

Hyperferritinemia is Associated with Serologic Antiphospholipid Syndrome in SLE Patients

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Abstract Ferritin may play a direct role on the immune system. We sought to determine if elevated levels of ferritin in lupus patients correlate with disease activity and organ involvement in a large cohort. Ferritin levels (gender and age adjusted) were assessed in 274 lupus serum samples utilizing the LIASON Ferritin automated immunoassay

method. Significant disease activity was determined if European Consensus Lupus Activity Index (ECLAM)>2 or Systemic Lupus Erythematosus Disease Activity Index (SLEDAI)>4. Utilizing an EXCEL database, we compared elevated ferritin levels to manifestations grouped by organ involvement, serology, and previous therapy. The patients

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were predominantly female (89%), median age was 37 years old, and disease duration was 10.6 ± 7.7 years. Hyperferritinemia was found in 18.6% of SLE patients. Compared to subjects with normal ferritin levels, a significantly greater proportion of patients with hyperferritinemia had thrombocytopenia (15.4% vs. 33.3%, $p=0.003$) and lupus anticoagulant (11.3% vs. 29.0%, $p=0.01$). Additionally, compared to normoferritinemic subjects, hyperferritinemic subjects had significantly higher total aCL (99.7 ± 369 vs. 30.9 ± 17.3 GPI, $p=0.02$) and aCL IgM antibody levels (75.3 ± 357.4 vs. 9.3 ± 10.3 GPI, $p=0.02$), and marginally lower aCL IgG antibody levels (9.2 ± 4.9 vs. 9.7 ± 3.9 GPI, $p=0.096$). While the ECLAM score significantly correlated with hyperferritinemia ($p=0.04$), the SLEDAI score was marginally associated with hyperferritinemia ($p=0.1$). Serositis was marginally associated with hyperferritinemia, but not with other manifestations. An association with serologic APS was encountered. Hyperferritinemia was associated with thrombocytopenia, lupus anticoagulant, and anti-cardiolipin antibodies suggest that it may be an early marker for secondary antiphospholipid syndrome in SLE patients.

Keywords Ferritin · Systemic lupus erythematosus · Antiphospholipid syndrome · Disease activity · Anti-cardiolipin antibodies · Lupus anticoagulant

Introduction

Iron Metabolism and Homeostasis

Iron metabolism in humans is 1–2 mg per day which is absorbed and excreted. Its homeostasis depends on the absorption from the gastrointestinal tract and involves molecular processes including protein products from interactions between hemochromatosis gene (HFE), transferrin (Tf), transferrin receptor (TfR), and the iron regulatory proteins (IRP) in the crypts of Lieberkuhn (tubular glands that line the small intestine), determining the rate of iron transport from the enterocyte into the circulation. HFE, Tf, and TfR interact among each other, while IRP interact with the mRNAs of TfR and ferritin. Most cells take up iron by way of the TfR and the transferrin to cell cycle. In short, the iron–TfR complex undergoes endocytosis, is incorporated in intracellular endosomes where it is acidified, is released from Tf and then co-transported out of the endosome by a divalent cation co-transporter (DCT1). The apotransferrin is transported to the cell surface and then to the circulation for further rounds of iron delivery. Iron can be targeted to the mitochondria. In non-erythroid cells, the iron is stored as ferritin and hemosiderin [1].

Ferritin is the major intracellular iron storage protein in all organisms. Ferritins share a unique protein nanocage structure containing polypeptide subunits. These cages incorporate Fe

atoms. Each apoferritin (iron-free ferritin) shell contains 24 polypeptide chains which are either a heavy subunit (H) or light subunit (L). H-ferritin is involved in detoxification of iron and intracellular iron transport and is found in the heart and pancreas, organs with low iron content. The L-subunit is involved in iron nucleation, mineralization, and long-term storage, and found in the liver and spleen, organs with high iron content [2, 3].

Iron and Ferritin

The primary role of ferritin is in homeostasis and regulation of iron sequestration. Iron has many functions. Iron in heme is responsible for the transport, binding, and release of oxygen to tissues. Iron is also important for enzymatic activity in the cell processes. In addition, iron can participate in the generation of hydroxyl radicals. In the state of iron toxicity, damage to tissues results from the generation of reactive species leading to damage of DNA, lipids, and proteins. Ferritin synthesis depends on the relationship between labile and chelatable iron. When iron levels are low, ferritin synthesis is decreased and reciprocally is true as well. The regulatory response of ferritin occurs posttranscriptionally leading to the recruitment of stored mRNA from monosomes to polysomes in the presence of iron. This process is mediated by iron responsive element (IRE) that has a stem-loop structure. To the stem loop bind two iron regulatory proteins 1 and 2 (IRP1 and IRP2) and lead to inhibition of mRNA translation [4].

The mechanisms of ferritin regulation include transcriptional regulation, via modulation of IRP proteins, or independent of IRP through mRNA and protein stability. Another mechanism includes the release of iron from sequestered intracellular compartments. The IRPs act in different ways. IRP1 exists in two forms: when iron is scarce, it assumes an open configuration, binds the IRE stem-loop, and represses ferritin translation. IRP2 is regulated by degradation; it is degraded rapidly when there is an abundance of iron through targeting a unique 73 amino acid sequence. Although altogether IRP1, IRP2, and IRE function simultaneously in ferritin synthesis inhibition, their roles may differ on tissues. Targeted deletions of IRPs in mice suggest that IRP2 is the primary posttranscriptional regulator of mammalian iron metabolism in response to the iron status. This concordant regulation of transferrin receptors and ferritin is designed so a cell can obtain iron when it is needed, and sequester iron when it is in excess [2, 4, 5].

For example, experimental IRP2 knockout mice present with intestinal and central nervous system aberrations of iron regulation not affecting IRP1. IRP1 is more abundant in the kidney, liver, intestine, and brain while IRP2 is more abundant in the pituitary and pro-B lymphocytic cell lines [4].

In addition to iron, ferritin synthesis is regulated by cytokines at various levels (transcriptional, posttranscriptional,

and translational) during development, cellular differentiation, proliferation, and inflammation. The cellular response by cytokines to infection stimulates the expression of ferritin genes. Tumor necrosis factor alpha (TNF- α) and interleukin-1 α (IL-1 α) alone or together induce the expression of the H chain of ferritin in mouse adipocytes, human muscle cells, and other cell types. Translation of ferritin is induced by IL-1 α , IL-6, or TNF- α in the HepG2 hepatic cell line, and iron is required for this regulation [5]. Ferritin expression is regulated by oxidative stress that acts either directly on gene expression or indirectly via modifications of IRPs activity. Expression of ferritin is also regulated by hormones, growth factors, second messengers, and hypoxia–ischemia and hyperoxia. Both oxidants and antioxidant response inducers regulate H-ferritin gene transcription [5].

Cytokines may also affect ferritin translation indirectly through their ability to induce nitric oxide synthase and hence increase NO to NO in turn causes the activation of both IRP1 and IRP2 (although effects on IRP2 appear to exhibit some cell type specificity), effects that may be particularly important in inflammatory conditions. Mechanisms hypothesized to underlie NO-mediated induction of IRP binding activity include cluster disassembly (IRP1), intracellular iron chelation (IRP1 and IRP2), or increased de novo synthesis (IRP2) [4]. Lipopolysaccharide (endotoxin), a component of the outer membrane of gram-negative bacteria, elicits a variety of reactions that involve ferritin [4].

Hormonal pathways are also involved in ferritin regulation. Thyroid hormone may regulate ferritin posttranscriptionally: T3 modulates the activity of IRP1, affecting its ability to bind to the ferritin IRE, possibly through induction of signal transduction cascades that result in phosphorylation of IRP1. T3 and TRH also induce the phosphorylation of IRP2. In addition to thyroid hormone, insulin, and insulin growth factor have also been implicated in regulation of ferritin at the mRNA level [4].

Macrophage ferritin accumulates during inflammation, when serum iron decreases and iron in specific cells increases, leading to ferritin with more iron/protein cage. Pathogen ferritins are the mini-ferritins that protect bacterial DNA when exposed to ferrous ions and hydrogen peroxide in vitro and confer cellular resistance to oxidative damage in vivo. During infection or inflammation, it is the relative “iron deficiency” created by the host redistribution of iron, with a deficit in serum and an increase in macrophages (the main stimulus for siderophore production), that is synthesized in response to environmental iron deficiency and associated with virulence [2].

Recent evidence points to immunological functions of ferritin located in the nucleus, cytoplasm, and mitochondria. In the nucleus, the DNA-binding protein family involved in homeostasis and the protection against oxidative stress has a structure similar to that of ferritin [6].

The expression of ferritin is under delicate control and is regulated at both the transcriptional and posttranscriptional levels by iron, cytokine release, T and B cell function, chemokine production, hormones, and oxidative stress.

H-ferritin binds signal elements involved in survival pathways. H-ferritin is a negative regulator of CXCL4. CXCL4 significantly upregulates monocytes, neutrophils, B cell subsets and plasma cells in murine models of lupus with nephritis which can be abrogated utilizing a blocking peptide [7]. CXCL4 enhances B cell survival, hence, propagating autoimmune mechanisms and with CXCL12 play an important role in human lupus as well [8].

The knockdown of H-ferritin prolongs the activation of downstream pathways. Nuclear ferritin levels increase following treatment with inflammatory cytokines such as TNF- α and IL-1 [6].

H-ferritin plays an important role in chemokine receptor-mediated signal transduction and migration contributing to the immunomodulatory activity of ferritin. Mutations in H-ferritin that inactivate its ferroxidase activity will abolish its myelo-suppressive activity [9].

The role of ferritin in the pathogenesis of autoimmunity is under investigation. The T cell immunoglobulin and mucin domain (TIM-2) gene family is involved in the regulation of immune responses. TIM-2 protein is expressed in B cells and renal tubule cells. The soluble ligand for TIM-2 is H-ferritin. TIM-2-deficient mice display increased inflammation and Th2 cytokine production in a mouse atopic model. Different mechanisms involving H-ferritin/TIM-2 interactions can inhibit the H-ferritin-mediated suppression of immune cells. In turn, the impaired immunosuppression may favor the loss of tolerance and the development of autoimmune diseases [10].

Ferritin and iron homeostasis have been implicated in the pathogenesis of many diseases [11–14], including diseases involved in iron acquisition, transport and storage (primary hemochromatosis) as well as in atherosclerosis [5], Parkinson's disease, Alzheimer disease, and Freidrich ataxia [1]. Genetic mutations of the ferritin IRE region as well as coding regions of ferritin cause some hereditary human diseases. Ferritin L IRE mutations cause the hereditary hyperferritinemia–cataract syndrome [11], which is an autosomal dominant disease characterized by elevated ferritin levels and early-onset bilateral cataracts. Neuro-ferritinopathy, a dominantly inherited movement disorder is characterized by decreased levels of ferritin and abnormal deposition of ferritin and iron in the brain, caused by a mutation in the C-terminus of the ferritin L gene [1]. Recently, a mutation of a hemochromatosis associated gene (HFE), C282Y, was associated with an increased risk of coronary artery disease and cardiovascular mortality in 3 cohort studies [5].

Hyperferritinemia in Clinical Disease

Hyperferritinemia is a marker for Adult Still's disease and macrophage activation syndrome (MAS) (a subset of hemophagocytic syndrome), both of which mechanistically are related to a cytokine storm involving interleukin-1 and interferon-gamma, tumor-necrosis factor (TNF)-alpha, with reduced function of cytotoxic T cells and natural killer cells [12, 13]. MAS is defined as expansion of T cells and macrophages leading to hemophagocytosis. While the syndrome is diagnosed based on findings in the bone marrow, these cells may be identified on occasion in other organs (liver, lymph nodes, skin, and lungs). The result is a systemic inflammatory response with amplified release of pro-inflammatory cytokines associated with increased morbidity and mortality. The clinical findings include cytopenia, liver dysfunction, and coagulopathy resembling DIC. Laboratory tests reveal elevated ferritin levels. MAS can be secondary to many autoimmune diseases. In the pediatric population systemic juvenile idiopathic arthritis, SLE, and Kawasaki disease are common. In the adult population, Still's disease, SLE, and vasculitis are common. The syndrome may be triggered by infections or certain drugs. In the hemophagocytic syndrome, also reported in SLE patients, ferritin levels are extremely elevated [12, 13].

The pathophysiology is due to mutations in the gene encoding perforin and another gene MUNC13-4 leading to defective cytolytic activity. Tests including low natural killer cells and increased interleukin-2 receptor levels are encountered.

The hemophagocytic macrophages in MAS express CD163 elucidating the role of hyperferritinemia in this syndrome. CD163 functions by binding hemoglobin-haptoglobin complexes and hence commencing the adaptations to oxidative stress induced by free heme and iron. Endocytosis of the hemoglobin-haptoglobin complex leads to increased production of ferritin that must sequester the free iron [15].

RA is an autoimmune disease involving predominantly the joints, associated with inflammation and elevated cytokine production of TNF- α and IL-1 γ . Serum levels of ferritin do not differ from those of controls, but do fluctuate within the normal range during disease activity. High concentrations of ferritin are found in the synovial fluid and synovial cells of RA patients [16, 17] and significant correlations between serum ferritin levels and disease activity by DAS28 score in RA patients was reported [18]. In our previous study of RA patients, the prevalence of hyperferritinemia was 4% and did not correlate with elevated titers of rheumatoid factor or anti-CCP antibodies [14].

Over the last few years, increased evidence has supported the role of iron dysregulation in the pathogenesis of multiple sclerosis (MS), as iron is essential for myelin

formation and oxidative phosphorylation. MS and its animal model, experimental allergic encephalomyelitis (EAE), are autoimmune disorders resulting in demyelination in the CNS. Data from several biochemical and pharmacological studies indicate that free radicals participate in the pathogenesis of EAE, and iron has been implicated as the catalyst leading to their formation [19]. In one study, apoferritin was injected to EAE mice in order to increase plasma ferritin levels and to evaluate disease activity. Additional mice received systemic injections of iron, which induces ferritin synthesis, in order to test the effects of exogenous iron on the disease course. Although plasma levels of ferritin were found to be elevated in both apoferritin and iron-injected EAE mice, only apoferritin treatment resulted in a reduction in disease activity. The suppressive effects of apoferritin administration suggest that the increase in endogenous ferritin levels that have been previously observed in the cerebrospinal fluid of chronic progressive MS patients with active disease might be functioning to limit the severity and spread of tissue damage [20]. Delivery of iron to the brain traditionally has been considered the responsibility of transferrin, where transferrin receptors are located primarily within gray matter areas. Another report provided evidence of ferritin binding sites primarily in white matter tracts in the brain. In brain tissue from MS patients, the normal pattern of transferrin and ferritin binding distributions is disrupted. Ferritin binding is absent in the lesion itself and in the immediate periplaque region within the white matter but returns to normal as the distance from the lesion becomes greater. These data suggest loss of ferritin binding is involved in or is a consequence of demyelination associated with MS [21–25]. In our previous study, 8% of MS patients had hyperferritinemia [14].

Thyroid hormone induces ferritin expression, and in one study, elevated ferritin levels in patients with subacute thyroiditis correlated with disease activity and decreased following steroid therapy. These levels were higher when compared to patients with Graves' disease and Hashimoto's thyroiditis [26].

In our previous study on ferritin levels in a cohort of autoimmune patients, hyperferritinemia was more frequently encountered in SLE patients compared to dermatomyositis, MS, and rheumatoid arthritis [14].

In this study, we expanded our cohort to assess the association of hyperferritinemia with lupus subsets and clinical activity.

Methods and Patients

Serum samples from 274 SLE patients were evaluated for elevated ferritin levels. The source of patients was from two

different European clinics: Hungary, $n=174$, Italy, $n=100$. Each clinic provided a database with demographic characteristics, and pertinent information regarding SLE which included clinical manifestations, serology, information regarding secondary antiphospholipid syndrome, and disease activity scores (European Consensus Lupus Activity Index—ECLAM and Systemic Lupus Erythematosus Disease Activity Index—SLEDAI).

Manifestations were further grouped by system involvement to simulate clinical disease and included cutaneous, joints, hematological, renal, and neuropsychiatric findings. Serology included ANA, antibodies to dsDNA, ENA (Ro/La/RNP/Sm), cardiolipin, β_2 GPI, lupus anticoagulant, and C3/C4.

The first group utilized the ECLAM score ($n=100$) for evaluation of disease activity while the second group utilized the SLEDAI score ($n=174$). Both are validated scores and widely utilized for the assessment of disease activity in SLE [19]. In order to relate to disease activity for the entire cohort, we arbitrarily chose cutoffs for disease activity: SLEDAI above 4 and ECLAM above 2. In either setting, a patient was termed as harboring significant disease activity. We evaluated the association of hyperferritinemia with medications utilized (corticosteroids, antimalarial, or others).

Diasorin Method

The LIASON ferritin method (DiaSorin S.p.A, Saluggia, Italy) is a chemiluminescent fully automated immunoassay. Monoclonal antibodies are utilized for the coating of solid phase (magnetic particles) and for the tracer. The tests were performed on the LIASON analyzer. Elevated levels of ferritin were gender and age adjusted. Normal ferritin levels were for premenopausal women (less than 45 years old): 4–104.2 ng/ml; postmenopausal women, 4.9–232.2 ng/ml; men, 18.2–341.2 ng/dl.

Statistical Analysis

Clinical manifestations were compared by composite score (which acquires a value of 0 or 1) using the chi-square test. Continuous variables were compared by composite score using ANOVA followed by the Bonferroni test, the t test for independent samples or the Mann–Whitney U as appropriate. To preserve the overall alpha of a given organ group, the grouped manifestation was tested by disease activity and if this was found to differ significantly, subsequent by-manifestation testing was performed.

Results

Of 274 lupus patients, 89% were female, the median age was 37 years old, and disease duration was 10.6 ± 7.7 years.

The mean ECLAM ($n=100$) was 3.2 ± 2.0 and the mean SLEDAI was 2.6 ± 2.8 . The mean ferritin levels were 153.6 ± 393.1 ng/ml.

Hyperferritinemia was found in 18.6% of the patients. Compared to subjects with normal ferritin levels, a significantly greater proportion of patients with hyperferritinemia had thrombocytopenia (15.4% vs. 33.3%, $p=0.003$) and lupus anticoagulant (11.3% vs. 29.0%, $p=0.01$). Additionally, compared to normoferritinemic subjects, hyperferritinemic subjects had significantly higher total aCL (99.7 ± 369 GPI vs. 30.9 ± 17.3 GPI, $p=0.02$) and aCL IgM antibody levels (75.3 ± 357.4 GPI vs. 9.3 ± 10.3 GPI, $p=0.02$), and marginally lower aCL IgG antibody levels (9.2 ± 4.9 GPI vs. 9.7 ± 3.9 GPI, $p=0.096$). Hyperferritinemic subjects also had marginally lower anti β_2 -GPI IgG levels (10.0 ± 15.3 vs. 11.2 ± 21.5 GPI, $p=0.1$).

Active disease was found in 74.8% of patients. While the ECLAM score was significantly higher in subjects with vs. without hyperferritinemia (4.25 ± 2.5 vs. 2.96 ± 1.8 , $p=0.04$), the SLEDAI score was marginally elevated in hyperferritinemic vs. normoferritinemic subjects (3.4 ± 3.2 vs. 2.4 ± 2.6 , $p=0.1$). No association was found between elevated concentrations of ferritin and cutaneous, joint, hematological, renal, or neuropsychiatric manifestations. An association between serologic antiphospholipid syndrome (APS) but not clinical APS was encountered.

Thrombocytopenia, lupus anticoagulant, and aCL antibodies were associated with hyperferritinemia in patients with active disease, especially aCL IgM (Figs. 1, 2, and 3).

Discussion

Some evidence for hyperferritinemia in lupus exists in the literature. In addition, some evidence pertains to elevated levels of ferritin in the urine of lupus nephritis patients [20], in pleural fluid [27], and in the cerebral spinal fluid of patients with neuropsychiatric lupus [27].

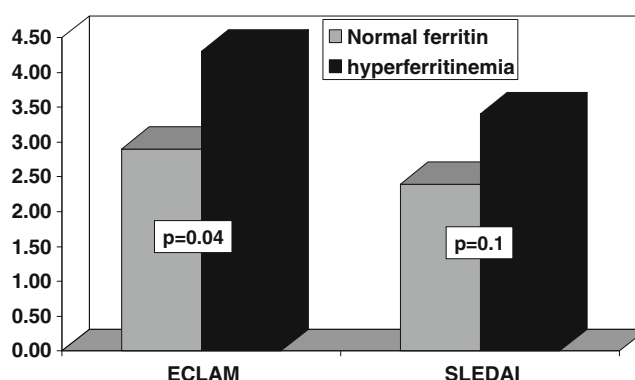


Fig. 1 SLE disease activity scores by ferritin status

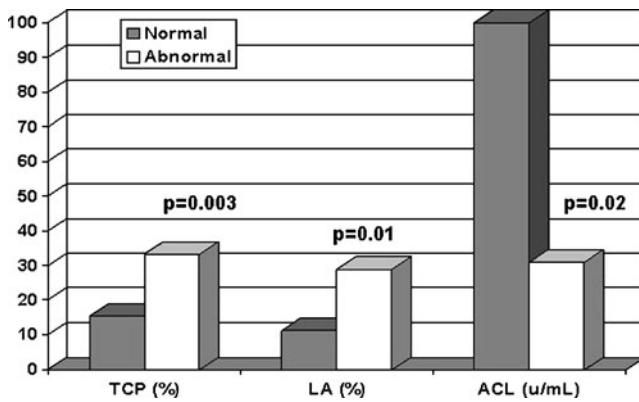


Fig. 2 Thrombocytopenia, lupus anticoagulant, and anti-cardiolipin antibodies by ferritin status in subjects with active disease. *TCP*, thrombocytopenia; *LA* lupus anticoagulant; *ACL* anti-cardiolipin antibodies

Elevated serum levels of ferritin were found in 128 SLE patients with active disease and correlated with serositis and hematological findings [28]. In another study on 72 SLE patients, elevated ferritin levels found in active disease decreased following treatment and correlated with a change in the SLEDAI score [29]. In a third study on 32 SLE patients, hyperferritinemia was associated with active disease, elevated titers of anti-dsDNA antibody titer, and low levels of CH50 [27]. Only one study did not find a correlation of ferritin levels with SLE disease course [30].

No formal studies have been performed investigating ferritin levels in APS prior to this one. Possible mechanisms may show the involvement of ferritin in atherosclerosis. Elevated ferritin levels are associated with an increased risk for atherosclerotic coronary artery disease. Recent proteomics and molecular biology studies have shown increased ferritin levels in diseased arteries and tissues [5]. Elevated nitric oxide induces free radicals, the oxidation of LDL, and increase in adhesion molecules. L-ferritin is detected in atherosclerotic plaques.

The pathogenic mechanisms leading to thrombosis in APS patients are not fully understood. Clearly, tissue factor expression by endothelial cells and monocytes occurs. Antiphospholipid antibodies induce tissue factor expression in monocytes from APS individuals by activation of the phosphorylation proteins (MEK1/ERK) and mitogen-activated protein (MAP) kinase dependent nuclear translocation and activation of NF- κ B/Rel proteins. Apolipoprotein E is linked to the pathophysiology of thrombosis. Hypoxia inducible factor (HIF-1 α) may also be linked to thrombosis as identified in a genomic and proteomic study [31]. Increased gene expression of hemoglobin may be implicated in recurrent fetal loss [32].

H-ferritin can suppress the proliferation of T cells in response to mitogens and impair the maturation of B cells in vitro and in vivo. This is further supported by the

inhibitory effect of a cloned human chimeric H-ferritin chain (placenta immunomodulator ferritin) that suppresses myelopoiesis and T cells by modulating the cytokine–chemokine network. The inhibitory effects of H-ferritin include signaling via specific receptors for H-ferritin on lymphocytes or its ability to induce IL-10 production on lymphocytes. H-ferritin can form a complex with the chemokine receptor CXCR4 leading to the activation of MAP kinase, important for cell proliferation, differentiation, and migration. H-ferritin can upregulate p53 and induce apoptosis [10, 33–35].

Activated monocytes and endothelial cells produce various cytokines and chemokines. Recent evidence purports to platelets harboring specific receptors for chemokines. Hence, chemokines may play a significant role in the thrombogenesis in APS, but yet needs to be investigated. In one study, chemokines CCL5 and CXCL1 induced by anti- β 2GPI antibodies had direct effects on platelets through platelet adhesion and aggregation [36].

CXCL12 attracts T cells, B cells, pre-B cells, and monocytes. Its receptor, CXCR4, is located on these types of cells and in addition, neutrophils and dendritic cells. Knockout experimental models validate B cell dysfunction, abnormal bone marrow myeloproliferation, cardiac, and cerebellar defects [36]. These chemokines activate important signaling pathways leading to the activation of STAT [8].

The non-iron-mediated roles for cytosolic ferritins involve the regulation of protein transcription, ApoB secretion, microtubule binding, and chemokine signaling [37]. Upregulation of ferritin gene expression can reduce CXCR4 neuroprotective functions.

Environmental xenobiotic pro-oxidants including experimental toxicants such as cigarette smoke, pesticide, and others that lead to the induction of antioxidant protective enzymes and are suggested to play a role in the induction of SLE may also be expected to induce ferritin as was demonstrated in experimental models and cell lines [4].

There were some caveats in this study. First, two different disease activity scores were utilized. We could not make a

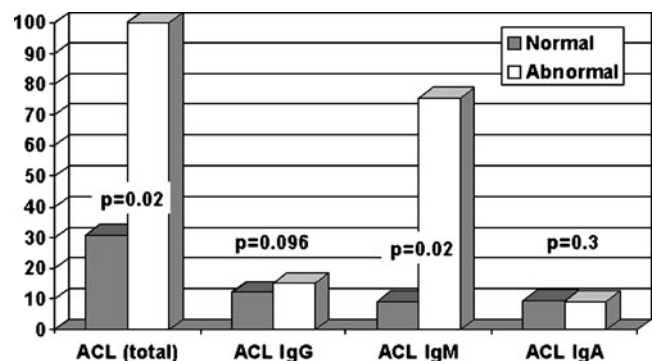


Fig. 3 Total ACL and subtypes by ferritin status in subjects with active disease

composite of the scores due to the differences in manifestations and weight of each manifestation in the score. However, we arbitrarily chose the cutoff points for disease activity among the disease activity scores. It could be that any disease activity (any score above 0) rather than mild–moderate activity as we chose would have lead to other results. We could not determine a subset of SLE patients recognizing a specific organ involvement. Moreover, no subset of APS patients was recognized. We did not perform serial samples in patients to compare changes within the individual for disease activity. Our patients did not harbor extremely elevated levels of ferritin. MAS was not determined in our study population.

Antibodies to cardiolipin are found in the circulation before the onset of SLE. This subset of patients has a more severe clinical course [38]. Hence, earlier detection of antiphospholipid antibodies in SLE patients and biomarkers such as elevated concentrations of serum ferritin may determine predictive primary prevention strategies in a subset of SLE patients for issues such as oral contraceptive use, pregnancy issues, and thromboembolic diversion. Future studies should include evaluation of these patients for the development of clinical APS. In addition, it would be interesting to assess hyperferritinemia in primary APS patients.

In conclusion, the pathogenesis of lupus remains complex and encompasses the innate and humoral pathways [39]. Drug-induced lupus following therapy with anti-TNF- α provides information that cytokine alteration can provoke disease [40]. Early detection of disease, recognition of the lupus-related syndromes including secondary APS may enable us to initiate the appropriate therapy at the commencement of the illness [41]. Ferritin plays multiple roles in the immune system. In this study, hyperferritinemia associated with thrombocytopenia, elevated lupus anticoagulant, and aCL antibodies, suggests that it may be an early marker for secondary APS in SLE patients.

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