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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 immunoassay 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

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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 concanavalin 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). The source and detailed secretory pathway of serum ferritin are not completely understood. Hepatocytes, macrophages and Kupffer cells have been shown to secrete ferritin (25). 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-1- β (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 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 CD4+ 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 deferoxamine (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 NFκB. Activation of NFκB 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 (HKa)(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, HKa, 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 HKa inhibits this process (78). ferritin, through a direct interaction with both HK and HKa, 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 HKa, thus reducing the levels of both of these angiogenic regulators.

Additionally, ferritin directly binds HKa. In fact, ferritin has a 10-fold higher affinity for HKa than HK. Ferritin binds within domain 5 of HKa. This domain is responsible for the anti-angiogenic properties of HKa and is exposed when HK is cleaved to release BK and form HKa. Though binding to the anti-angiogenic domain of HKa, ferritin antagonizes HKa's effects, leading to increased blood vessel growth. Indeed, in a mouse tumor model where HKa reduces tumor blood vessel growth, the addition of ferritin counteracts the effects of HKa, leading to significantly increased intratumor blood vessel density (82).

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/HKa 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). α_2M is a large plasma protein that can bind many ligands and remove them from blood circulation by α_2M receptor mediated endocytosis. The identification of α_2M 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) isoforms 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 marker 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 (Fe^{3+}) released from ferritin and hemosiderin is reduced to ferrous iron (Fe^{2+}) which, in the presence of super oxide and hydrogen peroxide (H_2O_2), can catalyze the formation of the hydroxyl radical ($\cdot OH$). 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

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 associated 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 allogeneic 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

1. Laufberger V. Sur la cristallisation de la ferritine. Bulletin de la Societe de chimie biologique 1937;19:1575–1582.
2. Worwood, M. Iron in Biochemistry and Medicine, II. A.a.W. Jacobs, M., editor. London: Academic Press; 1980. p. 204-244.
3. Addison GM, Beamish MR, Hales CN, Hodgkins M, Jacobs A, Llewellyn P. An immunoradiometric assay for ferritin in the serum of normal subjects and patients with iron deficiency and iron overload. J Clin Pathol 1972;25:326–329. [PubMed: 5063755]
4. Jacobs A, Miller F, Worwood M, Beamish MR, Wardrop CA. Ferritin in the serum of normal subjects and patients with iron deficiency and iron overload. Br Med J 1972;4:206–208. [PubMed: 5082548]
5. Jacobs A, Worwood M. Ferritin in serum. Clinical and biochemical implications. N Engl J Med 1975;292:951–956. [PubMed: 1090831]

6. Levi S, Arosio P. Mitochondrial ferritin. *Int J Biochem Cell Biol* 2004;36:1887–1889. [PubMed: 15203103]
7. Levi S, Corsi B, Bosisio M, Invernizzi R, Volz A, Sanford D, Arosio P, Drysdale J. A human mitochondrial ferritin encoded by an intronless gene. *J Biol Chem* 2001;276:24437–24440. [PubMed: 11323407]
8. Cai CX, Birk DE, Linsenmayer TF. Ferritin is a developmentally regulated nuclear protein of avian corneal epithelial cells. *J Biol Chem* 1997;272:12831–12839. [PubMed: 9139744]
9. Surguladze N, Patton S, Cozzi A, Fried MG, Connor JR. Characterization of nuclear ferritin and mechanism of translocation. *Biochem J* 2005;388:731–740. [PubMed: 15675895]
10. Torti FM, Torti SV. Regulation of ferritin genes and protein. *Blood* 2002;99:3505–3516. [PubMed: 11986201]
11. Orino K, Watanabe K. Molecular, physiological and clinical aspects of the iron storage protein ferritin. *Vet J* 2008;178:191–201. [PubMed: 17764995]
12. Arosio P, Levi S. Ferritin, iron homeostasis, and oxidative damage. *Free Radic Biol Med* 2002;33:457–463. [PubMed: 12160928]
13. Theil EC. Iron, ferritin, and nutrition. *Annu Rev Nutr* 2004;24:327–343. [PubMed: 15189124]
14. Worwood M, Brook JD, Cragg SJ, Hellkuhl B, Jones BM, Perera P, Roberts SH, Shaw DJ. Assignment of human ferritin genes to chromosomes 11 and 19q13.3---19qter. *Hum Genet* 1985;69:371–374. [PubMed: 3857215]
15. Lebo RV, Kan YW, Cheung MC, Jain SK, Drysdale J. Human ferritin light chain gene sequences mapped to several sorted chromosomes. *Hum Genet* 1985;71:325–328. [PubMed: 3000916]
16. Dugast IJ, Papadopoulos P, Zappone E, Jones C, Theriault K, Handelman GJ, Benarous R, Drysdale JW. Identification of two human ferritin H genes on the short arm of chromosome 6. *Genomics* 1990;6:204–211. [PubMed: 2307464]
17. Quaresima B, Tiano MT, Porcellini A, D'Agostino P, Faniello MC, Bevilacqua MA, Cimino F, Costanzo F. PCR analysis of the H ferritin multigene family reveals the existence of two classes of processed pseudogenes. *PCR Methods Appl* 1994;4:85–88. [PubMed: 7580890]
18. Andrews SC, Arosio P, Bottke W, Briat JF, von Darl M, Harrison PM, Lahlere JP, Levi S, Lobreaux S, Yewdall SJ. Structure, and evolution of ferritins. *J Inorg Biochem* 1992;47:161–174. [PubMed: 1431878]
19. Worwood M, Dawkins S, Wagstaff M, Jacobs A. The purification and properties of ferritin from human serum. *Biochem J* 1976;157:97–103. [PubMed: 962866]
20. Arosio P, Yokota M, Drysdale JW. Characterization of serum ferritin in iron overload: possible identity to natural apoferritin. *Br J Haematol* 1977;36:199–207. [PubMed: 871433]
21. Santambrogio P, Cozzi A, Levi S, Arosio P. Human serum ferritin G-peptide is recognized by anti-L ferritin subunit antibodies and concanavalin-A. *Br J Haematol* 1987;65:235–237. [PubMed: 3828232]
22. Cazzola M, Arosio P, Bellotti V, Bergamaschi G, Dezza L, Iacobello C, Ruggeri G, Zappone E, Albertini A, Ascari E. Immunological reactivity of serum ferritin in patients with malignancy. *Tumori* 1985;71:547–554. [PubMed: 4082287]
23. Lukina EA, Levina AA, Mokeeva RA, Tokarev Yu N. The diagnostic significance of serum ferritin indices in patients with malignant and reactive histiocytosis. *Br J Haematol* 1993;83:326–329. [PubMed: 8457481]
24. Ghosh S, Hevi S, Chuck SL. Regulated secretion of glycosylated human ferritin from hepatocytes. *Blood* 2004;103:2369–2376. [PubMed: 14615366]
25. Wesselius LJ, Nelson ME, Skikne BS. Increased release of ferritin and iron by iron-loaded alveolar macrophages in cigarette smokers. *Am J Respir Crit Care Med* 1994;150:690–695. [PubMed: 8087339]
26. Tran TN, Eubanks SK, Schaffer KJ, Zhou CY, Linder MC. Secretion of ferritin by rat hepatoma cells and its regulation by inflammatory cytokines and iron. *Blood* 1997;90:4979–4986. [PubMed: 9389717]
27. Cazzola M, Bergamaschi G, Tonon L, Arbustini E, Grasso M, Vercesi E, Barosi G, Bianchi PE, Cairo G, Arosio P. Hereditary hyperferritinemia-cataract syndrome: relationship between phenotypes and

- specific mutations in the iron-responsive element of ferritin light-chain mRNA. *Blood* 1997;90:814–821. [PubMed: 9226182]
28. Kannengiesser C, Jouanolle AM, Hetet G, Mosser A, Muzeau F, Henry D, Bardou-Jacquet E, Mornet M, Brissot P, Deugnier Y, Grandchamp B, Beaumont C. A new missense mutation in the L ferritin coding sequence associated with elevated levels of glycosylated ferritin in serum and absence of iron overload. *Haematologica* 2009;94:335–339. [PubMed: 19176363]
 29. Roy CN, Andrews NC. Anemia of inflammation: the hepcidin link. *Curr Opin Hematol* 2005;12:107–111. [PubMed: 15725899]
 30. Aguilar-Martinez P, Schved JF, Brissot P. The evaluation of hyperferritinemia: an updated strategy based on advances in detecting genetic abnormalities. *Am J Gastroenterol* 2005;100:1185–1194. [PubMed: 15842597]
 31. Li JY, Paragas N, Ned RM, Qiu A, Viltard M, Leete T, Drexler IR, Chen X, Sanna-Cherchi S, Mohammed F, Williams D, Lin CS, Schmidt-Ott KM, Andrews NC, Barasch J. Scara5 is a ferritin receptor mediating non-transferrin iron delivery. *Dev Cell* 2009;16:35–46. [PubMed: 19154717]
 32. Sibille JC, Kondo H, Aisen P. Interactions between isolated hepatocytes and Kupffer cells in iron metabolism: a possible role for ferritin as an iron carrier protein. *Hepatology* 1988;8:296–301. [PubMed: 3356411]
 33. Leimberg MJ, Prus E, Konijn AM, Fibach E. Macrophages function as a ferritin iron source for cultured human erythroid precursors. *J Cell Biochem* 2008;103:1211–1218. [PubMed: 17902167]
 34. Levy JE, Jin O, Fujiwara Y, Kuo F, Andrews NC. Transferrin receptor is necessary for development of erythrocytes and the nervous system. *Nat Genet* 1999;21:396–399. [PubMed: 10192390]
 35. Fargion S, Fracanzani AL, Brando B, Arosio P, Levi S, Fiorelli G. Specific binding sites for H-ferritin on human lymphocytes: modulation during cellular proliferation and potential implication in cell growth control. *Blood* 1991;78:1056–1061. [PubMed: 1831058]
 36. Chen TT, Li L, Chung DH, Allen CD, Torti SV, Torti FM, Cyster JG, Chen CY, Brodsky FM, Niemi EC, Nakamura MC, Seaman WE, Daws MR. TIM-2 is expressed on B cells and in liver and kidney and is a receptor for H-ferritin endocytosis. *J Exp Med* 2005;202:955–965. [PubMed: 16203866]
 37. Moss D, Fargion S, Fracanzani AL, Levi S, Cappellini MD, Arosio P, Powell LW, Halliday JW. Functional roles of the ferritin receptors of human liver, hepatoma, lymphoid and erythroid cells. *J Inorg Biochem* 1992;47:219–227. [PubMed: 1331322]
 38. Ramm GA, Britton RS, O'Neill R, Bacon BR. Identification and characterization of a receptor for tissue ferritin on activated rat lipocytes. *J Clin Invest* 1994;94:9–15. [PubMed: 8040296]
 39. Han J, Day JR, Connor JR, Beard JL. Gene expression of transferrin and transferrin receptor in brains of control vs. iron-deficient rats. *Nutr Neurosci* 2003;6:1–10. [PubMed: 12608731]
 40. Hulet SW, Heyliger SO, Powers S, Connor JR. Oligodendrocyte progenitor cells internalize ferritin via clathrin-dependent receptor mediated endocytosis. *J Neurosci Res* 2000;61:52–60. [PubMed: 10861799]
 41. Fisher J, Devraj K, Ingram J, Slagle-Webb B, Madhankumar AB, Liu X, Klinger M, Simpson IA, Connor JR. Ferritin: a novel mechanism for delivery of iron to the brain and other organs. *Am J Physiol Cell Physiol* 2007;293:C641–C649. [PubMed: 17459943]
 42. Kalgaonkar S, Lonnerdal B. Receptor-mediated uptake of ferritin-bound iron by human intestinal Caco-2 cells. *J Nutr Biochem* 2009;20:304–311. [PubMed: 18602806]
 43. Liao QK, Kong PA, Gao J, Li FY, Qian ZM. Expression of ferritin receptor in placental microvilli membrane in pregnant women with different iron status at mid-term gestation. *Eur J Clin Nutr* 2001;55:651–656. [PubMed: 11477463]
 44. Chakravarti S, Sabatos CA, Xiao S, Illes Z, Cha EK, Sobel RA, Zheng XX, Strom TB, Kuchroo VK. Tim-2 regulates T helper type 2 responses and autoimmunity. *J Exp Med* 2005;202:437–444. [PubMed: 16043519]
 45. Knickelbein JE, de Souza AJ, Tosti R, Narayan P, Kane LP. Cutting edge: inhibition of T cell activation by TIM-2. *J Immunol* 2006;177:4966–4970. [PubMed: 17015678]
 46. Todorich B, Zhang X, Slagle-Webb B, Seaman WE, Connor JR. Tim-2 is the receptor for H-ferritin on oligodendrocytes. *J Neurochem* 2008;107:1495–1505. [PubMed: 19014383]

47. Huggenvik JI, Craven CM, Idzerda RL, Bernstein S, Kaplan J, McKnight GS. A splicing defect in the mouse transferrin gene leads to congenital atransferrinemia. *Blood* 1989;74:482–486. [PubMed: 2752125]
48. Hayashi A, Wada Y, Suzuki T, Shimizu A. Studies on familial hypotransferrinemia: unique clinical course and molecular pathology. *Am J Hum Genet* 1993;53:201–213. [PubMed: 8317485]
49. Li L, Fang CJ, Ryan JC, Niemi EC, Lebron JA, Bjorkman PJ, Arase H, Torti FM, Torti SV, Nakamura MC, Seaman WE. Binding and uptake of H-ferritin are mediated by human transferrin receptor-1. *Proc Natl Acad Sci U S A*.
50. Theil EC, Liu XS, Tosha T. Gated Pores in the Ferritin Protein Nanocage. *Inorganica Chim Acta* 2008;361:868–874. [PubMed: 19262678]
51. Kidane TZ, Sauble E, Linder MC. Release of iron from ferritin requires lysosomal activity. *Am J Physiol Cell Physiol* 2006;291:C445–C455. [PubMed: 16611735]
52. De Domenico I, Ward DM, Kaplan J. Specific iron chelators determine the route of ferritin degradation. *Blood*. 2009
53. De Domenico I, Vaughn MB, Li L, Bagley D, Musci G, Ward DM, Kaplan J. Ferroportin-mediated mobilization of ferritin iron precedes ferritin degradation by the proteasome. *Embo J* 2006;25:5396–5404. [PubMed: 17082767]
54. Ruddell RG, Hoang-Le D, Barwood JM, Rutherford PS, Piva TJ, Watters DJ, Santambrogio P, Arosio P, Ramm GA. Ferritin functions as a proinflammatory cytokine via iron-independent protein kinase C zeta/nuclear factor kappaB-regulated signaling in rat hepatic stellate cells. *Hepatology* 2009;49:887–900. [PubMed: 19241483]
55. Schier WW, Roth A, Ostroff G, Schrift MH. Hodgkin's disease and immunity. *Am J Med* 1956;20:94–99. [PubMed: 13282926]
56. Izak G, Stupp Y, Manny N, Zajicek G, Weiss DW. The immune response in acute myelocytic leukemia: effect of the methanol extraction residue fraction of tubercle bacilli (MER) on T and B cell functions and their relation to the course of the disease. *Isr J Med Sci* 1977;13:677–693. [PubMed: 411767]
57. Hazard JT, Drysdale JW. Ferritinaemia in cancer. *Nature* 1977;265:755–756. [PubMed: 67563]
58. Matzner Y, Hershko C, Polliack A, Konijn AM, Izak G. Suppressive effect of ferritin on in vitro lymphocyte function. *Br J Haematol* 1979;42:345–353. [PubMed: 157770]
59. Broxmeyer HE, Williams DE, Geissler K, Hangoc G, Cooper S, Bicknell DC, Levi S, Arosio P. Suppressive effects in vivo of purified recombinant human H-subunit (acidic) ferritin on murine myelopoiesis. *Blood* 1989;73:74–79. [PubMed: 2910370]
60. Broxmeyer HE, Lu L, Bicknell DC, Williams DE, Cooper S, Levi S, Salfeld J, Arosio P. The influence of purified recombinant human heavy-subunit and light-subunit ferritins on colony formation in vitro by granulocyte-macrophage and erythroid progenitor cells. *Blood* 1986;68:1257–1263. [PubMed: 3490884]
61. Broxmeyer HE, Cooper S, Levi S, Arosio P. Mutated recombinant human heavy-chain ferritins and myelosuppression in vitro and in vivo: a link between ferritin ferroxidase activity and biological function. *Proc Natl Acad Sci U S A* 1991;88:770–774. [PubMed: 1992468]
62. Li R, Luo C, Mines M, Zhang J, Fan GH. Chemokine CXCL12 induces binding of ferritin heavy chain to the chemokine receptor CXCR4, alters CXCR4 signaling, and induces phosphorylation and nuclear translocation of ferritin heavy chain. *J Biol Chem* 2006;281:37616–37627. [PubMed: 17056593]
63. Harada T, Baba M, Torii I, Morikawa S. Ferritin selectively suppresses delayed-type hypersensitivity responses at induction or effector phase. *Cell Immunol* 1987;109:75–88. [PubMed: 2958143]
64. Gray CP, Franco AV, Arosio P, Hersey P. Immunosuppressive effects of melanoma-derived heavy-chain ferritin are dependent on stimulation of IL-10 production. *Int J Cancer* 2001;92:843–850. [PubMed: 11351305]
65. Recalcati S, Invernizzi P, Arosio P, Cairo G. New functions for an iron storage protein: the role of ferritin in immunity and autoimmunity. *J Autoimmun* 2008;30:84–89. [PubMed: 18191543]
66. McIntire JJ, Umetsu SE, Akbari O, Potter M, Kuchroo VK, Barsh GS, Freeman GJ, Umetsu DT, DeKruyff RH. Identification of Tapr (an airway hyperreactivity regulatory locus) and the linked Tim gene family. *Nat Immunol* 2001;2:1109–1116. [PubMed: 11725301]

67. Kuchroo VK, Umetsu DT, DeKruyff RH, Freeman GJ. The TIM gene family: emerging roles in immunity and disease. *Nat Rev Immunol* 2003;3:454–462. [PubMed: 12776205]
68. Rennert PD, Ichimura T, Sizing ID, Bailly V, Li Z, Rennard R, McCoon P, Pablo L, Miklasz S, Tarilonte L, Bonventre JV. T cell, Ig domain, mucin domain-2 gene-deficient mice reveal a novel mechanism for the regulation of Th2 immune responses and airway inflammation. *J Immunol* 2006;177:4311–4321. [PubMed: 16982865]
69. Kalantar-Zadeh K, Kalantar-Zadeh K, Lee GH. The fascinating but deceptive ferritin: to measure it or not to measure it in chronic kidney disease? *Clin J Am Soc Nephrol* 2006;1 Suppl 1:S9–S18. [PubMed: 17699375]
70. Zandman-Goddard G, Shoenfeld Y. Ferritin in autoimmune diseases. *Autoimmun Rev* 2007;6:457–463. [PubMed: 17643933]
71. Ganz T, Nemeth E. Iron sequestration and anemia of inflammation. *Semin Hematol* 2009;46:387–393. [PubMed: 19786207]
72. Hintze KJ, Theil EC. Cellular regulation and molecular interactions of the ferritins. *Cell Mol Life Sci* 2006;63:591–600. [PubMed: 16465450]
73. Weinberg ED, Miklossy J. Iron withholding: a defense against disease. *J Alzheimers Dis* 2008;13:451–463. [PubMed: 18487852]
74. Torti SV, Torti FM. Human H-kininogen is a ferritin-binding protein. *J Biol Chem* 1998;273:13630–13635. [PubMed: 9593701]
75. Sainz IM, Pixley RA, Colman RW. Fifty years of research on the plasma kallikrein-kinin system: from protein structure and function to cell biology and in-vivo pathophysiology. *Thromb Haemost* 2007;98:77–83. [PubMed: 17597995]
76. Sharma JN. The kallikrein-kinin system: from mediator of inflammation to modulator of cardioprotection. *Inflammopharmacology* 2005;12:591–596. [PubMed: 16259723]
77. Colman RW. Regulation of angiogenesis by the kallikrein-kinin system. *Curr Pharm Des* 2006;12:2599–2607. [PubMed: 16842160]
78. Guo YL, Colman RW. Two faces of high-molecular-weight kininogen (HK) in angiogenesis: bradykinin turns it on and cleaved HK (HKa) turns it off. *J Thromb Haemost* 2005;3:670–676. [PubMed: 15733059]
79. Folkman J. Fundamental concepts of the angiogenic process. *Curr Mol Med* 2003;3:643–651. [PubMed: 14601638]
80. Parthasarathy N, Torti SV, Torti FM. Ferritin binds to light chain of human H-kininogen and inhibits kallikrein-mediated bradykinin release. *Biochem J* 2002;365:279–286. [PubMed: 12071855]
81. Coffman LG, Brown JC, Johnson DA, Parthasarathy N, D'Agostino RB Jr, Lively MO, Hua X, Tilley SL, Muller-Esterl W, Willingham MC, Torti FM, Torti SV. Cleavage of high-molecular-weight kininogen by elastase and tryptase is inhibited by ferritin. *Am J Physiol Lung Cell Mol Physiol* 2008;294:L505–L515. [PubMed: 18192590]
82. Coffman LG, Parsonage D, D'Agostino R Jr, Torti FM, Torti SV. Regulatory effects of ferritin on angiogenesis. *Proc Natl Acad Sci U S A* 2009;106:570–575. [PubMed: 19126685]
83. Rashid KA, Hevi S, Chen Y, Le Caherec F, Chuck SL. A proteomic approach identifies proteins in hepatocytes that bind nascent apolipoprotein B. *J Biol Chem* 2002;277:22010–22017. [PubMed: 11934886]
84. Massover WH. Alpha 2-macroglobulin: a ferritin-binding protein. *Ann N Y Acad Sci* 1994;737:468–471. [PubMed: 7524423]
85. Santambrogio P, Massover WH. Rabbit serum alpha-2-macroglobulin binds to liver ferritin: association causes a heterogeneity of ferritin molecules. *Br J Haematol* 1989;71:281–290. [PubMed: 2466475]
86. Orino K, Ishiji T, Yamamoto S, Watanabe K. Characterization of bovine serum ferritin-binding proteins. *Comp Biochem Physiol A Mol Integr Physiol* 2004;137:375–381. [PubMed: 15123210]
87. Orino K, Yamamoto S, Watanabe K. Fibrinogen as a ferritin-binding protein in horse plasma. *J Vet Med Sci* 1993;55:785–787. [PubMed: 8286532]
88. Hevi S, Chuck SL. Ferritins can regulate the secretion of apolipoprotein B. *J Biol Chem* 2003;278:31924–31929. [PubMed: 12813058]

89. Watanabe K, Hayashi K, Miyamoto T, Tanaka M, Okano S, Yamamoto S. Characterization of ferritin and ferritin-binding proteins in canine serum. *Biometals* 2000;13:57–63. [PubMed: 10831225]
90. Sakamoto H, Kuboi T, Nagakura T, Hayashi S, Hoshi F, Mutoh K, Watanabe K, Orino K. Characterization of feline serum ferritin-binding proteins: the presence of a novel ferritin-binding protein as an inhibitory factor in feline ferritin immunoassay. *Biometals* 2009;22:793–802. [PubMed: 19326051]
91. Kirkali Z, Guzelsoy M, Mungan MU, Kirkali G, Yorukoglu K. Serum ferritin as a clinical marker for renal cell carcinoma: influence of tumor size and volume. *Urol Int* 1999;62:21–25. [PubMed: 10436426]
92. Hann HL, Stahlhut MW, Millman I. Human ferritins present in the sera of nude mice transplanted with human neuroblastoma or hepatocellular carcinoma. *Cancer Res* 1984;44:3898–3901. [PubMed: 6331659]
93. Selig RA, White L, Gramacho C, Sterling-Levis K, Fraser IW, Naidoo D. Failure of iron chelators to reduce tumor growth in human neuroblastoma xenografts. *Cancer Res* 1998;58:473–478. [PubMed: 9458092]
94. Marcus DM, Zinberg N. Measurement of serum ferritin by radioimmunoassay: results in normal individuals and patients with breast cancer. *J Natl Cancer Inst* 1975;55:791–795. [PubMed: 1185803]
95. Elliott RL, Elliott MC, Wang F, Head JF. Head, Breast carcinoma and the role of iron metabolism. A cytochemical, tissue culture, and ultrastructural study. *Ann N Y Acad Sci* 1993;698:159–166. [PubMed: 8279755]
96. Weinstein RE, Bond BH, Silberberg BK. Tissue ferritin concentration in carcinoma of the breast. *Cancer* 1982;50:2406–2409. [PubMed: 7139533]
97. Rossiello R, Carriero MV, Giordano GG. Distribution of ferritin, transferrin and lactoferrin in breast carcinoma tissue. *J Clin Pathol* 1984;37:51–55. [PubMed: 6323544]
98. Good MF, Powell LW, Halliday JW. Iron status and cellular immune competence. *Blood Rev* 1988;2:43–49. [PubMed: 3289653]
99. Weinberg JB, Hibbs JB Jr. Endocytosis of red blood cells or haemoglobin by activated macrophages inhibits their tumoricidal effect. *Nature* 1977;269:245–247. [PubMed: 563513]
100. Walker EM Jr, Walker SM. Effects of iron overload on the immune system. *Ann Clin Lab Sci* 2000;30:354–365. [PubMed: 11045759]
101. Bradbear RA, Bain C, Siskind V, Schofield FD, Webb S, Axelsen EM, Halliday JW, Bassett ML, Powell LW. Cohort study of internal malignancy in genetic hemochromatosis and other chronic nonalcoholic liver diseases. *J Natl Cancer Inst* 1985;75:81–84. [PubMed: 2989605]
102. Niederau C, Fischer R, Sonnenberg A, Stremmel W, Trampisch HJ, Strohmeyer G. Survival and causes of death in cirrhotic and in noncirrhotic patients with primary hemochromatosis. *N.Engl.J.Med* 1985;313:1256–1262. [PubMed: 4058506]
103. Kabat GC, Rohan TE. Does excess iron play a role in breast carcinogenesis? An unresolved hypothesis. *Cancer Causes Control* 2007;18:1047–1053. [PubMed: 17823849]
104. Buss JL, Torti FM, Torti SV. The role of iron chelation in cancer therapy. *Curr Med Chem* 2003;10:1021–1034. [PubMed: 12678674]
105. Kratz A, Ferraro M, Sluss PM, Lewandrowski KB. Case records of the Massachusetts General Hospital. Weekly clinicopathological exercises. Laboratory reference values. *N Engl J Med* 2004;351:1548–1563. [PubMed: 15470219]
106. Finch CA, Bellotti V, Stray S, Lipschitz DA, Cook JD, Pippard MJ, Huebers HA. Plasma ferritin determination as a diagnostic tool. *West J Med* 1986;145:657–663. [PubMed: 3541387]
107. Guyatt GH, Oxman AD, Ali M, Willan A, McIlroy W, Patterson C. Laboratory diagnosis of iron-deficiency anemia: an overview. *J Gen Intern Med* 1992;7:145–153. [PubMed: 1487761]
108. Hallberg L, Bengtsson C, Lapidus L, Lindstedt G, Lundberg PA, Hulten L. Screening for iron deficiency: an analysis based on bone-marrow examinations and serum ferritin determinations in a population sample of women. *Br J Haematol* 1993;85:787–798. [PubMed: 7918045]
109. Rushton DH, Barth JH. What is the evidence for gender differences in ferritin and haemoglobin? *Crit Rev Oncol Hematol*. 2009
110. Heath AL, Fairweather-Tait S, Worwood M. Reference limits for haemoglobin and ferritin. If it's not broken, don't fix it. *Bmj* 2001;323:806–807. author reply 807–808. [PubMed: 11669079]

111. Morison IM, Ferguson EL. Reference limits for haemoglobin and ferritin. Differences in haemoglobin concentrations reflect physiological differences. *Bmj* 2001;323:807–808. [PubMed: 11669080]
112. Jarjour IT, Jarjour LK. Low iron storage in children and adolescents with neurally mediated syncope. *J Pediatr* 2008;153:40–44. [PubMed: 18571533]
113. Krieger J, Schroeder C. Iron, brain and restless legs syndrome. *Sleep Med Rev* 2001;5:277–286. [PubMed: 12530992]
114. Clardy SL, Earley CJ, Allen RP, Beard JL, Connor JR. Ferritin subunits in CSF are decreased in restless legs syndrome. *J Lab Clin Med* 2006;147:67–73. [PubMed: 16459164]
115. Frauscher B, Gschliesser V, Brandauer E, El-Demerdash E, Kaneider M, Rucker L, Poewe W, Hogl B. The severity range of restless legs syndrome (RLS) and augmentation in a prospective patient cohort: association with ferritin levels. *Sleep Med* 2009;10:611–615. [PubMed: 19200780]
116. Earley CJ, Connor JR, Beard JL, Malecki EA, Epstein DK, Allen RP. Abnormalities in CSF concentrations of ferritin and transferrin in restless legs syndrome. *Neurology* 2000;54:1698–1700. [PubMed: 10762522]
117. Kalantar-Zadeh K, Regidor DL, McAllister CJ, Michael B, Warnock DG. Time-dependent associations between iron and mortality in hemodialysis patients. *J Am Soc Nephrol* 2005;16:3070–3080. [PubMed: 16033854]
118. Kirchbaum B. Profiling hemodialysis patients with high ferritin levels. *Clin Nephrol* 2001;56:117–123. [PubMed: 11522088]
119. Kirschbaum B. Serial ferritin concentrations in hemodialysis patients receiving intravenous iron. *Clin Nephrol* 2002;57:452–456. [PubMed: 12078949]
120. Kalantar-Zadeh K, Rodriguez RA, Humphreys MH. Association between serum ferritin measures of inflammation, nutrition and iron in haemodialysis patients. *Nephrol Dial Transplant* 2004;19:141–149. [PubMed: 14671049]
121. Wish JB. Assessing iron status: beyond serum ferritin and transferrin saturation. *Clin J Am Soc Nephrol* 2006;1 Suppl 1:S4–S8. [PubMed: 17699374]
122. Lee MH, Means RT Jr. Extremely elevated serum ferritin levels in a university hospital: associated diseases and clinical significance. *Am J Med* 1995;98:566–571. [PubMed: 7778572]
123. Olive A, Junca J. Elevated serum ferritin levels: associated diseases and clinical significance. *Am J Med* 1996;101:121–122. author reply 122.
124. Koduri PR, Shah PC, Goyal V, Mehta P. Elevated serum ferritin levels: associated diseases and clinical significance. *Am J Med* 1996;101:121–122. author reply 122. [PubMed: 8686709]
125. Evensen KJ, Swaak TJ, Nossent JC. Increased ferritin response in adult Still's disease: specificity and relationship to outcome. *Scand J Rheumatol* 2007;36:107–110. [PubMed: 17476616]
126. Sharkey RA, Donnelly SC, Connelly KG, Robertson CE, Haslett C, Repine JE. Initial serum ferritin levels in patients with multiple trauma and the subsequent development of acute respiratory distress syndrome. *Am J Respir Crit Care Med* 1999;159:1506–1509. [PubMed: 10228118]
127. Fisman DN. Hemophagocytic syndromes and infection. *Emerg Infect Dis* 2000;6:601–608. [PubMed: 11076718]
128. Henter JI, Horne A, Arico M, Egeler RM, Filipovich AH, Imashuku S, Ladisch S, McClain K, Webb D, Winiarski J, Janka G. HLH-2004: Diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. *Pediatr Blood Cancer* 2007;48:124–131. [PubMed: 16937360]
129. Arceci RJ. When T cells and macrophages do not talk: the hemophagocytic syndromes. *Curr Opin Hematol* 2008;15:359–367. [PubMed: 18536575]
130. Kaito K, Kobayashi M, Katayama T, Otsubo H, Ogasawara Y, Sekita T, Saeki A, Sakamoto M, Nishiwaki K, Masuoka H, Shimada T, Yoshida M, Hosoya T. Prognostic factors of hemophagocytic syndrome in adults: analysis of 34 cases. *Eur J Haematol* 1997;59:247–253. [PubMed: 9338623]
131. Coffernils M, Soupart A, Pradier O, Feremans W, Neve P, Decaux G. Hyperferritinemia in adult onset Still's disease and the hemophagocytic syndrome. *J Rheumatol* 1992;19:1425–1427. [PubMed: 1433011]
132. Emmenegger U, Frey U, Reimers A, Fux C, Semela D, Cottagnoud P, Spaeth PJ, Neftel KA. Hyperferritinemia as indicator for intravenous immunoglobulin treatment in reactive macrophage activation syndromes. *Am J Hematol* 2001;68:4–10. [PubMed: 11559930]

133. Castillo L, Carcillo J. Secondary hemophagocytic lymphohistiocytosis and severe sepsis/ systemic inflammatory response syndrome/multiorgan dysfunction syndrome/macrophage activation syndrome share common intermediate phenotypes on a spectrum of inflammation. *Pediatr Crit Care Med* 2009;10:387–392. [PubMed: 19325510]
134. Chapman RW, Morgan MY, Laulicht M, Hoffbrand AV, Sherlock S. Hepatic iron stores and markers of iron overload in alcoholics and patients with idiopathic hemochromatosis. *Dig Dis Sci* 1982;27:909–916. [PubMed: 7117074]
135. Erhardt A, Hauck K, Haussinger D. [Iron as comorbid factor in chronic hepatitis C]. *Med Klin (Munich)* 2003;98:685–691. [PubMed: 14685669]
136. Eisenbach C, Gehrke SG, Stremmel W. Iron, the HFE gene, and hepatitis C. *Clin Liver Dis* 2004;8:775–785. vii-viii. [PubMed: 15464655]
137. Lin TJ, Liao LY, Lin SY, Lin CL, Chang TA. Influence of iron on the severity of hepatic fibrosis in patients with chronic hepatitis C. *World J Gastroenterol* 2006;12:4897–4901. [PubMed: 16937477]
138. Metwally MA, Zein CO, Zein NN. Clinical significance of hepatic iron deposition and serum iron values in patients with chronic hepatitis C infection. *Am J Gastroenterol* 2004;99:286–291. [PubMed: 15046219]
139. Won JE, Jeong SH, Chung JI, Lee JH, Hwang SH, Kim JW, Lee SH, Kim N, Park YS, Lee DH, Kim H. Hepatic iron, serum ferritin, HFE mutation, and hepatic fibrosis in chronic hepatitis C. *Intervirolgy* 2009;52:239–246. [PubMed: 19602897]
140. Sezer S, Ozdemir FN, Guz G, Ozdemir BH, Sengul S, Arat Z, Turan M, Demirhan B, Haberal M. Ferritin levels can be a good predictor for HCV viremia in potential renal transplant candidates. *Transplant Proc* 2001;33:2796–2798. [PubMed: 11498163]
141. Beaton M, Guyader D, Deugnier Y, Moirand R, Chakrabarti S, Adams P. Noninvasive prediction of cirrhosis in C282Y-linked hemochromatosis. *Hepatology* 2002;36:673–678. [PubMed: 12198660]
142. Guyader D, Jacquelinet C, Moirand R, Turlin B, Mendler MH, Chaperon J, David V, Brissot P, Adams P, Deugnier Y. Noninvasive prediction of fibrosis in C282Y homozygous hemochromatosis. *Gastroenterology* 1998;115:929–936. [PubMed: 9753496]
143. Morrison ED, Brandhagen DJ, Phatak PD, Barton JC, Krawitt EL, El-Serag HB, Gordon SC, Galan MV, Tung BY, Ioannou GN, Kowdley KV. Serum ferritin level predicts advanced hepatic fibrosis among U.S. patients with phenotypic hemochromatosis. *Ann Intern Med* 2003;138:627–633. [PubMed: 12693884]
144. Fletcher LM, Dixon JL, Purdie DM, Powell LW, Crawford DH. Excess alcohol greatly increases the prevalence of cirrhosis in hereditary hemochromatosis. *Gastroenterology* 2002;122:281–289. [PubMed: 11832443]
145. Waalen J, Felitti VJ, Gelbart T, Beutler E. Screening for hemochromatosis by measuring ferritin levels: a more effective approach. *Blood* 2008;111:3373–3376. [PubMed: 18025154]
146. Asberg A, Thorstensen K, Irgens W, Hveem K. Screening for hemochromatosis. *Blood* 2008;111:3896. author reply 3897. [PubMed: 18362213]
147. Screening for hemochromatosis: recommendation statement. *Ann Intern Med* 2006;145:204–208. [PubMed: 16880462]
148. Adamkiewicz TV, Abboud MR, Paley C, Olivieri N, Kirby-Allen M, Vichinsky E, Casella JF, Alvarez OA, Barredo JC, Lee MT, Iyer RV, Kutlar A, McKie KM, McKie V, Odo N, Gee B, Kwiatkowski JL, Woods GM, Coates T, Wang W, Adams RJ. Serum ferritin level changes in children with sickle cell disease on chronic blood transfusion are non-linear, and are associated with iron load and liver injury. *Blood*. 2009
149. Busca A, Falda M, Manzini P, D'Antico S, Valfre A, Locatelli F, Calabrese R, Chiappella A, D'Ardia S, Longo F, Piga A. Iron overload in patients receiving allogeneic hematopoietic stem cell transplantation: quantification of iron burden by superconducting quantum interference device (SQUID) and therapeutic effectiveness of phlebotomies. *Biol Blood Marrow Transplant*. 2009
150. Kataoka K, Nannya Y, Hangaishi A, Imai Y, Chiba S, Takahashi T, Kurokawa M. Influence of pretransplantation serum ferritin on nonrelapse mortality after myeloablative and nonmyeloablative

- allogeneic hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 2009;15:195–204. [PubMed: 19167679]
151. Kim YR, Kim JS, Cheong JW, Song JW, Min YH. Transfusion-associated iron overload as an adverse risk factor for transplantation outcome in patients undergoing reduced-intensity stem cell transplantation for myeloid malignancies. *Acta Haematol* 2008;120:182–189. [PubMed: 19129689]
152. Mahindra A, Bolwell B, Sobecks R, Rybicki L, Pohlman B, Dean R, Andresen S, Sweetenham J, Kalaycio M, Copelan E. Elevated ferritin is associated with relapse after autologous hematopoietic stem cell transplantation for lymphoma. *Biol Blood Marrow Transplant* 2008;14:1239–1244. [PubMed: 18940678]
153. Pullarkat V, Blanchard S, Tegtmeier B, Dagus A, Patane K, Ito J, Forman SJ. Iron overload adversely affects outcome of allogeneic hematopoietic cell transplantation. *Bone Marrow Transplant* 2008;42:799–805. [PubMed: 18762767]
154. Sucak GT, Yegin ZA, Ozkurt ZN, Aki SZ, Karakan T, Akyol G. The role of liver biopsy in the workup of liver dysfunction late after SCT: is the role of iron overload underestimated? *Bone Marrow Transplant* 2008;42:461–467. [PubMed: 18604240]
155. Armand P, Kim HT, Cutler CS, Ho VT, Koreth J, Ritz J, Alyea EP, Antin JH, Soiffer RJ. A prognostic score for patients with acute leukemia or myelodysplastic syndromes undergoing allogeneic stem cell transplantation. *Biol Blood Marrow Transplant* 2008;14:28–35. [PubMed: 18158958]
156. Altes A, Remacha AF, Sarda P, Baiget M, Sureda A, Martino R, Briones J, Brunet S, Canals C, Sierra J. Early clinical impact of iron overload in stem cell transplantation. A prospective study. *Ann Hematol* 2007;86:443–447. [PubMed: 17279415]
157. Miceli MH, Dong L, Graziutti ML, Fassas A, Thertulien R, Van Rhee F, Barlogie B, Anaissie EJ. Iron overload is a major risk factor for severe infection after autologous stem cell transplantation: a study of 367 myeloma patients. *Bone Marrow Transplant* 2006;37:857–864. [PubMed: 16532017]
158. Altes A, Remacha AF, Sarda P, Sancho FJ, Sureda A, Martino R, Briones J, Brunet S, Canals C, Sierra J. Frequent severe liver iron overload after stem cell transplantation and its possible association with invasive aspergillosis. *Bone Marrow Transplant* 2004;34:505–509. [PubMed: 15286693]
159. Storey JA, Connor RF, Lewis ZT, Hurd D, Pomper G, Keung YK, Grover M, Lovato J, Torti SV, Torti FM, Molnar I. The transplant iron score as a predictor of stem cell transplant survival. *J Hematol Oncol* 2009;2:44. [PubMed: 19852846]
160. Holbein BE. Iron-controlled infection with *Neisseria meningitidis* in mice. *Infect Immun* 1980;29:886–891. [PubMed: 6159328]
161. Ballantyne GH. Rapid drop in serum iron concentration as a host defense mechanism. A review of experimental and clinical evidence. *Am Surg* 1984;50:405–411. [PubMed: 6465688]
162. Reid D, Snell G, Ward C, Krishnaswamy R, Ward R, Zheng L, Williams T, Walters H. Iron overload and nitric oxide-derived oxidative stress following lung transplantation. *J Heart Lung Transplant* 2001;20:840–849. [PubMed: 11502406]
163. Rao KV, Anderson WR. Hemosiderosis and hemochromatosis in renal transplant recipients. Clinical and pathological features, diagnostic correlations, predisposing factors, and treatment. *Am J Nephrol* 1985;5:419–430. [PubMed: 3909817]
164. Herget-Rosenthal S, Gerken G, Philipp T, Holtmann G. Serum ferritin and survival of renal transplant recipients: a prospective 10-year cohort study. *Transpl Int* 2003;16:642–647. [PubMed: 12732929]