Serum Ferritin: Past, Present and Future

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Abstract

Background—Serum ferritin was discovered in the 1930’s, and was developed as a clinical test in the 1970’s. Many diseases are associated with iron overload or iron deficiency. Serum ferritin is widely used in diagnosing and monitoring these diseases.

Scope of Review—In this chapter, we discuss the role of serum ferritin in physiological and pathological processes and its use as a clinical tool.

Major Conclusions—Although many aspects of the fundamental biology of serum ferritin remain surprisingly unclear, a growing number of roles have been attributed to extracellular ferritin, including newly described roles in iron delivery, angiogenesis, inflammation, immunity, signaling and cancer.

General Significance—Serum ferritin remains a clinically useful tool. Further studies on the biology of this protein may provide new biological insights.

Historical perspective

Ferritin was discovered in 1937 by the French scientist Laufberger, who isolated a new protein from horse spleen that contained up to 23% by dry weight of iron (1). The appearance of ferritin in human serum was documented several years thereafter (2). However, quantification of serum ferritin awaited the purification of ferritin and anti-ferritin antibodies and the development of sensitive immunosassay techniques. In 1972, using an immunoradiometric assay, Addison et al. convincingly demonstrated that ferritin could be reliably detected in human serum (3). To determine the relationship between serum ferritin level and total body iron stores, the authors measured serum ferritin in a normal population, patients with iron deficiency and individuals with iron overload. They demonstrated that serum ferritin was elevated in patients with iron overload.

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overload and decreased in patients with iron deficiency diseases (4). In 1975 Jacobs and Worwood suggested that the assay of serum ferritin might provide a “useful and convenient method of assessing the status of iron storage.” (5). Serum ferritin continues to be measured to this day, although it is now known that many additional factors, including inflammation, infection and malignancy -- all of which may elevate serum ferritin -- complicate the interpretation of this value (see below). What is most surprising is that despite this long history of clinical use, fundamental aspects of the biology of serum ferritin are still unclear. For example its tissue of origin, secretory pathway, receptor interactions and cellular effects remain topics of active debate. In this chapter, we will discuss recent studies on serum ferritin and its roles in iron delivery, immunity, inflammation, angiogenesis, and cancer as well as its current use as a clinical tool.

1. Serum ferritin: basic biology

Ferritin is present in most tissues as a cytosolic protein, although a mitochondrial form has recently been described (6,7) and nuclear localization and functions have been proposed (8) (9). Ferritin plays an important role in the storage of intracellular iron, and has been the subject of extensive recent reviews (10–13). Ferritin is a 24-subunit protein that is composed of two types of subunits, termed H and L. H refers to the original isolation of isoforms of ferritin from human heart, which are rich in the H subunit, or to its electrophoretic migration as the heavier of the two subunits. L refers to ferritin isolated from human liver, which is rich in a lighter subunit. The ratio of H to L subunits within the assembled ferritin protein varies depending on tissue type and developmental stage. Genes encoding the H and L subunits of human ferritin are located on chromosomes 11q and 19q respectively (14). Both H and L ferritin also have multiple pseudogenes (15–17). Amino acid sequence similarity between ferritin H and L subunits in mammals is about 50%; sequence conservation between subunit types is even greater (among mammalian H subunits, there is approximately 90% homology; among L subunits, approximately 80% homology) (11,18).

Serum ferritin is relatively iron-poor (19,20). Based on its ability to bind concanavilin A, serum ferritin is believed to be glycosylated (21). It is composed primarily of the L subunit type, as measured by immunological cross reactivity with anti-ferritin L antibodies (21,22) (23,24). Serum ferritin is encoded by the ferritin L gene. Despite the absence of a conventional secretory signal on ferritin L, it appears and serum ferritin L and tissue ferritin L are encoded by the same gene. Thus, ferritin L was secreted from hepatocytes transfected with ferritin L cDNA via a classic secretory pathway (24). Nevertheless, due to lack of a signal peptide sequence that mediates ferritin secretion, the mechanisms of how ferritin enters the secretory pathway require further characterization. Ferritin secretion into the medium of cultured cells is increased by iron and the cytokines interleukin-1β (IL-1) and tumor necrosis factor-α (TNF-α) (26). This enhanced secretion was blocked by co-treatment with dichlorofuranosylbenzimidazole (DRB), a specific transcriptional inhibitor, suggesting that these cytokines transcriptionally upregulate ferritin and its secretion.

In rare cases, hyperferritinemia arises from hereditary disorders that do not cause iron overload. This includes mutations in the gene for tissue ferritin L, and this has been used as evidence that serum ferritin is encoded by this gene. For example, individuals with hyperferritinemia-cataract syndrome who have mutations that increase production of tissue ferritin L also show increased levels of serum ferritin (27). Recently a new missense mutation in the ferritin L coding sequence was identified and shown to be associated with hyperferritinemia without overall iron overload (28). The mutation, which mapped to the amino terminus of the L ferritin subunit and did not cause clinical symptoms, was proposed to cause hyperferritinemia by increasing ferritin secretion (28). Hereditary hyperferritinemia also results from mutations in
the ferroportin and ceruloplasmin genes, as well as genes causing genetic hemochromatosis such as **HAMP, HFE, TFR2**, and **HJV** (see (29) for review). Although the extent of iron overload differs among these patients, in these cases, the increase in serum ferritin is secondary to an increase in systemic iron (30).

The decreased serum iron, increased macrophage iron, and decreased dietary iron absorption of anemia of inflammation are explained by increases in hepcidin expression induced by inflammatory cytokines; the increased serum iron, depleted macrophage iron, and accelerated dietary iron absorption in hereditary hemochromatosis result from aberrant regulation of hepcidin expression from genetic defects.

### 2. Extracellular ferritin in physiological and pathological processes

Due to difficulties in isolating serum ferritin in quantity, few if any experiments have directly assessed effects of exogenous administration of serum ferritin. However, several investigators have studied the effects of exogenous tissue ferritin on cells. It is uncertain whether this accurately models serum ferritin, or whether it instead models paracrine effects of ferritin released from adjacent cells. Despite this uncertainty, several interesting observations have been made using tissue ferritin as a model, including the identification of ferritin receptors and the discovery of proliferative and signaling responses to ferritin.

#### 2. A. Extracellular ferritin as an iron delivery system

Studies have shown that extracellular ferritin can function as an iron carrier to provide iron to cells. Compared to transferrin, which carries a maximum of 2 iron atoms, a single ferritin molecule can sequester up to 4500 iron atoms, thus making it potentially a very effective iron delivery system. Serum ferritin, which is believed to be iron poor, carries much less iron than this (31), but could nevertheless make a significant impact on iron delivery. Sibille et al. studied ferritin release by Kupffer cells loaded with iron (32). Their results showed that about 50% of the iron content of these cells was released to the culture medium within 24 hours in the form of ferritin. When this conditioned medium was used to culture isolated hepatocytes, released ferritin was quickly taken up by the cells. The authors calculated that one hepatocyte could accumulate over 160,000 iron molecules per minute via this efficient mechanism. This study demonstrates that exogenous ferritin can function as a highly efficient iron delivery mechanism.

Although erythroid cells take up iron primarily via the transferrin-transferrin receptor pathway, it has also been shown that ferritin secreted by macrophages can function as an iron source for erythroid precursor cells (33). Using a two-phase culture protocol, the authors of this study showed that in the absence of transferrin, monocyte-derived macrophages provided enough iron for the proliferation of erythroid precursor cells. Although the exact pathway that mediates ferritin uptake by erythroid cells has not been not characterized, receptor-mediated endocytosis might be involved in this process. However, since a primary defect in the development of Tfr knockout mice is a failure of erythropoiesis (34), it is likely that the transferrin-mediated pathway plays the primary role in iron delivery to the developing erythrocyte.

In order for extracellular ferritin to carry out a physiological role, a cell surface receptor must be envisioned. Indeed, saturable binding of ferritin to a variety of different cell types has been observed for many years. Fargion et al. identified a saturable binding site for ferritin on the surface of human lymphocytes (35). Binding was specific to H ferritin, not L ferritin. Further studies showed that most B cells and about 30% of CD+ and CD8+ T-lymphocytes possessed this binding ability. The binding of ferritin to lymphocytes was shown to decrease cell proliferation. Specific and saturable binding of ferritin has also been observed in liver cells, brain oligodendrocytes, enterocytes, and erythroid precursor cells (36). Studies using
recombinant human ferritin indicated that at least two different types of ferritin receptors are present on liver cells (37). The first type of ferritin receptor had similar binding affinities for ferritin H and L, while the second type of receptor showed a specific binding for H ferritin. When H ferritin was added to the culture medium, cells expressing H receptors showed decreased proliferation and colony formation. Interestingly, a specific H ferritin receptor is also present on activated, but not quiescent, liver lipocytes (38). The activated lipocytes can internalize ferritin via this receptor. As activated lipocytes are responsible for increased collagen production and liver cirrhosis in many iron overload diseases, the authors speculate that the H ferritin receptor on the surface of activated lipocytes may mediate the transfer of iron from outside to lipocytes and thus activate them.

Binding of exogenous ferritin to cell surface receptors has also been implicated as an important iron delivery pathway in the brain. Although the transferrin-transferrin receptor pathway is the main iron import system in most cells, TfR mRNA is not detectable in white matter tracts, even in rats that are fed iron deficient diets (39). As iron is required for oligodendrocytes to produce myelin, and these cells contain more iron than any other cells in the central nervous system, other iron uptake systems that are independent of transferrin must be present. Connor and co-workers identified an H ferritin receptor on the cell surface of oligodendrocytes that could take up ferritin via receptor-mediated endocytosis (40,41). They proposed that iron delivered by ferritin is the major source of iron for oligodendrocytes.

Other studies have demonstrated binding of ferritin to other cell types, although specificity for H- or L-ferritin was not explicitly examined. Thus experiments using intestinal Caco-2 cells indicated that enterocytes possess a ferritin receptor and absorb ferritin via a receptor-mediated (42). A ferritin receptor is also present on placental membranes (43). Interestingly, in pregnant women with mild or moderate iron deficiency, ferritin receptor binding sites are much more abundant than in pregnant women with normal iron status.

Although many studies have identified ferritin binding sites on cells, the first cell surface receptor for ferritin to be cloned was mouse T cell immunoglobulin-domain and mucin-domain 2 (TIM-2). TIM-2 is a transmembrane protein expressed in liver, kidney, T cells and B cells (36,44). There is no known human ortholog of TIM-2, although TIM-1 shares sequence homology with TIM-2. TIM-2 has been shown to inhibit T cell activation (45). TIM-2 was identified as a ferritin H receptor in a screen for TIM-2 ligands (36). The authors demonstrated that TIM-2 specifically bound ferritin H and not ferritin L. The interaction between ferritin H and TIM-2 on the cell surface cause internalization of ferritin H into endosomes. This study is consistent with a role for TIM-2 in delivering iron-containing ferritin into cells. Todorich et al. demonstrated that TIM-2 is expressed on oligodendrocytes and that its expression level is responsive to iron challenge, as iron repletion decreased its expression while iron chelation increased its expression(46). Since there is no detectable Tf-TfR pathway for iron delivery in oligodendrocytes, ferritin-TIM2 was suggested to be the primary mechanism for iron uptake by these cells.

Recently Li et al. identified another cell surface receptor for ferritin, Scara5 (31). Scara5 is a scavenger receptor that can bind various ligands. In contrast to TIM-2, which is a ferritin H receptor, Scara5 preferentially binds ferritin L. Scara5 plays an important role in kidney organogenesis, presumably by delivering iron to cells. Identification of Scara5 grew out of the observation that despite the embryonic lethality of a TfR1 knockout, some organogenesis still occurs in early embryos. In addition, hypotransferrinemic mice that produce less than 1% of serum transferrin of normal mice show normal organogenesis(47), and patients with familial hypotransferrinemia also have normal organ development(48). These studies led the authors to speculate that there must be other mechanisms responsible for cellular iron uptake besides Tf-TfR. Using murine chimeric embryos composed of unlabeled TfR1 wild type cells and
TfR−/− cells tagged with green fluorescent protein, they demonstrated two independent iron delivery systems during kidney organogenesis. They showed that the ureteric bud takes up iron via the classic TfR1 pathway, while capsular cells take up iron via a TfR1-independent pathway, which was identified as a ferritin L receptor, Scara5. The authors further showed that iron-containing ferritin bound to Scara 5 and underwent endocytosis, releasing iron into the cytoplasm. It will be interesting to determine mechanisms that dictate cell type specificity for transferrin-dependent and ferritin-dependent iron delivery, and to explore the role of Scara5 in the adult animal.

A human ferritin receptor was recently identified (49). Using expression cloning, Li et al. identified human TfR1 as a cell surface receptor for H ferritin. No binding to L ferritin was observed. The binding of H ferritin to TfR1 was independent of HFE and was only partially inhibited by diferric transferrin, suggesting that binding sites for transferrin and ferritin on the receptor do not entirely overlap. The binding of H ferritin to TfR1 induces H ferritin to enter endosomes and lysosomes, and accounts for most of the binding of H ferritin to the cell surface.

Mechanisms by which iron is released from ferritin for intracellular use are currently being investigated. It has been suggested that iron may exit the protein through gated pores (50). Using defereroxamine (DFO) as a iron chelator, Kidane et al. demonstrated that lysosome-dependent ferritin degradation is required for iron release (51). Domenico et al. confirmed this result and described an additional route for iron release following treatment with the more permeant iron chelators deferriprone and desferasirox, which were found to induce ferritin degradation in the proteasome and iron release from ferritin before its degradation (52,53). It will be interesting to identify pathways of iron trafficking following its release from ferritin.

2. B. Ferritin as a signaling molecule

Very recently, Ruddell et al. proposed a new role for extracellular ferritin as a pro-inflammatory signaling molecule in hepatic stellate cells (54). They observed that cells treated with ferritin activated a pathway comprising PI3 kinase phosphorylation, protein kinase C zeta activation and MAP kinase activation, ultimately culminating in activation of NFkB. Activation of NFkB in turn enhanced the expression of pro-inflammatory mediators, including interleukin 1 beta, iNOS and others. Interestingly, this function was independent of the iron content of ferritin, suggesting that exogenous ferritin may subsume roles entirely independent of its classic role as an iron binding protein.

2. C. Serum ferritin in immunity

For many years, it has been known that patients with hematologic malignancies, such as Hodgkin’s disease and acute leukemia, have impaired cell-mediated immunity(55,56). These patients also exhibit elevated levels of serum ferritin. This suggested a possible relation between serum ferritin and immunity(57). Early in vitro studies indicated that ferritin modulates body immune function by inhibiting lymphocyte function (58). When human lymphocytes were treated with splenic ferritin, lymphocyte cell activation by phytohaemagglutinin (PHA) and concanavalin A (Con A) was inhibited (35). Later in vivo studies also suggested that ferritin inhibits immunity. Broxmeyer et al. injected mice with recombinant human ferritin H and studied the effect of ferritin on hematopoiesis(59,60). They found that ferritin H decreased the proliferation and the number of granulocyte-macrophage, erythroid and multipotential progenitor cells significantly. Interestingly, the myelosuppressive function of ferritin H depended on its ferroxidase activity, as mutations in ferritin H that inactivate its ferroxidase activity abolished its myelosuppressive activity(61). Consistent with this result, ferritin L, which lacks ferroxidase activity, also had no effect on myelopoiesis. Hemin, an iron source, reversed the suppression by ferritin H (61). As iron is required for cell
proliferation and differentiation, including lymphoid and myeloid cells, the authors attributed the myelosuppressive activity of ferritin to its inhibition of cellular transferrin iron uptake.

Chemokines are a family of proteins with chemotactic and activating effects on various leukocyte lineages that play important roles in T helper cell responses, hematopoiesis, hemostasis and angiogenesis. Li et al. observed that the chemokine CSCL12 induced binding of ferritin heavy chain to the CXC chemokine receptor 4 (CXCR4) both in vitro and in vivo. Ferritin H overexpression repressed CXCR4-mediated ERK1/2 activation, while ferritin H knockdown enhanced ERK 1/2 activation. This study indicated that ferritin H plays an important role in chemokine receptor mediated signal transduction and migration (62), effects which may contribute to the immunomodulatory activity of ferritin.

Ferritin H has also been shown to suppress immune activity in humans in vivo. Harada et al. determined that ferritin can selectively inhibit the delayed-type hypersensitivity (DTH) response (63). The effect of ferritin on DTH was studied by footpad reaction using different antigens. Results indicated that ferritin suppresses DTH, while having no effect on antibody mediated inflammatory responses. Although the precise mechanisms by which ferritin H inhibits immune responses are largely unknown, it has been suggested that ferritin H can contribute to immune suppression at least in part by inducing IL-10 production in lymphocytes (64). (IL-10 has been shown to inhibit IL-2 production as well as lymphocyte proliferation). This study explored factors secreted by melanoma cells that enable them to evade the immune system in the tumor microenvironment (64). A cDNA library from the MM200 melanoma cell line was immunoscreened with anti-sera from a melanoma patient, and an immunoreactive plaque was identified. Restriction enzyme analysis and DNA sequence analysis confirmed that this plaque encoded ferritin H. The same study show that suppression of lymphocytes by ferritin H was dependent on IL-10 production, as a monoclonal antibody against IL-10 attenuated ferritin H suppressive function.

The signaling pathways that mediate the anti-immune function of ferritin H are not completely understood. However, the identification of TIM-2 as a specific cell surface receptor for ferritin H makes it tempting to speculate that there may be a link between the immune suppressive function of ferritin H and TIM-2. TIM-2 is a member of the T cell immunoglobulin and mucin-domain (TIM) gene family, which is involved in the regulation of immune responses(65). The TIM gene family is found within the TAPR locus (T cell and airway phenotype regulator) on mouse chromosome 11 and human chromosome 5 (66). Genetic variations in the TAPR locus are associated with various immune-related diseases. A number of polymorphisms have been found in human TIM-1 and TIM-3 and these polymorphisms are associated with asthma and other allergic diseases(67). The mouse TIM gene family consists of eight members (TIM-1 to TIM-8), in human, however, the TIM family seems to include only three members (TIM-1, TIM-2 and TIM-4). There is no human orthologue of mouse TIM-2. However, given to its close sequence homology, human TIM-1 may share the same or at least some of the functions of murine TIM-2. In contrast to TIM-1, which is expressed on Th1 cell surface and regulates Th1 immune responses, TIM-2 is mainly expressed in differentiated Th2 cells and negatively regulates Th2 cell responses (44,45). Knockout TIM-2 mice were generated recently (68). TIM-2 deficient mice display increased inflammation and Th2 cytokine production in a mouse atopic model. These results indicate that TIM-2 is a negative regulator of Th2 immune responses. However, despite these suggestive relationships, whether ferritin H plays its immunosuppression function via the activation of TIM-2 receptor has not been studied.

2.D. Ferritin in Inflammation

Serum ferritin is widely recognized as an acute phase reactant and marker of acute and chronic inflammation, and is nonspecifically elevated in a wide range of inflammatory conditions, including chronic kidney disease(69), rheumatoid arthritis and other autoimmune disorders.
(70), acute infection, and malignancy. The elevated ferritin in these states reflects increased total body iron storage, but paradoxically, these stores are sequestered and not available for hematopoiesis, a process which contributes to the widely recognized anemia of inflammation (71). This relative iron deficiency in inflammation and malignancy is presumed to have developed as a defense mechanism to restrict serum iron from utilization by pathogens and tumors (70–73). Still’s disease and hemophagocytic syndrome represent two clinical entities in which serum ferritin elevations are particularly remarkable, as discussed in Section 3 of this review.

2.E. Ferritin and angiogenesis

The search for binding partners of ferritin in human serum led to the identification of high molecular weight kininogen (HK) as a ferritin interacting protein (74). HK is a 120 kDa abundant plasma protein which was initially described as a co-factor in the intrinsic coagulation cascade. HK is cleaved by the serine protease kallikrein to produce two independently active proteins: bradykinin (BK) and two-chain high molecular weight kininogen (HKα)(75). BK is a 9 amino acid rapid acting peptide which induces NO release, pain and vasodilation (76). BK is also a pro-angiogenic peptide. In contrast, the other byproduct of HK cleavage, HKα, is anti-angiogenic(77,78).

Angiogenesis, the process of creating new blood vessels from pre-existing vessels, is a key step in multiple physiologic and pathologic processes ranging from wound healing to the menstruation cycle to tumor growth and metastasis(79). The process of angiogenesis is regulated by a balance of multiple pro and anti-angiogenic factors. Interestingly, the two HK cleavage products have opposing roles in angiogenesis: BK promotes vessel formation while HKα inhibits this process (78). Ferritin, through a direct interaction with both HK and HKα, is a newly defined angiogenic regulator.

Deletion mapping and solid phase binding assays revealed that ferritin directly interacts with the light chain of HK with a Kd of 140 nM (80). Ferritin decreases the cleavage of HK by kallikrein and by two inflammatory proteases, neutrophil elastase and mast cell tryptase (80, 81). Though decreasing the cleavage of HK, ferritin decreases the production of BK and HKα, thus reducing the levels of both of these angiogenic regulators.

Additionally, ferritin directly binds HKα. In fact, ferritin has a 10-fold higher affinity for HKα than HK. Ferritin binds within domain 5 of HKα. This domain is responsible for the anti-angiogenic properties of HKα and is exposed when HK is cleaved to release BK and form HKα. Though binding to the anti-angiogenic domain of HKα, ferritin antagonizes HKα’s effects, leading to increased blood vessel growth. Indeed, in a mouse tumor model where HKα inhibits this process (78), ferritin, through a direct interaction with both HK and HKα, is a newly defined angiogenic regulator.

As described below, serum ferritin levels rise significantly during inflammation and certain malignancies—times when angiogenesis, both physiologic and pathologic, occurs. The pro-angiogenic activity of ferritin exerted through its ability to bind HK/HKα may provide a rationale for this increase: serum ferritin levels may rise in order to function as an angiogenic modulator, working to increase new blood vessel growth. This may represent a physiologic response in the setting of inflammation and wound healing, and may also represent a pathologic response in the setting of tumor growth.

2.F. Interaction between ferritin and other plasma proteins

In addition to HK, several other ferritin binding partners in serum and/or plasma have been identified including apolipoprotein B(83), α-2-macroglobulin(α 2M)(84,85), anti-ferritin
autoantibody(86), and fibrinogen(87). By binding to apolipoprotein B, ferritin
posttranslationally inhibits its secretion (88). α2M is a large plasma protein that can bind many
ligands and remove them from blood circulation by α2M receptor mediated endocytosis. The
identification of α2M as a ferritin binding protein indicated a potential pathway for cellular
uptake and/or clearance of ferritin from circulation(84). In addition, ferritin autoantibodies and
a ferritin immune complex were identified in canine serum, which may contribute to the
clearance of circulating ferritin (89,90).

2.G. Ferritin in Cancer

Serum ferritin is elevated in many malignancies (57,91). In some cases, this overall increase
in circulating ferritin is also associated with a shift in the composition of ferritin to more H-
rich species (22). For example, serum ferritin in malignant histiocytosis consists mainly of
ferritin H (23). Mechanisms underlying these changes are unclear. However, in neuroblastoma,
an increase in serum ferritin has been directly linked to secretion of ferritin by the tumor. In
these studies, human ferritins were detected in the sera of nude mice transplanted with human
neuroblastoma (92). However, no difference in the ratio of acidic (H-rich) to basic (L-rich)
isoferitins was detected in the sera of patients with neuroblastoma, suggesting that the amount
or composition of ferritin secreted by tumors is not sufficient to change the overall composition
of serum ferritin (93).

Preoperative serum ferritin levels were elevated in with newly diagnosed breast cancer, locally
recurrent, and metastatic disease(94). Tissue ferritin in cytosol extracts from mammary
carcinomas showed up to a 10-fold increase from benign breast tissues, and electron
microscopy showed that the ferritin was abundant in malignant epithelium, but was sparse in
benign epithelium and connective tissue(95,96). However, another study detected ferritin
primarily in stroma and in histiocytes surrounding neoplastic cells, suggesting that raised serum
ferritin concentrations in breast carcinoma patients might be attributed to stromal reaction
rather than to tumor synthesis(97).

It is known that excess iron alters the distribution of T-lymphocyte subsets and suppresses the
action of helper T (CD4) cells(98), as well as the tumoricidal action of macrophages and
monocytes(99). In hereditary hemochromatosis patients, iron overload increases the numbers
and activities of suppressor T (CD8) cells and decreases the numbers and activities of CD4
cells resulting in increased CD8:CD4 ratios(100). Thus, it is thought that the excess iron may
impair surveillance for cancer cells by these mechanisms. However, two cohort studies of
malignancy in hemochromatosis patients showed no increased risk of breast cancer, though
the number of women with hemochromatosis was quite small(101,102).

No studies to date have demonstrated that ferritin contributes to the etiology of cancer rather
than merely being a serum maker for the presence of cancer. However, Kabat and Rohan have
pointed out that the higher risk of breast cancer in women who are post-menopausal is
consistent with the hypothesis that increased iron storage may contribute to carcinogenesis.
They proposed that iron overload and the disruption of iron homeostasis may contribute to the
development of breast cancer, and reviewed the evidence for this hypothesis(103). Oxidative
stress is induced by a reactive oxygen species, the formation of which is mediated by free iron.
Ferric iron (Fe3+) released from ferritin and hemosiderin is reduced to ferrous iron (Fe2+)
which, in the presence of super oxide and hydrogen peroxide (H2O2), can catalyze the
formation of the hydroxyl radical (*OH). The hydroxyl radical is a powerful oxidizing agent
which can promote lipid peroxidation, mutagenesis, DNA strand breaks, activation of
oncogenes, and tumor suppressor gene inhibition. Conflicting evidence exists regarding the
contribution of lipid peroxidation products in breast cancer. It is postulated that iron interacts
with known agents in breast carcinogenesis, particularly estradiol, ethanol and ionizing

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radiation. Iron overload favors the production of reactive oxygen species, lipid peroxidation, and DNA damage.

If indeed future studies show that excess body iron levels contribute to the development of breast cancer, it may be feasible to reduce this risk by the use of natural or synthetic chelating agents(104).

3. Ferritin as a clinical tool

Ferritin is a valuable tool for the clinician, both for the evaluation of common disease states, such as iron-deficiency anemia, and for evaluation of hereditary and acquired iron-overload conditions, such as hereditary hemochromatosis and chronic transfusion therapy. Serum ferritin is usually part of panel of several blood tests routinely ordered to diagnose and manage these conditions, and is arguably the single most useful marker in most populations, though some caveats apply, as discussed below. Elevated serum ferritin levels can also be a diagnostic clue to very rare but devastating autoimmune or inflammatory disorders, such as hemophagocytic syndrome and Still’s disease.

Iron Deficiency Anemia

Iron deficiency anemia is a condition which is extremely common in both developed and undeveloped countries; serum ferritin, which indirectly reflects total body iron stores, is routinely ordered in the evaluation of anemia. Low serum ferritin is highly specific for iron deficiency anemia, and is much less invasive than the gold standard method of obtaining a bone marrow biopsy to assess stainable iron. Reference ranges for serum ferritin vary across laboratories, but levels of 30 to 300 ng/ml are considered normal for men, and 10–200 ng/ml for women(105). It is widely accepted that a serum ferritin less than 12 ng/ml indicates depletion of iron stores. Only two conditions other than iron deficiency can lower serum ferritin: hypothyroidism and ascorbate deficiency; neither condition is easily confused with iron deficiency anemia(106). A systematic overview of the diagnostic values used in the evaluation of iron deficiency anemia showed that serum ferritin was by far the most powerful test for the diagnosis of iron deficiency, outperforming red cell protoporphyrin, transferrin saturation, mean cell volume, or red cell distribution(107), with an area under the receiving operating characteristic curve of 0.95(107). Test properties differed for populations of patients with inflammatory, liver or neoplastic disease, but when appropriately interpreted was useful even across this range of patients. The authors concluded that serum ferritin concentration should be the only blood test ordered to evaluate suspected iron deficiency anemia, and that the traditional cutoff point dividing normal and abnormal (typically between 12 and 20 ng/ml) was too low to detect iron deficiency anemia even in the general population, but especially for those with inflammatory or liver disease). Using pretest probabilities and likelihood ratios, they suggested that a level higher than approximately 40 ng/ml should be used to exclude iron deficiency in most patients, whereas a level higher than 70 ng/ml was more appropriate to exclude iron deficiency in patients with inflammation or liver disease. In another study, 25% of women with absent stainable bone marrow iron (the gold standard test for diagnosis of iron deficiency) had serum ferritin levels greater than 15 ng/ml (previously considered the low end of the normal range of ferritin, (108) confirming that iron deficiency can exist even with ferritin levels within the normal range. It has been suggested that gender differences in ferritin normal ranges are based on large populations that contain significant numbers of women who are iron-deficient because of poor diet and menstrual blood loss, and that reference values should be based on iron-replete populations(109). However, the need for changes in the normal range has been disputed(110,111).
Neurologic Associations with Low Serum Ferritin

Low serum ferritin was recently shown to be associated with neurally mediated syncope in children and adolescents (112), and is a potentially important factor in the pathophysiology of this common problem. Breath-holding spells, which are a form of neurally mediated syncope in early childhood, have long been recognized as an indicator of iron deficiency in very young children. In adults, restless legs syndrome is frequent in anemic and pregnant patients, suggesting that serum ferritin may have a role in the pathophysiology of this syndrome. However, available evidence supports a more complex relationship between serum ferritin, CSF ferritin, and symptoms(113–116).

Ferritin in chronic kidney disease

In patients with chronic kidney disease serum ferritin is a less robust marker of bioavailable iron. Hyperferritinemia is a misleading marker of iron stores in such patients as reviewed in (117). Although almost half of all patients on maintenance hemodialysis have a serum ferritin >500 ng/ml, this high level does not represent iron that is bioavailable for erythropoiesis. Inflammation was the probable cause of increased ferritin level in about one-third of hemodialysis patients (118,119). In addition, hemodialysis patients with a serum ferritin >800 ng/ml had a higher CRP level and a worse malnutrition-inflammation score(120). Guidelines for such patients from the Kidney Disease Outcomes Quality Initiative (K/DOQI)) propose a serum ferritin level of 800 ng/ml as an upper limit for intravenous iron therapy. Absolute iron deficiency is defined using another laboratory measure (transferrin saturation <20%) or serum ferritin <100 ng/ml, both of which correlate with absence or near absence of bone marrow stainable iron(121).

Ferritin in Clinical inflammatory Conditions and Trauma

Serum ferritin levels of greater than 1000 ng/ml are a nonspecific marker of illness, including infections and cancer. The frequency of hyperferritinemia was examined in a hospitalized population over a one year period. 6.7% of patients were shown to have a level greater than 1000 ng/ml, and was associated with liver disease, renal disease, human immunodeficiency virus infection, systemic infections, chronic transfusion, and sickle cell syndromes(122). No syndrome to account for an elevated ferritin level was seen in 8% in this series. The highest levels occurred in patients with sickle cell disease and in the chronically transfused. Similar results were seen in other series(123,124), with extremely high levels of ferritin (mean level of 45,000 ng/ml) suggesting reactive hemophagocytic syndrome.

Ferritin is elevated in Still’s disease. An exaggerated ferritin response with levels above 5 times the normal upper level was 100% specific in predicting chronic disease course in patients with Adult type of Still’s disease, and ferritin was useful in distinguishing these patients from clinically similar rheumatoid arthritis patients(125).

Initial serum ferritin levels in patients with multiple trauma correlated with the degree of initial trauma injury and predicted subsequent development of acute respiratory distress syndrome, but not length of ventilation or mortality. There was a significant association between serum ferritin levels of products of endothelial activation(126).

Ferritin in the evaluation of the acutely ill patient

Ferritin is an important diagnostic clue in the evaluation of critically ill patients. Hemophagocytic syndrome (HPS), also referred to as hemophagocytic lymphohistiocytosis (HLH)(127) and its phenotypic variants including macrophage activation syndrome are characterized by the presence of five of the following eight diagnostic criteria: 1) fever, 2) cytopenia of two cell lines, 3) hypertriglyceridemia and/or hypofibrinogenemia, 4)
hyperferritinemia (>500 ng/ml), 5) hemophagocytosis, 6) elevated soluble interleukin-2 receptor (CD25), 7) decreased natural killer-cell activity, and 8) splenomegaly(128). A very elevated ferritin is sometimes observed as an incidental finding when iron studies are sent to evaluate anemia in critically ill patients, and should prompt consideration of an inflammatory syndrome such as severe sepsis/systemic inflammatory response syndrome, multiorgan dysfunction syndrome/macrophage activation syndrome. Hemophagocytic lymphohistiocytosis represents a complex group of disorders which may be inherited or acquired which are characterized by exaggerated and life-threatening inflammatory responses; the final common pathway results from impaired natural killer cell function(129).

Elevated ferritin was shown to be a risk factor associated with death in an analysis of 34 cases of HPS in adults. (130). Macrophages have been implicated as the major source of hyperferritinemia in reactive Macrophage Activation Syndrome, (131) and hyperferritinemia was an effective indicator for administration of intravenous immunoglobulin in this disorder (132).

Recently, the overlap of these 8 nonspecific criteria for acquired HLH and the clinical and laboratory manifestations of sepsis/SIRS/MODS and macrophage activation syndrome were examined, and a spectrum of inflammation was proposed to reconcile these clinically similar entities. The authors urged extreme caution when considering chemotherapy and bone marrow transplantation, the recommended treatments for HLH and severe or persistent HLH, respectively(133).

Ferritin in non-hemochromatotic liver disease

Serum ferritin concentrations reflect iron stores in alcoholics with minimal liver disease, but do not reflect iron stores (as measured by liver iron concentration) in alcoholics with significant liver disease(134). Hepatitis C viral infection is often association with an elevation of iron parameters, especially where there is coincidence of hemochromatosis mutations(135,136). Iron is considered an important co-morbid factor in Hepatitis C disease progression to advanced liver fibrosis or even cirrhosis. Serum ferritin correlated with ALT, iron, and transferrin saturation, and hepatic iron stain(137). Serum ferritin has been shown to independently predict severe hepatic fibrosis in patients with chronic Hepatitis C infection (138), though this finding was not replicated in recent Korean series(139). Ferritin is the most predictive laboratory parameter showing the degree of liver damage in HCV-infected hemodialysis patients(140).

Ferritin in hemochromatosis

Serum ferritin robustly predicts the risk of cirrhosis, the main clinical manifestation of hemochromatosis. Several studies have shown that cirrhosis of the liver occurs only rarely in hemochromatosis patients with serum ferritin levels of less than 1000 micrograms/liter(141–144). A recent study of nearly 30,000 white subjects showed that only 59 had serum ferritin levels of greater than 1000 micrograms/liter, of which 24 had homozygous mutant or compound heterozygous mutant HFE genotypes. Based on these findings, a new screening strategy to detect only those who are at the highest risk for serious clinical manifestations was proposed : it was suggested that screening for hemochromatosis with serum ferritin levels is a more cost-effective screening strategy and will detect the majority of patients who will be clinically affected, especially given the low rate of rise of ferritin over time(145). However, this proposal is controversial, based on cost and suboptimal ferritin limit(146). A strategy to detect only those who are at the highest risk for serious clinical manifestations. Presently, the US Preventive Services Task recommends against routine genetic screening for hereditary hemochromatosis in the asymptomatic general population, concluding that the potential harms of screening (unnecessary surveillance, labeling, unnecessary invasive work-up, anxiety, and potentially unnecessary treatments) outweigh the potential benefits and costs for HFE.
hemochromatosis (147). Further prospective evaluation of serum ferritin level as screening strategy with follow-up of liver biopsies and treatment is warranted (145).

**Ferritin in acquired iron overload conditions**

Elevated serum ferritin also predicts end-organ involvement in non-hereditary iron overload conditions, such as transfusion-associated iron overload in myelodysplastic syndromes, thalassemias and hemoglobinopathies. The natural history of transfusion-associated iron-overload was examined in chronically transfused children with sickle cell disease who were observed for up to 10 years in two consecutive stroke prevention trials. An analysis of ferritin levels, estimated transfusion iron overload (TIL) (estimated from the cumulative blood volume in patients who received simple transfusions prior to the start of chelation therapy), and liver iron concentrations (LIC) (assayed by inductively coupled plasma-mass spectrometry analytical chemistry method on liver biopsy specimens) done on children enrolled in two trials for stroke prevention showed serum ferritin changes that were non-linear compared to TIL or LIC (148). Levels less than 1500 ng/ml indicated mostly acceptable iron overload; levels greater than or equal to 3000 ng/ml were specific for significant iron-overload and were associated with liver injury. (Accurate assessment of iron levels is required in those with levels between 1500 and 3000 ng/ml.) Of note, serum ferritin rose rapidly with transfusion initially, then slowed after reaching 1500–2500 ng/ml, despite evidence of increasing iron load. Serum ferritin levels greater than 3000 ng/ml were associated with both increased LIC and liver injury, as estimated by ALT levels. The authors proposed an approach to monitor iron-overload that utilized a combination of methods, including calculation of transfusion iron overload, frequent measurement of ferritin, and serial ALTs, with optimal iron load assessment to include periodic tissue iron determination, especially in outpatients with intermediately elevated serum ferritin levels.

**Ferritin in the post-transplant setting**

Several studies have shown that the presence of iron overload prior to either allogeneic or autologous hematopoietic stem cell transplant is associated with complications and decreased survival, though there is no consensus regarding a definition of iron overload in these patients. High ferritin level (>1000 ng/ml) was an independent risk factor for the occurrence of liver dysfunction, as measured by abnormal liver function tests, in series of Italian patients undergoing allogeneic hematopoietic stem cell transplant. In addition, the rate of proven/probable invasive fungal disease was significantly higher among patients with hyperferritinemia as compared to patients with normal ferritin levels (13% vs 0%). (149). Elevated pretransplantation serum ferritin alone (defined as ferritin > 598 ng/ml) predicted worse survival and nonrelapse mortality in a large series of Japanese patients who underwent allogenic HSCT for hematologic malignancies. Patients in the high ferritin group were significantly more likely to die of infection and organ failure (150). Disease-free and overall survival was lower in a group of transfusion associated iron-overloaded patients who underwent reduced-intensity stem cell transplantation in Korea (151). Ferritin >685 ng/ml was associated with a higher incidence of relapse and relapse mortality, but not of nonrelapse mortality in a large series of patients who underwent autologous hematopoietic stem cell transplantation for Hodgkin or non-Hodgkin lymphoma (152). Elevated serum ferritin was associated with increased risk of death, increased day 100 mortality, increased incidence of acute graft versus host disease and increased risk of blood stream infection (153). There was a significant correlation between serum ferritin levels and histologically proven liver iron overload in hepatocytes in the post-HSCT setting and hepatic dysfunction (154).

Pretransplantation ferritin was incorporated into a simple prognostic scoring system for patients with acute leukemia or myelodysplastic syndromes (155). Iron burden as measured by a ferritin level of >1500 ng/ml predicted severe mucositis, bacteremia, and days with fever in patients.
with autologous but not allogeneic transplants(156). Iron overload was a major risk factor for severe infection after autologous stem cell transplantation (157) and was possibly associated with invasive aspergillosis(158).

Serum ferritin >1000 ng/ml was one measure incorporated into another scoring system for iron overload in patients prior to autologous or allogeneic stem cell transplantation; the score also assigned points for transfusion of greater than 25 red cell units and a semi-quantitative bone marrow iron stain. This score was more closely associated with survival than any available single iron parameter. Iron overload predicted decreased transplant survival primarily attributable to an increase in early treatment-related deaths and lethal infections(159). The most intriguing findings were that excess mortality occurred early, not late, and that iron overload did not predict tumor recurrence, but seemed to predispose to infectious deaths. This finding parallels quite old observations that bacteria proliferate in iron-rich vs iron-poor conditions, as shown in a mouse model of iron-controlled infection (160) and as reviewed in(161).

Though iron overload is less common in recipients of solid organs, owing to less frequent red cell transfusions compared to patients with hematopoietic malignancies, the issue has been addressed after solid organ transplantation. Ferritin concentrations in bronchoalveolar lavage fluid were significantly elevated in lung transplant recipients compared to normal controls, and suggested that the allograft could be subjected to iron-generated oxidative stress(162). Serum ferritin was reliable in predicting the histological diagnosis of hepatic hemosiderosis in renal allograft recipients(163). Ten year follow-up of renal transplant recipients with serum ferritin levels >1,100 ng/ml suggested that multiple blood transfusions (>40 units) prior to transplantation conferred a 3-fold relative risk for mortality (164).

**Future directions**

Despite its clear utility as a clinical tool to assess body iron stores, much of the biology of serum ferritin remains as elusive today as when it was first discovered. For example, cellular mechanisms involved in the secretion of ferritin, which does not contain a canonical leader sequence, remain unknown. This will be important to unravel, particularly as it is becoming clear that extracellular ferritin can subsume many functions unrelated to its classic role as an intracellular iron storage protein. The delineation of precise relationships between ferritin secretion and immunomodulation, iron delivery, and triggering of signaling pathways all will require further investigation. The study of ferritin isolated from the serum of normal, non-hemochromatotic individuals may shed additional light on the biochemistry of this protein. Finally, identification of cell types responsible for the secretion of human ferritin, and further studies on the cells and receptors targeted by ferritin action may bring us closer to an understanding of this multifunctional protein.

**References**


