Serum ferritin

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Introduction
Ferritin provides the primary form of iron storage in the body. It consists of an apoprotein shell (mol. wt. 480,000) which encloses a core of iron in the form of ferric hydroxy-phosphate, which may contain up to 4500 atoms of iron [1]. Ferritin is a soluble protein but is degraded to an insoluble derivative, haemosiderin, which accumulates in lysosomes, and is the 'stainable iron' referred to by pathologists and haematologists. Normally much of storage iron in the body (approx. 1 g in men and less in women) is in ferritin but with increasing iron overload the proportion present as haemosiderin increases.

The ferritin in human tissues contains two types of subunit: H and L [2]. H subunits have a molecular weight of 21,000 and are found in the more acidic isoforms (pI 4.5–5.0) in heart [3, 4], red blood cells [5, 6], lymphocytes and monocytes [7], the HeLa cell [8] and other tissues. L subunits have a molecular weight of 19,000 and predominate in the more basic isoforms (pI 5.3–5.8) of liver, spleen and placenta. Variation in the ratio of H to L subunits explains the charge heterogeneity of ferritin, which is most readily demonstrated by isoelectric focusing [3]. The isoelectric point of ferritin is not significantly affected by its iron content, which varies from tissue to tissue and with the iron content of the tissue. Purified preparations of ferritin usually contain a small proportion of molecules in the form of dimers, trimers etc. [9] but the significance of this polymerization (in terms of haemosiderin formation, for example) is not known. The H rich isoforms are immunologically distinguishable from the L rich forms and polyclonal [10] and monoclonal [11] antibodies which are specific for heart ferritin have been raised. H and L sub-units are only 55% homologous [12] and are coded for by genes on chromosomes 11 and 19q13.3–qter respectively [13, 14]. However, there are multiple gene copies of H sequences [12, 15] and H genes have been found on at least nine different chromosomes [16]. Presumably some of these sequences will be found to be pseudo-genes. Less is known about the L genes in man, although there are multiple gene copies in the rat [17].

The major function of ferritin is clearly that of providing a store of iron which may be used for haem synthesis when required. In vitro, iron uptake requires an oxidizing agent and iron release a reducing agent [1]. This function of iron storage is largely carried out by the L rich isoforms of liver and spleen. However, other biological activities have been postulated: vasopressor activity [18], inhibition of granulocyte-macrophage colony formation in vitro [19], inhibition of lymphocyte transformation in vitro [20]. In addition to soluble tissue ferritins there may be a glycosylated, secreted form (see later) and even membrane bound ferritin [21, 22].

Ferritin in plasma
Ferritin was first detected in plasma by assaying vasodepressor activity with a rat mesoappendix assay [18]. The first direct demonstration of its presence in plasma (serum) was reported by Reissman & Dietrich in 1956 [23]. They precipitated ferritin with rabbit anti-human ferritin and measured the amount of iron in the precipitate. Ferritin was found in serum from patients with hepatic necrosis but was not found in normal sera, or serum from patients with iron overload but without hepatic necrosis. However, after the development of a sensitive immunoradiometric assay for ferritin it was also detected in normal serum [24]. It was shown that ferritin concentrations in normal subjects (15–300 µg/l) were related to the amount of storage iron in the
body [25], that concentrations were low in patients with iron deficiency anaemia (<15 μg/l) and high in patients with iron overload [26]. This relationship with the level of storage iron is the unique contribution of the serum ferritin assay to the work of the clinical laboratory.

The assay is widely used in haematology and clinical chemistry laboratories and many kits (RIA, IRMA or ELISA) are available commercially. The International Committee for Standardisation in Haematology (Expert Panel on Iron) has prepared a reference reagent of human liver ironisation in Haematology (Expert Panel on Iron) which provides a standard for the serum ferritin assay (for further information contact the author). However, before considering the clinical use of the assay I shall summarize what is known about the biochemistry and physiology of plasma ferritin.

Biochemistry and physiology of plasma ferritin

The following comments refer to studies with normal serum (plasma) or serum from patients with iron overload. Immunologically plasma ferritin resembles liver or spleen ferritin. It is detected with antibodies to ferritins rich in L subunits. Only low concentrations of H rich iso-ferritins are usually measurable with antibodies to heart or HeLa cell ferritin (however, see later for a summary of conflicting reports on this topic and for the possible presence of such ferritins in patients with cancer). Plasma ferritin from patients with iron overload has a low iron content (0.02–0.07 μg of Fe/μg of protein [27, 28] compared with >0.2 μg of Fe/μg of protein [4] in the liver and spleen of such patients). On isoelectric focusing native and purified serum ferritin displays a wide range of iso-ferritins including iso-ferritins of similar pI to those found in liver and heart [27, 29], yet it behaves as a relatively basic iso-ferritin on anion exchange chromatography [30, 31]. The reason for this discrepancy seems to be that the micro-heterogeneity of plasma ferritin is due to glycosylation rather than variation in the ratio of H to L subunits. About 60% of ferritin from normal serum binds to the lectin concanavalin A [32], whereas liver, spleen or heart ferritin show little or no binding. On incubation with neuraminidase the acidic iso-ferritins of serum are converted to the more basic iso-ferritins [33], whereas the pI of tissue iso-ferritins is unaffected. Finally it has been shown the ferritin from the plasma of patients with iron overload has an additional subunit (the G subunit), which stains for carbohydrate and has an apparent molecular weight of 23 000 [28].

The origin of plasma ferritin is not certain. The presence of glycosylation suggests secretion of ferritin, possibly from phagocytic cells degrading haemoglobin. When there is tissue damage another mechanism becomes important, direct release of cellular ferritin through damaged cell membranes [34], and in patients with ferritinemia due to liver necrosis very little of the plasma ferritin binds to concanavalin A [32]. However, the differences between plasma and tissue ferritins may also be determined by differential clearance. On injection of 131I-labelled plasma ferritin into normal subjects it was found that labelled ferritin was removed only slowly from the plasma [35]. Radioactivity decreased to 50% after 30 h and the concanavalin A binding ferritin was cleared more slowly than the non-concanavalin A binding fraction. However, when 131I-labelled spleen ferritin was injected clearance was very rapid (T1/2 approx. 9 min), with uptake and degradation of ferritin by the liver [36]. Rapid clearance may be initiated by interaction with another component of plasma. Labelled heart ferritin, and to a lesser extent spleen ferritin, interacted with serum to form a complex which precipitated in the presence of polyethylene glycol, but purified plasma ferritin did not form a precipitate [37]. It is not known how such findings relate to the demonstration of ‘ferritin receptors’ on several cell types, including liver parenchymal cells [38]. It seems that although many iso-ferritins may be released into the plasma the only ones which normally accumulate are L24 molecules and glycosylated molecules which are rich in L subunits but contain little iron. This low iron content may result from the lack of opportunity to acquire iron during secretion. It should be noted that the clearance studies with labelled purified ferritins do not confirm estimates of plasma ferritin turnover derived from measurements of plasma ferritin during exchange transfusions. In premature babies Siimes et al. [39] calculated plasma ferritin half-lives from 3 to 34 min, and in two patients with lysinuric protein intolerance values of 95 and 65 min were calculated [40]. For these patients it was suggested that prolonged survival of ferritin in the plasma resulted from reduced uptake by the liver.

Clinical use of the serum ferritin assay (for a review see [41])

Since the first demonstration of a relationship between serum ferritin concentration and the level of iron stores there have been many subsequent examinations of this relationship [41]. In adults
serum ferritin concentrations are usually between 15 and 300 µg/l. Mean values are lower in women than in men, reflecting their lower iron stores caused by the iron losses of menstruation and childbirth. There are changes during development which also reflect changes in storage iron levels. In cord blood the mean value is about 200 µg/l and concentrations increase in the first few weeks of life when iron stores are increasing due to the destruction of foetal haemoglobin. As the iron is used to form adult haemoglobin serum ferritin concentrations decline to a mean value of 20-30 µg/l which persists through childhood and indicates the low levels of storage iron found during this period of rapid growth. After the menopause mean values in men and women become similar. The relationship between the level of storage iron and serum ferritin concentration has been shown directly by quantitative phlebotomy and examination of stainable iron in the bone marrow and indirectly by the inverse relationship between serum ferritin concentration and iron absorption from a standard dose of radioactive iron. In patients with simple iron deficiency anaemia (a low haemoglobin concentration associated with a low serum iron concentration, raised total iron binding capacity, and/or a response to iron therapy) serum ferritin concentrations are almost always less than 15 µg/l. Serum ferritin concentrations are high in patients with the clinical and pathological features of idiopathic haemochromatosis and are usually in the range 500-5000 µg/l. However, concentrations may be lower in patients presenting early in the course of the disease when the total body iron content may be within the normal range. When screening for the disease (particularly among other members of the family of a patient) it is important to include measurements of serum iron concentration and total iron binding capacity, as a rise in the percentage saturation of transferrin is one of the earliest signs of the disease and often occurs before a significant rise in serum ferritin concentration [42].

In patients with iron overload due to blood transfusion serum ferritin concentrations are high [43-45]. Cazzola et al. [44] found that much of the iron was deposited in parenchymal tissues and concluded that most of the serum ferritin entered the circulation by leakage from the cytosol of iron-loaded liver parenchymal cells. In patients who were ascorbic acid replete there was an excellent relationship between the number of units of blood transfused or liver iron concentration and serum ferritin concentration. In patients who were deficient in ascorbic acid the relationships were much weaker [45]. Studies in guinea-pigs [46] have demonstrated that ascorbic acid deficiency in the animals reduces both serum iron and ferritin concentrations and the same applies in man [45].

In hospital patients anaemia is usually secondary to another disease process, for example infection, inflammation or malignancy. Such patients usually have a low serum iron concentration (as in simple iron deficiency anaemia) but the total iron binding capacity will be either normal or depressed. The serum ferritin concentration is usually elevated, reflecting the raised iron stores in such patients, but a low serum ferritin concentration is a valuable indicator of depleted iron stores. However, some patients with an absence of stainable iron in the bone may have serum ferritin concentrations of up to 100 µg/l instead of < 15 µg/l. There may be a number of reasons: (1) the unreliability of the assessment of stainable iron; (2) the presence of storage iron elsewhere in the body; (3) the direct response of ferritin synthesis to inflammation or infection, which makes it one of the ‘acute phase response’ proteins [47]. In practice ferritin concentrations of <100 µg/l in anaemic patients with chronic disease should be taken to indicate depleted iron stores (i.e. insufficient storage iron for haemoglobin regeneration). Despite these limitations serum ferritin has proved to be a useful way of assessing iron stores in hospital patients, including those with renal disease undergoing treatment by dialysis. In the latter group the serum ferritin assay alerted physicians to the danger of inducing iron overload by over-use of parenteral and oral iron to compensate for iron losses during the dialysis procedure (see, for example, [48-53]).

However, there are other important causes of ferritininaemia, liver damage being the best understood [34]. The high concentration of ferritin in the liver means that cellular damage causes ferritinaemia. If liver damage is suspected the only safe interpretation of the serum ferritin concentration is that a concentration in the normal range indicates that iron stores are not raised [55]. More information is required before an elevated concentration can be ascribed to iron overload (consideration of liver function tests, serum iron concentration or, finally, liver biopsy). Although pathological processes usually cause ferritinaemia there have been a few reports [56, 57] which indicate that normal serum concentrations may be found when excess iron is ‘sequestered’ in unusual sites (i.e. not in liver or bone marrow).

Serum ferritin and cancer

High concentrations of serum ferritin are seen in most patients with pancreatic carcinoma, lung
cancer, hepatoma and neuroblastoma, although in most cases of cancer of the oesophagus, stomach and colon serum ferritin concentrations are within the normal range [41]. Serum ferritin concentrations are usually elevated in patients with breast cancer with metastatic disease, but the assay has proved to be of little value in predicting the development of metastatic disease. Patients with untreated acute leukaemia generally have elevated serum ferritin concentrations but concentrations are lower in patients with chronic leukaemia. In Hodgkin's disease ferritin concentrations increase from stages 1 to 4 but are not related to the histological type of disease.

The relationships between tumour and plasma ferritin may be illustrated by the case of hepatoma. Tumours seem to contain less ferritin/g of tissue than normal liver and the ferritin may have a lower iron content [4, 58, 59]. There may be an increase in the ratio of H to L subunits, which is revealed by isoelectric focusing [4, 58-61] or direct analysis [59]. However, the increase is relatively small, being from 19 to 26% in the study reported by Whittaker et al. [59]. The presence of a 'tumour-specific' subunit [60] has not been confirmed [59]. Serum ferritin concentrations, measured with antibodies to liver or spleen ferritin, are often higher than in patients with cirrhosis of the liver but are not as high as in patients with hepatitis [62, 63]. However, attempts to measure serum ferritin concentrations with antibodies to H rich isoferritins from the heart or the HeLa cell have yielded very different results: Jones et al. [64] found very low concentrations and Niitsu et al. [65] recorded higher concentrations than for anti-liver ferritin in many cases. Whittaker et al. [59] failed to raise a specific antibody to H subunit enriched ferritin from hepatoma. Although there may be quantitative and qualitative abnormalities in the synthesis of ferritin in malignant cells, convincing evidence that corresponding changes in serum ferritin can be detected has not yet been provided. The conflicting reports resulting from the application of a number of assays for 'H rich isoferritins' have recently been reviewed [66]. The high serum ferritin concentrations in malignancy may therefore be due to the associated anaemia with accumulation of iron in cells of the reticuloendothelial system, to tissue necrosis, causing direct release of cytosolic ferritin which may also be a consequence of therapy and to changes in liver function causing changes in the rate of ferritin clearance.

Amongst the various 'blood tests' for the level of storage iron in the body the serum ferritin assay is the most direct. However, its use in the hospital laboratory requires an understanding of both iron metabolism and of the factors other than the level of iron stores, such as liver damage or inflammation, which influence serum ferritin concentrations. The assay provides a valuable way of studying the incidence of iron deficiency and iron overload in the community but again malnutrition and chronic disease limit its value in many parts of the third world. Although the practical use of the assay is well understood the way in which ferritin normally enters the plasma and the clearance mechanisms remain largely a mystery. There is also continuing controversy about changes in the serum ferritin phenotype in patients with cancer. With the present interest in the genetics of human ferritin it is likely that these topics will soon be understood.

References


54. Reference deleted.


