. (*165*)	Fernandez-Rodriguez et al. (*166*)
Test												
Mean cell volume	–	–	0.32/0.93	L	0.86	–	–	0.42/0.83	–	–	–	–
Mean cell Haemoglobin	–	–	–	–	–	0.71/0.71	–	–	–	–	–	–
% hypo	–	–	–	–	–	0.77/0.90	–	–	–	–	–	–
Reticulocyte haemoglobin concentration	–	–	0.73/0.74	–	–	–	–	–	–	–	–	–
Serum iron concentration	–	–	–	L	0.68	L	–	–	NS	–	–	–
Total iron binding capacity	–	–	–	L	0.84[c]	L	–	–	–	–	–	< 0.65[b]
Percentage saturation	–	–	0.65/0.70	L	0.79[d]	L	–	0.38–0.89	–	–	–	< 0.65[b,c]
Serum ferritin concentration	0.79/0.97	0.870	0.52/0.93	90/75	0.89	0.86/0.90	1.00/0.81	0.25/0.99	0.94/0.95	0.60/0.90	0.92/0.98	0.83b (0.75/0.75)
Red cell ferritin concentration	–	–	–	L	–	–	–	–	–	0.82/0.83	–	0.68[b]
Zinc protoporphyrin	0.74/0.94		–	–	–	L	–	–	–	–	–	–
Serum transferrin receptor concentration	0.63/0.81	0.704	–	–	0.98	L	1.00/0.84	0.71/0.74	0.61/0.68	–	0.92/0.84	0.69[b]
Serum transferrin receptor concentration to log ferritin concentration	0.74/0.97	0.865	–	–	1.00	–	1.00/0.97	0.67/0.93	–	–	–	–

L, Lower sensitivity/specificity than serum ferritin, individually or in combination. The combination of ferritin and erythrocyte sedimentation rate or C-reactive protein did not improve efficiency.

[a] Adults, iron stores determined by staining for iron in bone marrow). Optimum diagnostic thresholds selected vary.

[b] Area under Receiver operating characteristics (ROC) curve

[c] Transferrin concentration (equivalent to TIBC, see text)

[d] Transferrin Index (equivalent to % saturation, see text)

Conventional red cell parameters such as the haemoglobin concentration, mean cell volume (MCV), mean cell haemoglobin (MCH), and reticulocyte counts do not distinguish between the presence or absence of bone marrow iron in patients with chronic disease.The serum iron concentration is almost invariably low in patients with chronic disease and although the TIBC (or transferrin concentration) is higher in patients with no storage iron, neither this measurement nor the transferrin saturation derived from the serum iron and TIBC, provide useful means of identifying patients with iron deficiency. In patients with chronic disease the serum ferritin concentration reflects the concentration of storage iron, but the concentration of ferritin is higher than in healthy subjects. For this reason it is necessary to set a threshold of 30–50 µg/l of ferritin in order to distinguish between the presence and absence of storage iron. Even when this threshold is applied the sensitivity of diagnosis is low.

Combinations of serum ferritin, ESR or CRP either in a discriminant analysis (*151*) or logistic regression (*152*) provide only marginal improvement in the ability to detect a lack of storage iron.

The serum transferrin receptor concentration is usually raised in patients with chronic disease and an absence of storage iron in the bone marrow although there is disagreement as to whether or not the assay is superior in predictive power than the serum ferritin concentration. Several studies show that the ratio of sTfR/$\log_{10}$ SF is superior to either test on its own. The use of the log of serum ferritin in this ratio decreases the influence of the acute phase response on the ferritin component of the ratio. Although the $\log_{10}$ (sTfR/SF) ratio is an excellent measure of iron stores in healthy subjects (*153*) its use may not be not appropriate for clinical diagnosis of iron deficiency. When the assay of sTfR is easily done on an immunoanalyser the sTfR/log SF ratio may provide the best means of identifying the co-occurrence of iron deficiency in chronic disease. However if this ratio is to gain wide acceptance, this will also require the standardization of units and ranges for the various sTfR assays.

Measurements of the percentage of hypochromic erythrocytes or the reticulocyte haemoglobin concentration also provide some power to identify people with iron deficiency, but their sensitivity and specificity are not sufficiently high enough to distinguish between the presence and absence of storage iron in acute or chronic disease.

Patients with a functional iron deficiency will require parenteral iron therapy in order to respond to erythropoietin with an acceptable rise in haemoglobin concentration. The percentage of hypochromic erythrocytes has been shown to be a good predictor of a response (*154*) and Table 5). Fishbane et al. (*155*) concluded that the reticulocyte haemoglobin concentration (CHr) was a markedly more stable analyte than serum ferritin or transferrin saturation, and that it predicted functional iron deficiency more efficiently. Fishbane et al. (*155*) did not include the percentage of hypochromic cells in their analysis.

16.3 Iron deficiency in infancy and childhood

Diagnostic thresholds for iron deficiency and iron deficiency anaemia in infants are not universally agreed upon. There are rapid changes in iron status in the first year of life as the fetal haemoglobin is replaced by haemoglobin A. The serum ferritin concentration is a less useful guide to iron deficiency than in adults partly because of the changes in concentration that continue for the first 18 months (*167*) and the low concentrations generally found in children. In a study of healthy, breastfed infants

TABLE 5
The threshold of assays used to predict a response to treatment with intravenous iron in haemodialysis patients treated with erythropoietin, and the efficiency of the threshold in predicting a response[a]

Assay	Threshold	Efficiency (%)[b]
Hypochromic erythrocytes	>6%	89.6
Reticulocyte haemoglobin concentration	≤29 pg	78.4
Soluble transferrin receptor concentration	>1.5 mg/l	72.4
Erythrocyte zinc protoporphyrin concentration	>52 µmol/mol haem	73.0
Transferrin saturation	19%	70.4
Serum ferritin concentration	<50 µg/l	64.0

[a] Not all cell counters detect hypochromic red cells but this study indicates that the other tests have insufficient predictive power to justify cost beyond the blood count.
[b] Number of true positive and negative results/total number of tests
Adapted from Tessitore et al. (*146*), with permission of the publisher.

in Honduras and Sweden, Domellof et al. (*168*) evaluated the change in haemoglobin concentration in response to oral iron supplementation. At 4–6 months of age the initial haemoglobin concentration did not predict a response to iron therapy. At 6 months of age the haemoglobin concentration, the MCV and the ZPP concentration predicted the response, but SF and sTfR did not. Table 9 shows the proposed thresholds of ferritin concentration to diagnose iron deficiency in infants. In some studies the ferritin concentration has been assayed in addition to MCV, zinc protoporphyrin, transferrin saturation or serum transferrin receptor, in order to apply a multiple parameter approach to diagnosing iron deficiency and iron deficiency anaemia (see for example (*169*,*170*).

Margolis et al. (*171*) found that, in children age 6 months to 17 years, the best predictor of a response was the initial haemoglobin concentration, although the sensitivity of this parameter was only 66% and the specificity 60%. The concentration of SF, TS and erythrocyte protoporphyrin had even lower efficiencies, while a combination of the various measures made little improvement. Hershko et al. (*172*) studied children aged 1–6 years from villages in the Golan Heights and concluded that erythrocyte protoporphyrin 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. The concentration of ZPP provides a useful indicator of iron-deficient erythropoiesis although high values may indicate lead poisoning rather than iron deficiency. The small volume of blood needed to measure ZPP is also an advantage in paediatric practice.

A recent report confirms the effect of low level infection on measures of iron status. Abraham et al. (*64*) studied 101 healthy, 11 month old infants. On the morning of blood sampling, slight clinical signs of an respiratory tract infection were observed in 42 infants. Extensive blood analyses were done, including a highly sensitive assay for CRP. While the concentration of CRP measured using routine methods gave values of <6 mg/l for all infants, the highly sensitive assay gave values that were higher for many of the infants with symptoms of infection. The serum iron concentration was low in these children and was significantly correlated with the CRP concentration. In those children for whom a second blood sample was taken, the serum ferritin concentration was higher in the sample with the higher CRP concentration and the serum iron concentration was reduced, but the sTfR and transferrin concentrations were unchanged.

16.4 Treatment of iron deficiency anaemia

Oral iron therapy given at conventional doses of 60 mg of iron, 3 times daily, has little immediate effect on the serum ferritin concentration, which rises slowly as the haemoglobin concentration increases. However with double doses there is a rapid rise in serum ferritin concentration in a few days to within the normal range, an increase that probably does not represent an increase in storage iron (*173*). Intravenous iron causes a rapid rise in serum ferritin concentration to a concentration that may be above the normal range, but it gradually drops back to normal (*174*).

16.5 Screening blood donors for iron deficiency

A number of studies have shown that regular blood donation reduces storage iron levels (see reviews by Skikne et al. (*175*) and Milman and Kirchhoff (*37*)). The conventional screening test for anaemia, the "copper sulphate" test, lacks specificity so that donors may be deferred unnecessarily. Despite the availability of the serum ferritin assay for 30 years there has been little attention to the fundamental relationship between storage iron levels and the ability to donate blood. Screening blood donors by routinely assaying serum ferritin may make it possible to predict the development of iron deficiency anaemia (37) and may identify donors with high iron stores who may give blood more frequently than is usually permitted. However the assay has low predictive power to identify donors who are homozygous for HFE gene C282Y (see below).

16.6 Pregnancy

In early pregnancy the serum ferritin concentration usually provides a reliable indication of the presence of iron deficiency. Haemodilution in the second and third trimesters of pregnancy reduces the concentration of all measures of iron status and means that the threshold values for iron deficiency established in non-pregnant women are not appropriate. In principle the calculation of values as ratios, such as ZPP µmol/mol haem, transferrin saturation or sTfR/ferritin, should be more diagnostically reliable. In a study of healthy, non-anaemic women supplemented with iron (176), the serum iron, TS and SF concentrations fell from the first to the third trimester and increased after delivery while TIBC increased during pregnancy and fell after delivery. The sTfR concentration showed a substantial increase of about 2-fold during pregnancy, a change that probably reflects increased erythropoiesis (*176*). In contrast, Carriaga et al. (*177*) reported that the mean sTfR concentration of pregnant women in their third trimester did not differ from that in non-pregnant women, and that the sTfR concentration was not influenced by pregnancy per se. Choi et al. (*176*) suggest that the use of different assays and the different ages of subjects in the control groups may explain this discrepancy. In practice the Institute of Medicine (USA) has recommended that iron deficiency in the first and second trimester can be identified by a haemoglobin concentration <110 g/l and a ferritin concentration <20 µg/l (*178*). Women with low iron stores only should receive lower doses of oral iron than those who are also iron deficient and anaemic. In the third trimester all women should receive iron supplements. Other US guidelines recommend supplementation for all women throughout pregnancy (*179,180*).

16.7 Genetic haemochromatosis

Iron overload in haemochromatosis begins with enhanced iron absorption leading to an increase in plasma iron concentration and transferrin saturation followed by an increasing iron concentration in liver parenchymal cells. As the serum ferritin concentration reflects the iron concentration of macrophages (*10*), and macrophages accumulate little iron initially, the serum ferritin concentration might be expected to remain normal during the early stages of iron accumulation. The transferrin saturation is therefore the most efficient test for detecting iron accumulation in genetic haemochromatosis (*181*). The use of serum ferritin to detect genetic haemochromatosis in populations is discussed later.

16.8 Secondary iron overload

The major aims of diagnostic tests are to estimate the degree of iron overload and to monitor the success of chelation therapy in removing iron from the body. The only reliable methods are the quantification of the amount of iron in the liver using biopsy samples, an invasive procedure, or by measuring magnetic susceptibility (*182*) which can only be done in three countries. In practice, the serum ferritin concentration provides a combined index of storage iron concentration and liver damage, and gives useful information to monitor the progress of treatment. The aim of treatment with chelating agents is to reduce both the tissue iron concentration and tissue damage. The reduction of the concentration of ferritin in serum below 1 000 µg/l is a realistic aim. This topic has recently been reviewed (*183*).

17. Population studies

17.1 Distribution of serum ferritin concentration in people in the United States of America and Europe

The first studies of serum ferritin concentrations in normal subjects including neonates, infants, children and adults, were drawn from populations in the USA, Canada, UK and Denmark (*1*). The sample sizes were usually relatively small and criteria to exclude people with iron deficiency or chronic disease etc. were not usually applied. The distribution of values has been summarized in several ways including the mean and standard deviation, although the typically right-skewed distributions of serum ferritin means that such parameters are inappropriate. Other ways of presenting the distribution include the median and range, median, 5th and 95th percentiles and geometric means. Much larger sample sizes were made available from the National Health and Nutrition Examination Surveys (NHANES) II and III. These surveys were designed to assess the health and nutritional status of the civilian, non-institutionalised population of the USA.

17.1.1 Age, sex and race

Figure 2 shows the distribution of the serum ferritin concentration and transferrin saturation of 20 040 men and women aged 17 to over 90 years using data from NHANES III (*184*). The analysis was limited to white (n = 8477), black (n = 5484) and Hispanic (n = 5304) subjects as only 775 people were of 'other' races. The values shown are the geometric means for each age group. The minimum number of unweighted observations in each 10 year age category (except for 17–19 years) was 30

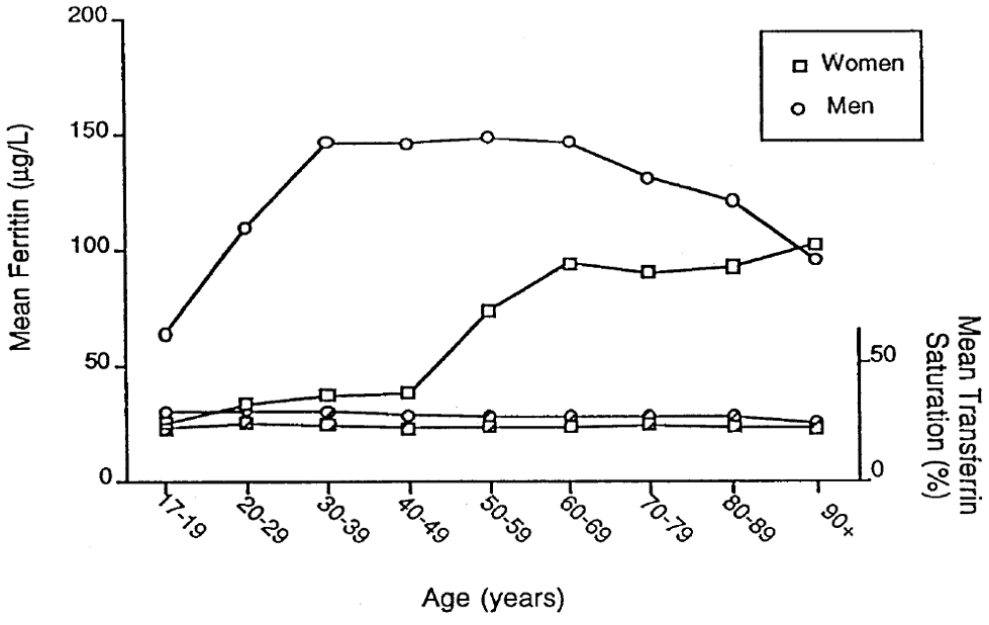
and analyses were weighted to adjust for the probability of selection due to sample design and non-response.

Figure 3 shows the distribution of the serum ferritin concentration by age and race. The geometric mean ferritin concentration in men was always higher in blacks than in whites or Hispanics. In women, the values for blacks were higher than the other groups after the menopause. It is unlikely that the higher mean values of serum ferritin concentration in US blacks are due to a greater intake of dietary iron. Popkin et al. (*185*) and Zacharski et al. (*184*) suggested that these high ferritin concentrations may indicate that higher iron stores contribute to the higher mortality and morbidity rates among blacks when compared with whites for many common diseases. However, it may be that higher morbidity is the cause of the higher ferritin concentrations later in life.

An alternative approach to examining the distribution of the serum ferritin concentration in a population was described by Custer at al. (*47*). Data were collected on over 964 000 patients unselected by race and medical condition. Over 98% of samples came from physicians' offices or blood-drawing stations. Information included results on 29 tests as well as age and sex. They analysed the distribution of serum ferritin according to age and sex for all subjects and then for a subgroup of 22 464 males and 37 450 females who had normal values for the other 28 assays. The concentration of ferritin was lower in the normal subgroup than in all subjects combined. Table 6 shows the 2.5th, 50th and 97.5th percentiles of the ferritin concentration for the subgroup of normal males and females.

FIGURE 2

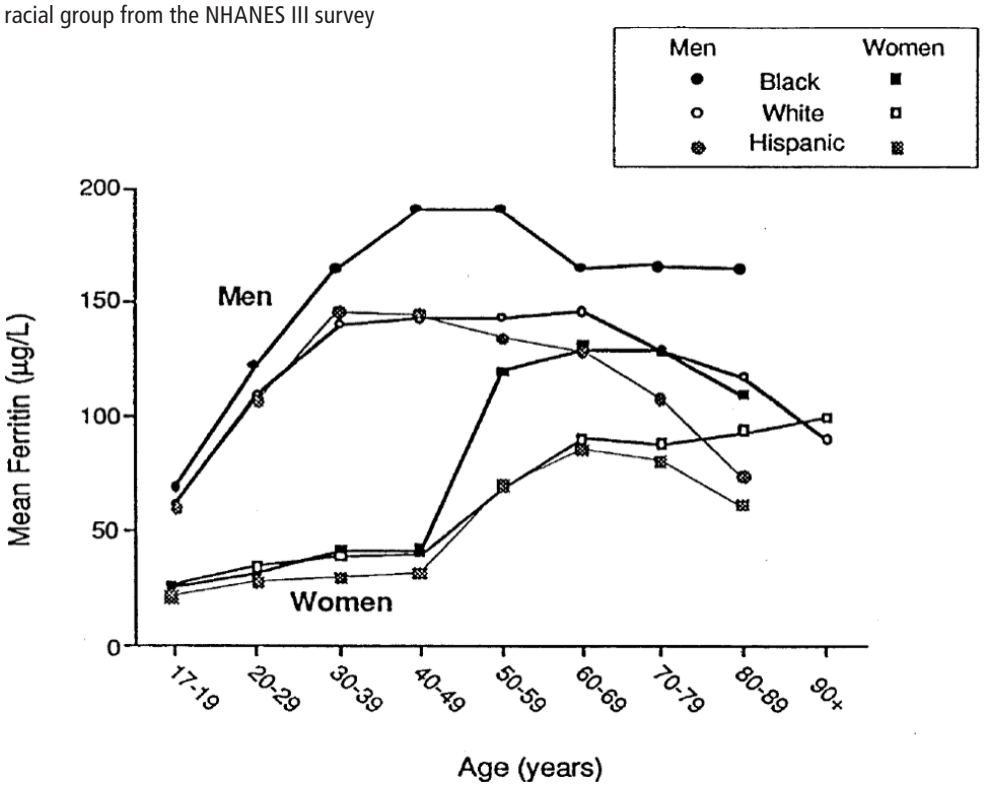
The distribution of mean serum ferritin concentration (left y-axis) and transferrin saturation (right y-axis) by age group for American men and women participating in NHANES III surveys



Adapted from Zacharski et al. (*184*), with permission of the publisher.

FIGURE 3

Distribution of mean serum ferritin concentration by age group for American men and women by age and racial group from the NHANES III survey



Adapted from Zacharski et al. (*184*), with permission of the publisher.

TABLE 6

The values of serum ferritin concentration (µg/l) by percentiles in males and females with normal laboratory test results for indicators of iron deficiency (other than ferritin)

Age groups (years)	Males				Females			
	Number	2.5%	50%	97.5%	Number	2.5%	50%	97.5%
12–<16	129	11	36	117	563	7	24	110
16–<20	616	17	56	171	1 143	6	27	100
20–<24	720	20	90	259	1 711	8	32	128
24–<28	1 085	25	105	311	2 175	7	35	140
28–<32	1 670	26	117	343	2 599	7	39	174
32–<36	1 508	23	123	385	3 007	7	38	174
36–<40	1 581	24	127	399	3 085	6	38	190
40–<44	1 734	19	122	427	3 241	7	38	193
44–<48	1 734	19	127	450	3 013	7	37	216
48–<52	1 657	19	128	481	2 457	7	41	232
52–<56	1 550	19	121	477	2 101	10	49	264
56–<60	1 463	20	127	486	1 826	13	60	308
60–<64	1 432	17	122	441	1 896	13	75	346
64–<68	1 541	14	120	488	1 873	13	80	369
68–<72	1 451	14	113	522	1 947	12	81	379
72–<76	1 148	15	111	485	1 718	12	78	381
76–<80	813	14	98	441	1 310	11	79	382
80–<90	766	16	107	474	1 523	13	76	369
Total	22 338				37 188			

Adapted from Custer et al. (*47*), with permission of the publisher.

17.1.2 Genes modifying iron status

Mutations of the HFE gene are common in Northern European populations (10–20% carrying C282Y and 20–30% H63D). From 0.25–1 % of the population will be homozygous for HFE C282Y and are at risk from iron overload (*186*). About 2 % will be compound heterozygotes (C282Y/H63D) and are at lesser risk. However, although most subjects who are homozygous for C282Y will accumulate excess iron the clinical penetrance is low and for compound heterozygotes is even lower. In population surveys, slightly but significantly higher values for serum iron and transferrin saturation have been found in heterozygotes for either C282Y (*187–190*) or H63D (*187,189,190*) with subjects lacking these mutations. The differences in ferritin levels were smaller and not significant except that Jackson et al. (*190*) found higher levels of serum ferritin in men heterozygous for C282Y. In compound heterozygotes and subjects homozygous for H63D there are greater differences (*189–191*). In heterozygotes for C282Y (*188,189*) and H63D (*189*) Hb levels were slightly higher than in subjects lacking mutations. Beutler et al. (*189*) noted a lower prevalence of anaemia among women carrying either mutation, but the differences were small and only significant if all subjects carrying mutations were compared with those lacking mutations. Serum ferritin concentrations in haemochromatosis may not accurately reflect tissue iron concentrations during the early stages of iron accumulation, particularly in heterozygotes. Serum ferritin concentrations are related to the levels of ferritin iron in macrophages and in haemochromatosis the iron initially accumulates in hepatic parenchymal cells. Edwards et al. (*192*) noted that liver iron concentrations in heterozygote family members were above the reference range although serum ferritin concentrations were not elevated. Thus iron accumulation may be underestimated in heterozygotes. Tables 7 and 8 illustrate population data for blood donors from South Wales.

Rare causes of genetic haemochromatosis include mutations in the HAMP (hepcidin) gene (*193,194*), transferrin receptor 2 (*195*), ferroportin 1 (*196–198*) and HFE2 (*199*) genes. Other genes may modify the phenotype involved in haemochromatosis. It was found by Langlois et al. (*200*) that men with the 2-2 haplotype for haptoglobin had a higher serum ferritin concentration than those with HP 1-1 or 2-1 but others have not confirmed this finding (*201–203*). Until now, no mutations in genes coding for iron transport or storage have been linked to iron deficiency.

TABLE 7
The mean (SD) of indicators of iron status for different genotypes among male blood donors

Genotype[a] Number of donors (%)	Serum iron concentration (µmol/l)	Unsaturated iron binding capacity (µmol/l)	Total iron binding capacity (µmol/l)	Transferrin saturation (%)	Serum ferritin concentration (µg/l)[b]
All donors 4952 (100%)	16.7 (6.4)	39.2 (11.2)	55.8 (10.6)	30.0 (12.1)	81 (24–196)
HHCC 2896 (58.5%)	16.0 (6.1)	40.8 (11.0)	56.8 (10.6)	28.3 (11.0)	79 (23–193)
HDCC 1167 (23.6%)	17.4 (6.5)***	38.2 (10.5)***	55.6 (10.4)**	31.8 (11.7)***	81 (23–197)
HHCY 644 (13.0%)	17.3 (6.0)***	35.8 (10.0)***	53.1 (10.0)*	33.1 (11.5)***	82 (29–201)*
DDCC 105 (2.1%)	19.1 (6.9)***	34.4 (10.5)***	53.5 (10.1)**	36.4 (13.1)***	99 (33–210)***
HDCY 111 (2.2%)	20.2 (6.9)***	31.2 (12.1)***	51.4 (11.6)***	40.5 (15.5)***	107 (24–260) ***
HHYY 29 (0.59%)	27.5 (9.9)***	15.6 (8.4)***	43.1 (8.3)***	63.6 (17.8)***	154 (50–410)***

Significance of difference from wild-type donors * $p < 0.01$, ** $p < 0.001$, *** $p < 0.0001$ (Mann-Whitney test for significance)
[a] HHCC, wild-type; HHYY, homozygous C282Y; DDCC etc, homozygous H63D
[b] Median (95% range).
Adapted from Jackson et al. (*190*), with permission of the publisher.

TABLE 8
The mean (SD) of indicators of iron status for different genotypes among female blood donors

Genotype[a] Number of donors (%)	Serum iron concentration (µmol/l)	Unsaturated iron binding capacity (µmol/l)	Total iron binding capacity (µmol/l)	Transferrin saturation (%)	Serum ferritin concentration (µg/l)[b]
All donors 5372 (100%)	14.3 (6.3)	42.9 (12.9)	56.8 (11.2)	25.4 (12.4)	44 (9–130)
HHCC 3100 (57.7%)	13.8 (6.1)	44.2 (12.1)	58.1 (11.4)	24.6 (10.9)	44 (9–126)
HDCC 1277 (23.8%)	14.7 (6.2)**	41.5 (12.2)***	56.2 (10.9)***	27.0 (12.0)***	43 (10–127)
HHCY 682 (12.7%)	14.6 (6.1)*	40.7 (12.5)**	55.1 (11.5)***	27.3 (12.1)***	44 (8–116)
DDCC 135 (2.5%)	15.6 (7.1)**	38.4 (11.8)***	54.0 (11.1)***	29.5 (13.8)***	46.5 (11–140)
HDCY 138 (2.6%)	16.6 (6.8)***	34.2 (10.5)***	50.8 (8.6)***	33.2 (14.0)***	50 (10–180)*
HHYY 41 (0.76%)	21.4 (8.4)**	23.4 (12.6)***	44.3 (10.4)***	49.9 (20.5)***	65 (8–238)**

Significance of difference from wild-type donors * $p < 0.01$, ** $p < 0.001$,*** $p < 0.0001$ (Mann-Whitney test for significance)
[a] HHCC, wild-type; HHYY, homozygous C282Y; DDCC etc, homozygous H63D
[b] Median (95% range)
Adapted from Jackson et al. (*190*), by permission of the publisher.

17.2 Prevalence of iron deficiency

Iron deficiency has generally been defined as an absence of iron stores while iron deficiency anaemia as the same but with a haemoglobin concentration below a defined threshold. The absence of stored iron may simply be defined as a serum ferritin concentration <15 µg/l (see below). However an analysis of NHANES II data revealed that for those subjects with either a low ferritin concentration or transferrin saturation, the prevalence of anaemia was only slightly greater than in those with normal test results. Iron deficiency was therefore defined as two abnormal results out of a panel of three: free erythrocyte protoporphyrin concentration, serum ferritin concentration, and transferrin saturation (*42*). Not surprisingly the results produced by the two diagnostic approaches were very different. Hallberg et al. (*148*) estimated the prevalence of iron deficiency among women using a serum ferritin concentration of <15 µg/l to be about 30% in the USA (NHANES II) and 33% in Sweden. Using the results of "two of three tests abnormal" approach on data from NHANES II and III, about 10% of young women were iron deficient but only 2–5% had iron deficiency anaemia (*3*).

These different diagnostic approaches may not entirely explain the very different prevalences of iron deficiency reported in Sweden and the USA. Among pre-menopausal women surveyed in Denmark in both 1984 and 1994 approximately 11% were iron deficient (defined as a serum ferritin concentration <16 µg/l) while 2.7% had iron deficiency anaemia – prevalences very much closer to those in the USA than for Sweden. Throughout Europe between 11 and 45 % of menstruating women were reported to have a serum ferritin concentration below thresholds varying from 10 to 17 µg/l (*204*).

17.3 Longitudinal and intervention studies

For the last 30 years, serum ferritin has been an important tool for investigating changes in storage iron concentration. However attempts to measure the changes in iron stores over several years or a decade have been bedevilled by changes in assay methods, by changes in survey procedures (*205*), or changes in other confounding factors such as the blood lead concentration (*206,207*). Milman et al. (*207,208*) have compared changes in iron stores and the prevalence of iron deficiency and iron overload in a cohort of Danish men and women studied in 1984 and 1994. In 1987 the fortification of flour with iron was abolished, thereby reducing daily iron intake significantly. Surprisingly perhaps, the prevalence of iron deficiency or iron deficiency anaemia (ferritin <13 µg/l and a haemoglobin concentration <5th percentile) did not increase, but in elderly women and men the prevalence of iron overload (ferritin >300 µg/l) increased. Changes in the ferritin assay procedure required the correction of values to permit valid comparisons to be made. Changes in diet, alcohol consumption and smoking habits were probably responsible for these findings.

17.4 Iron stores in people in developing countries

In many regions where iron deficiency is of great public health importance malaria, hookworm and other parasitic infections are endemic. Whether or not serum ferritin is a valid indicator of iron stores depends on the type and degree of infection. Where malaria is "holo-endemic" serum ferritin appears to be little affected by parasite load (209). However in malarial disease a high ferritin concentration results from the destruction of red blood cells, an acute phase response, suppressed erythropoiesis, and ferritin released from damaged liver or spleen cells (*210*). In adults with hookworm infection both the haemoglobin and serum ferritin concentrations are inversely correlated with the intensity of infection (*211*). These authors also found that serum ferritin was a valuable indicator of iron stores in populations infected with the helminths *Ascaris lumbricoides*, *Trichuris trichiura* and *Schistosoma mansoni*. To assess the value of iron status indicators in a population in which malnutrition, *Plasmodium falciparum* malaria and helminths were highly endemic, Stoltzfus et al. (*212*) examined the relationship between iron status indicators and erythropoiesis. The concentration of serum ferritin, erythrocyte protoporphyrin and sTfR were all significantly influenced by *Plasmodium* spp. infection, and the concentration of erythrocyte protoporphyrin and serum ferritin were also influenced by fever. The authors concluded that it would be "nearly impossible" to estimate the prevalence of iron deficiency in this population, except by a trial of therapeutic iron supplementation. A subsequent trial revealed that oral iron supplementation led to an increased concentration of SF and EP but had only a small effect on anaemia (*213*). The authors suggested that concurrent helminth infections may stimulate inflammatory immune responses in young children, with harmful effects on protein metabolism and erythropoiesis. Thus serum ferritin may be a valuable indicator of iron stores in some populations with chronic infection, but only after determining the infecting organisms and the relationship between the burden of infection and serum ferritin.

17.5 Detection of subjects with genetic haemochromatosis

Despite the first hopes that the serum ferritin assay would provide an effective way of detecting subjects with haemochromatosis early in their disease, it soon became clear that this was not the case. The first indication of iron accumulation is provided by measuring transferrin saturation (*181*) for reasons described above. The sensitivity and specificity of methods to detect nascent haemochromatosis can now be examined by studying subjects found to be homozygous for HFE C282Y, because 90% of patients with genetic haemochromatosis have this genotype in northern European populations (*214*). Beutler et al. (*215*) identified 152 subjects homozygous for C282Y among 41 038 individuals attending a clinic in California. Of these, 75% of men and 54% of women had a ferritin concentration greater than 250 µg/l and 200 µg/l respectively. Similar percentages had a raised transferrin saturation (75% and 40%). The mean age of these individuals was 57 years. In a prior analysis of the first 10 198 subjects (*189*) the sensitivity and specificity of serum ferritin and transferrin saturation to detect people with C282Y homozygosity was examined. Using optimal thresholds of ferritin >200 µg/l for women, ferritin >250 µg/l for men and a transferrin saturation of >45%, the sensitivities were 70% and 70%, and the specificities were 90% and 89% respectively.

In blood donors with a mean age of 38 years, Jackson et al. (*190*) identified 72 subjects who were homozygous for HFE C282Y from a total of 10 500 donors tested. Using

a transferrin saturation threshold of >50%, which was the 95th percentile of the value of first-time donors, the sensitivity of diagnosis was 45% and 86% for females and males respectively with a specificity of 97% and 95% respectively. Using a threshold serum ferritin concentration in women of >130 µg/l (the 95th percentile) the sensitivity of diagnosis was 22% ; for men a threshold of >210 µg/l gave a sensitivity of 34%. In both cases the specificity was high at 98%. The most powerful single diagnostic measure examined, an unsaturated iron binding capacity threshold of <20 µmol/l, showed a positive predictive value of 14% for men and 18% for women, with negative predictive values of >99.5% for both sexes. Although a combination of raised transferring saturation and serum ferritin concentrations gave a positive predictive value of 50% this was at the expense of sensitivity which only reached 22%. Of the 69 homozygotes for C282Y tested for iron status, only 15 had both a raised transferrin saturation and serum ferritin concentration.

18. Threshold values of ferritin to determine iron status

There are two approaches to establishing a threshold value of an indicator at which iron deficiency or iron overload is probable. The first is to identify subjects with iron deficiency or haemochromatosis. A threshold may be established from the range of values found in iron deficient or iron loaded patients. This approach has rarely been applied although the ferritin concentration during iron deficiency anaemia provides an example.

The second approach is to measure the concentration of the substance in healthy subjects not likely to be either iron deficient or iron loaded, and to calculate appropriate threshold values based on either 90 or 95% confidence intervals. This requires selecting subjects in order to exclude those with iron deficiency and possibly iron overload.

Thresholds for iron deficiency will be described for infants, children, adolescents and adults. In the case of iron overload, values are only available for adults.

18.1 Iron deficiency

Thresholds have been identified by examining the highest concentration found in patients with iron deficiency anaemia classified as microcytic, who have either an absence of stainable iron in their bone marrow or show a response to therapeutic iron (*1,148*). The suggested upper limit was 15 µg/l in each case. Table 9 shows thresholds derived from the analysis of iron replete populations. There are rapid changes in storage iron concentrations in the first 6 months of life. The WHO thresholds (4) appear to reflect results from clinical studies rather than an analysis of population distributions. The WHO report suggests that a ferritin concentration of <30 µg/l in children less than 5 years old in the presence of infection indicates depleted iron stores, but there is no consensus about this value in the literature. No threshold was suggested for adults with infection or inflammation.

18.2 Iron overload

The thresholds suggested for a serum ferritin concentration during iron overload have varied widely. WHO (*4*) concluded that thresholds of >200 µg/l for men and >150 µg/l for women were appropriate. In the UK values of >300 µg/l for men and elderly women, and >200 µg/l for young women, have been suggested (*216*). Thresholds

TABLE 9
Suggested thresholds for age groups to classify individuals as iron deficient during epidemiological studies

Age	Serum ferritin concentration (µg/l)	Reference
4 months	<20	Domellof et al. (*168*)[a]
6 months	<9	Domellof et al. (*168*)[a]
9 months	<5	Domellof et al. (*168*)[a]
1–2 years	<10	Looker et al. (*3*)[b]
3–5 years	<10	Looker et al. (*3*)[b]
<5 years	<12	WHO (*4*)
>5 years	<15	WHO (*4*)
6–11 years	<12	Looker et al. (*3*)
12–15 years	<12	Looker et al. (*3*)
<16 years	<12	Looker et al. (*3*)

[a] Data derived from studies of iron replete, breast-fed infants.
[b] Data from NHANES III.

from 200 µg/l (217) to 400 µg/l (*218*) have been applied to men. For pre-menopausal women 200 µg/l has been commonly selected as a threshold although Asberg et al. (*219*) used a value of >100 µg/l. Where good data are available age related thresholds should be applied (*47*).

19. Using ferritin to determine the iron status of populations

There have been two general approaches to assessing iron deficiency in populations. Iron deficiency has been defined as a serum ferritin concentration of <15 µg/l in studies conducted by Hallberg et al. (*148*) and Milman et al. (*207*) in Scandinavia. In the USA the criteria set to classify an iron deficiency in NHANES II and III have been two of three abnormal values for transferrin saturation, serum ferritin concentration and erythrocyte protoporphyrin concentration (*205*).

The first approach may over-estimate the frequency of iron deficiency. This is particularly critical in paediatric practice where the low concentration of serum ferritin is associated with a high coefficient of variation in the assay. The second approach tends to under-estimate the frequency, unless thresholds are carefully selected (e.g. a threshold at the 12th percentile for transferrin saturation instead of the 5th percentile for data from NHANES III).

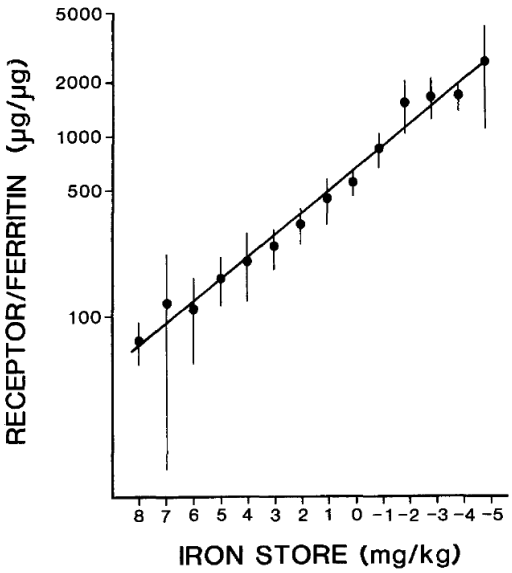
20. Using the ratio of serum transferrin receptor to serum ferritin to measure iron stores

Recently Cook et al. (*153*) have demonstrated that in healthy subjects the concentration of stored iron may be estimated from the ratio of sTfR/SF (reported in µg/l for both assays). The relationship between the sTfR/SF ratio and the concentration of stored iron was estimated in a study in which serial measurements of serum transferrin receptor and serum ferritin were made during repeated phlebotomy of 14 healthy subjects (*220*). There was a close, linear relationship between the logarithm of the sTfR/SF ratio and stored iron expressed as mg per kg body weight (Figure 4). The value of the ratio may be negative, which represents a deficit in iron required to maintain a normal haemoglobin concentration.

The equation of the regression line derived from this study has been applied to three situations: 1) estimating iron stores in the US population; 2) estimating changes in body iron after supplementation in pregnant Jamaican women; and 3) estimating changes in body iron during an iron fortification trial in anaemic Vietnamese women.

The results for people studied in the US population survey (a subset of NHANES III) confirmed a previous analysis (*221*), and the supplementation studies gave results similar to those found with earlier studies using radioactive iron or the "multiple measurement" approach. In the Viet Nam study the efficacy of the fortification strategy was demonstrated by studying only 30 subjects for 3 months. Fortification trials have previously required hundreds of participants and taken years to conduct (see references in (*153*)). The sTfR/SF ratio provided estimates of stored iron for each person in the survey, and their degree of anaemia due to iron deficiency could be calculated from the tissue iron deficit.

FIGURE 4
The variation in the serum transferrin receptor to serum ferritin concentration ratio with body iron. Positive values of body iron represent storage iron and negative values represent iron deficiency. Bars represent the standard error of the mean.



Adapted from Skikne et al. (*220*), with permission of the publisher.

Although a significant advance on previous methods, there are some qualifications: it will be difficult to validate the relationship between sTfR/SF ratio and body iron stores in children and pregnant women, two groups with the highest incidence of iron deficiency, by a similar process of quantitative phlebotomy, although non-invasive methods such as Superconducting Quantum Interference Device (SQUID) (*182*) may be applicable; and the ratio will be influenced by chronic disease because serum ferritin is one component of the ratio. The serum transferrin concentration is less influenced by infection than the ferritin concentration although there is debate about the reliability of measuring sTfR to estimate iron status during chronic disease and in populations where malaria is endemic. At present the only valid approach is to identify subjects with inflammation, infection or chronic disease and remove them from the analysis.

Currently the lack of standardization in assay ranges and units of measurement, and the lack of reference samples, prevents the wider use of the sTfR assay.

21. Recommendations for future surveys of iron status

There are four important principles:

1. Use the best possible approach based on current knowledge. Define anaemia based on the haemoglobin concentration. In principle, the serum ferritin concentration provides an index of the concentration of stored iron, but its use is limited because the ferritin concentration increases during acute and chronic disease. Neither erythrocyte protoporphyrin nor transferrin saturation provide a better index of stored iron concentration than serum ferritin concentration, although

they do identify iron-deficient erythropoiesis, but the value of TS is reduced by high biological variation. Rather than measuring lots of parameters to define iron deficiency, the sTfR/SF ratio should be further evaluated (see below).

2. An important feature of current work is to be able to compare results with previous studies and to detect trends. For the UK and Scandinavia this compatibility may require measurements of haemoglobin and serum ferritin in adults. For comparison with previous studies of infants and children in the USA (NHANES) and other international studies, transferrin saturation and zinc protoporphyrin concentration should be measured.
3. Standardized and reliable assays calibrated against reference material should be used.
4. Markers of inflammation or infection should be included. At the moment the reliable assessment of iron status is not possible in the presence of inflammation or infection, and it is necessary to exclude such subjects. The measurement of the concentration of C-reactive protein (CRP) provides indicator of acute disease whereas other proteins, such as alpha-1-acid glycoprotein (AGP), may provide a marker of chronic infection. An alternative is to measure the erythrocyte sedimentation rate (ESR). However none of these will identify minor infections that may increase the ferritin concentration for long periods (*63*) and a health questionnaire should be completed for each subject to identify possible infection. The use of highly sensitive assays for CRP may be valuable to detect sub-clinical infections. The ideal marker of infection has not been identified but ideally would have a similar response to disease as ferritin, but without being influenced by iron.

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