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Authors

Rambod, Mehdi

Kovesdy, Csaba P

Kalantar-Zadeh, Kamyar

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Combined High Serum Ferritin and Low Iron Saturation in Hemodialysis Patients: The Role of Inflammation

Mehdi Rambod,*† Csaba P. Kovesdy,‡ and Kamyar Kalantar-Zadeh*†

*Harold Simmons Center for Kidney Disease Research and Epidemiology and †Division of Nephrology and Hypertension, Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance, California; and ‡Salem Veterans Affairs Medical Center, Salem, Virginia

Background: Serum ferritin, frequently used as a marker of iron status in individuals with chronic kidney disease, is also an inflammatory marker. The concurrent combination of high serum ferritin and low iron saturation ratio (ISAT) usually poses a diagnostic dilemma. We hypothesized that serum ferritin ≥500 ng/ml, especially in the seemingly paradoxical presence of ISAT level <25%, is more strongly associated with inflammation than with iron in maintenance hemodialysis (MHD) patients.

Design, setting, and participants: In 789 MHD patients in the Los Angeles area, the association of serum ferritin ≥500 ng/ml with inflammatory markers, including IL-6 (IL-6) and C-reactive protein levels, and malnutrition-inflammation score (MIS) was examined.

Results: After multivariate adjustment for case-mix and other measures of malnutrition-inflammation complex, MHD patients with serum ferritin ≥500 ng/ml and ISAT <25% had higher odds ratio for serum C-reactive protein ≥10 mg/L. The area under the receiver operating characteristic curves for the continuum of ISAT and IL-6 in detecting a serum ferritin ≥500 ng/ml were identical (0.57 *versus* 0.56, $P = 0.7$). The combination of IL-6 with ISAT yielded a higher area under the receiver operating characteristic curve (0.61) than either ISAT or IL-6 alone ($P = 0.03$ and $P = 0.02$, respectively).

Conclusion: In MHD patients, ferritin values above 500 ng/ml, especially in paradoxical conjunction with low ISAT, are associated with inflammation. Strategies to dissociate inflammation from iron metabolism to mitigate the confounding impact of inflammation on iron and to improve iron treatment responsiveness may improve anemia management in chronic kidney disease.

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Anemia is common in individuals with chronic kidney disease (CKD), including those undergoing maintenance hemodialysis (MHD) treatment and may be associated with poor outcome, including higher death risk (1). With widespread administration of erythropoiesis stimulating agent (ESA) since the early 1990s, anemia management has become one of the core components of the nephrology practice striving to achieve an adequate and stable hemoglobin level. Not infrequently, however, anemia is hyporesponsive to ESA resulting from various conditions, especially iron deficiency (2,3) and inflammation (4), leading to hemoglobin variability and adverse outcomes (5).

The most commonly used markers of iron management in CKD patients are iron saturation ratio (ISAT), also known as transferrin saturation ratio, and serum ferritin (6). Whereas serum ferritin is the main storage molecule for iron (7), it also is an acute phase reactant; *i.e.*, its serum concentration tends to increase moderately in the presence of inflammation (3,7,8), which occurs commonly in MHD patients (9). Moreover, inflammation is closely related to protein-energy wasting in dialysis patients (10) and the simultaneous combination of these two conditions, also referred to as malnutrition-inflammation-cachexia syndrome (MICS), is observed frequently in CKD patients (9). Concurrent to the poor clinical outcomes, MICS may also lead to moderate hyperferritinaemia and refractory anemia in the form of ESA hyporesponsiveness (4). Hence, the latest update of the National Kidney Foundation Kidney Disease and Dialysis Outcome Quality Initiative guidelines removed the upper limit of serum ferritin of 800 ng/ml to withhold iron supplementation and suggested to individualize iron treatment strategies if serum ferritin is >500 ng/ml (6). Nevertheless, the mention of 500 ng/ml cutoff levels in the said guidelines has led to some confusion among nephrologists who may not be sure whether iron treatment should be withheld in patients with such moderately high ferritin levels (11). One of the challenging areas of this decision-making process is dealing with MHD patients with serum ferritin ≥500 ng/ml but relatively low ISAT, *e.g.*, ISAT <25%. Even though a recent randomized controlled trial showed that such patients may still benefit from intravenous iron supplementation (12), the condi-

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Correspondence: Dr. Kamyar Kalantar-Zadeh, Harold Simmons Center for Kidney Disease Research and Epidemiology, Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, 1124 West Carson Street, C1-Annex, Torrance, CA 90502. Phone: 310-222-3891; Fax: 310-782-1837; E-mail: kamkal@ucla.edu

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tions that may lead to such a seemingly paradoxical combination have not been well studied. We hypothesized that, in MHD patients, moderately high ferritin levels, especially if combined with low ISAT, is more strongly associated with inflammation than with iron stores. Therefore, in the present study, we examined the relative contribution of inflammation and iron stores to high serum ferritin in a large and contemporary cohort of MHD patients, in that we first compared various clinical and paraclinical characteristics of the paradoxical high-ferritin/low-ISAT group with other MHD patients. Then, using several

Table 1. Baseline demographic, clinical, and laboratory variables in total and according to the four categories of serum ferritin (ng/ml) and iron saturation ratio (ISAT) (%) in 789 maintenance hemodialysis patients

	Ferritin <500 and ISAT <25 (n = 129, 16%)	Ferritin <500 and ISAT ≥25 (n = 271, 34%)	Ferritin ≥500 and ISAT <25 (n = 100, 13%)	Ferritin ≥500 and ISAT ≥25 (n = 289, 37%)	P (ANOVA)[a]
Demographic					
age, years	53.7 ± 15.6	52.0 ± 15.8	56.5 ± 12.2	53.9 ± 13.9	0.07
women, %	49	41	52	50	0.12
race, % black	43	26	45	29	<0.001
ethnicity, % Hispanic	42	57	36	52	0.001
primary insurance, % Medicare	57	49	49	53	0.6
diabetes mellitus, %	57	53	55	57	0.7
Charlson comorbidity score	1.7 ± 1.6	1.7 ± 1.5	2.1 ± 1.7	2.1 ± 1.6	0.047
Malnutrition-inflammation score	4.9 ± 4.2	4.4 ± 3.4	5.9 ± 3.6	5.2 ± 3.4	0.003
Body composition					
body mass index, kg/m^2	27.9 ± 7.1	26.0 ± 5.6	27.4 ± 6.3	26.2 ± 6.0	0.013
triceps skinfold, mm	20.0 ± 12.0	16.4 ± 8.8	18.8 ± 9.5	17.3 ± 9.9	0.007
biceps skinfold, mm	11.4 ± 9.4	9.2 ± 7.5	10.6 ± 8.3	9.6 ± 7.4	0.06
mid-arm muscle circumference, cm	27.0 ± 4.2	26.0 ± 4.5	26.2 ± 4.0	25.3 ± 4.5	0.6
near infrared-measured body fat, %	28.6 ± 11.5	25.0 ± 10.1	28.2 ± 11.8	26.6 ± 10.7	0.009
Hemodialysis treatment measures					
dialysis vintage < 6 months, %	39	29	14	12	<0.001
dialysis vintage, months	21.7 ± 29.3	24.2 ± 30.0	39.6 ± 39.0	38.2 ± 35.0	<0.001
dialysis dose, Kt/V single pool	1.56 ± 0.34	1.64 ± 0.33	1.54 ± 0.29	1.61 ± 0.27	0.01
residual urea clearance, ml/min	0.37 ± 1.1	0.37 ± 1.1	0.82 ± 7.2	0.87 ± 5.7	0.5
nPNA or nPCR, g/kg per day	1.02 ± 0.23	1.07 ± 0.25	1.04 ± 0.22	1.08 ± 0.24	0.11
erythropoietin dose, 1000 units/wk	21.5 ± 15.6	12.2 ± 9.2	19.7 ± 15.5	11.8 ± 11.1	<0.001
Biochemical measurements					
serum albumin, g/dl	3.79 ± 0.39	3.93 ± 0.39	3.84 ± 0.35	3.92 ± 0.36	0.002
transthyretin (prealbumin), mg/dl	26.1 ± 9.0	27.6 ± 8.9	27.1 ± 10.8	29.7 ± 10.1	0.002
creatinine, mg/dl	9.70 ± 3.3	10.1 ± 3.3	10.6 ± 3.3	10.5 ± 3.3	0.07
ferritin, ng/ml	215 ± 148	256 ± 141	892 ± 320	915 ± 392	<0.001
TIBC, mg/dl	228 ± 46	213 ± 34	198 ± 35	198 ± 38	<0.001
iron saturation ratio, %	20.4 ± 3.8	35.6 ± 9.4	20.6 ± 3.5	38.7 ± 11.5	<0.001
iron, mg/dl	46.0 ± 11.2	75.6 ± 22.2	40.8 ± 10.1	76.64 ± 28.9	<0.001
calcium, mg/dl	9.3 ± 0.8	9.3 ± 0.7	9.4 ± 0.6	9.4 ± 0.7	0.008
phosphorus, mg/dl	6.0 ± 1.4	5.7 ± 1.5	6.3 ± 1.7	5.6 ± 1.4	0.06
bicarbonate, mg/dl	22.3 ± 3.0	22.2 ± 2.9	22.0 ± 2.9	22.2 ± 2.8	0.8
total homocysteine, μmol/l	22.2 ± 9.4	22.8 ± 10.2	26.8 ± 17.5	24.4 ± 9.5	0.006
C-reactive protein, mg/l	8.1 ± 7.8	4.6 ± 7.0	7.2 ± 6.0	5.3 ± 6.5	<0.001
IL-6, pg/ml	18.0 ± 32.9	15.4 ± 53.8	18.9 ± 23.7	18.8 ± 48.3	0.8
TNF-α, pg/ml	10.7 ± 16.6	8.7 ± 12.7	8.6 ± 12.0	8.6 ± 8.8	0.4
blood hemoglobin, g/dl	11.9 ± 1.0	12.3 ± 0.9	11.7 ± 1.1	12.1 ± 0.9	<0.001
WBC, ×1000 cell/μl	7.5 ± 2.2	6.8 ± 1.8	8.0 ± 2.3	7.2 ± 2.0	<0.001
lymphocyte, % of total WBC	21.7 ± 8.1	22.3 ± 7.9	19.1 ± 7.2	23.8 ± 7.4	<0.001

Values are mean SD. Kt/V, dialysis dose; TIBC, total iron binding capacity; nPCR, normalized protein catabolic rate; IL-6, Interleukin 6; TNF-α, tumor necrosis factor-α.

[a]P values for dialysis dose (vintage), ferritin, CRP, IL-6, and TNF-α are based on the logarithmic values of these measures.

inflammatory markers and pro-inflammatory cytokines, we examined the extent to which inflammation is responsible for the moderately high serum ferritin concentrations ≥500 ng/ml, especially when combined with ISAT <25%.

Materials and Methods

Patient Population

We studied MHD patients who were participating in the Nutritional and Inflammatory Evaluation in Dialysis (NIED) Study (13). The original patient cohort was derived from a pool of approximately 3000 MHD outpatients over 5 yr in eight DaVita outpatient dialysis facilities in the South Bay Los Angeles area (see NIED Study website: www.NIEDStudy.org for more details and previous publications) (14–16). Inclusion criteria were outpatients who had been undergoing MHD for at least 8 wk, were 18 yr or older and who signed the Institutional Review Board approved consent form. Patients with acute infection or an anticipated life expectancy of less than 6 mo (for example, due to a metastatic malignancy or advanced HIV/AIDS disease) were excluded. From October 1, 2001, through December 31, 2006, 893 MHD patients signed the informed consent form and underwent the periodic evaluations of the NIED Study. For this study, data including baseline serum ferritin, iron and total iron binding capacity (TIBC), were available in 789 MHD patients. The medical chart of each MHD patient was thoroughly reviewed by a collaborating physician, and data pertaining to underlying kidney disease, cardiovascular history, and other comorbid conditions were extracted. A modified version of the Charlson comorbidity index, *i.e.*, without the age and kidney disease components, was used to assess the severity of comorbidities (17).

Table 2. Unadjusted and multivariate adjusted Pearson's correlation coefficient of baseline serum ferritin and other relevant variables in 789 maintenance hemodialysis patients

Variable	Unadjusted	Case-mix[a] adjusted	Case-mix + albumin + inflammatory markers (full model)[b]
General variables			
age	0.09[d]	0.05[c]	0.04
modified Charlson comorbidity score	0.14[f]	0.12[f]	0.12[e]
Malnutrition-inflammation score (MIS)	0.16[f]	0.15[f]	0.14[f]
Nutritional variables			
body mass index	−0.04	−0.06[c]	−0.08[d]
serum creatinine	0.02	0.02	0.02
triceps skinfold	−0.04	−0.08[d]	−0.09[d]
biceps skinfold	−0.03	−0.04	−0.06[c]
mid-arm muscle circumference	0.03	0.03	0.03
near infrared measured body fat	0.01	−0.06[c]	−0.08[d]
lean weight	−0.09[d]	−0.09[d]	−0.11[e]
nPNA (nPCR)	0.06[c]	0.08[d]	0.07[c]
Laboratory variables			
serum albumin	−0.05	−0.02	−0.02
prealbumin (transthyretin)	0.07[c]	0.11[e]	0.15[f]
creatinine	0.02	0.02	0.03
iron	0.11[e]	0.14[f]	0.17[f]
iron saturation ratio	0.22[f]	0.23[f]	0.26[f]
TIBC (transferrin)	−0.27[f]	−0.21[f]	−0.21[f]
calcium	0.07[d]	0.04	0.04
phosphorus	−0.07[d]	−0.07[c]	−0.07[d]
homocysteine	0.03	0.00	0.01
C-reactive protein (logarithm)	0.08[d]	0.06[c]	0.04
IL-6 (logarithm)	0.12[e]	0.06[c]	0.03
TNF-α (logarithm)	0.06[c]	0.04	0.03
erythropoietin dose	0.00	−0.01	−0.03

nPCR, normalized protein catabolic rate; IL-6, interleukin 6; TNF-α, tumor necrosis factor-α.

[a]Case-mix variables include age, gender, race/ethnicity, diabetes, dialysis vintage, insurance (Medicare), marital status, modified Charlson comorbidity score, dialysis dose (Kt/V), and residual urea clearance (KRU).

[b]Full model consist of case-mix variables, albumin, and three inflammatory markers: C-reactive protein, interleukin-6, and tumor necrosis factor-α.

[c]$P = 0.20$ to 0.05.

[d]$P = 0.05$ to 0.01.

[e]$P = 0.01$ to 0.001.

[f]$P < 0.001$.

Anthropometric Measures

Body weight assessment and anthropometric measurements were performed while patients were undergoing a hemodialysis treatment or within 5 to 20 min after termination of the treatment. Biceps skinfold and triceps skinfold thicknesses were measured with a conventional skinfold caliper using standard techniques as described previously (18–20).

Near Infrared Interactance

To estimate the percentage of body fat and fat-free body mass, near infrared (NIR) interactance was measured at the same time as the anthropometric measurements (20,21). A commercial NIR interactance sensor with a coefficient of variation of 0.5% for total body fat measurement (portable Futrex 6100, Gaithersburg, MD, www.futrex.com) was used. NIR measurements were performed by placing, for several seconds on the upper aspect of the arm without a vascular access, a Futrex sensor, and entering the required data (date of birth, gender, weight, and height) of each patient. NIR measurements of body fat appear to correlate significantly with other nutritional measures in MHD patients.

Malnutrition-Inflammation Score

The Malnutrition-Inflammation Score (MIS) was assessed based on its 10 components at 4 levels of severity from 0 (normal) to 3 (severely abnormal) and included five nutritional history criteria (weight change, dietary intake, gastrointestinal symptoms, functional capacity, and comorbid conditions), two physical examination components (subcutaneous body fat and signs of muscle wasting), body mass index (>20, 18 to 19.99, 16 to 17.99, and <16 kg/m^2), and serum albumin (≥4.0, 3.5 to 3.9, 3.0 to 3.4, and <3.0 g/dl) and TIBC concentrations (≥250, 200 to 249, 150 to 200, and <150 mg/dl). The sum

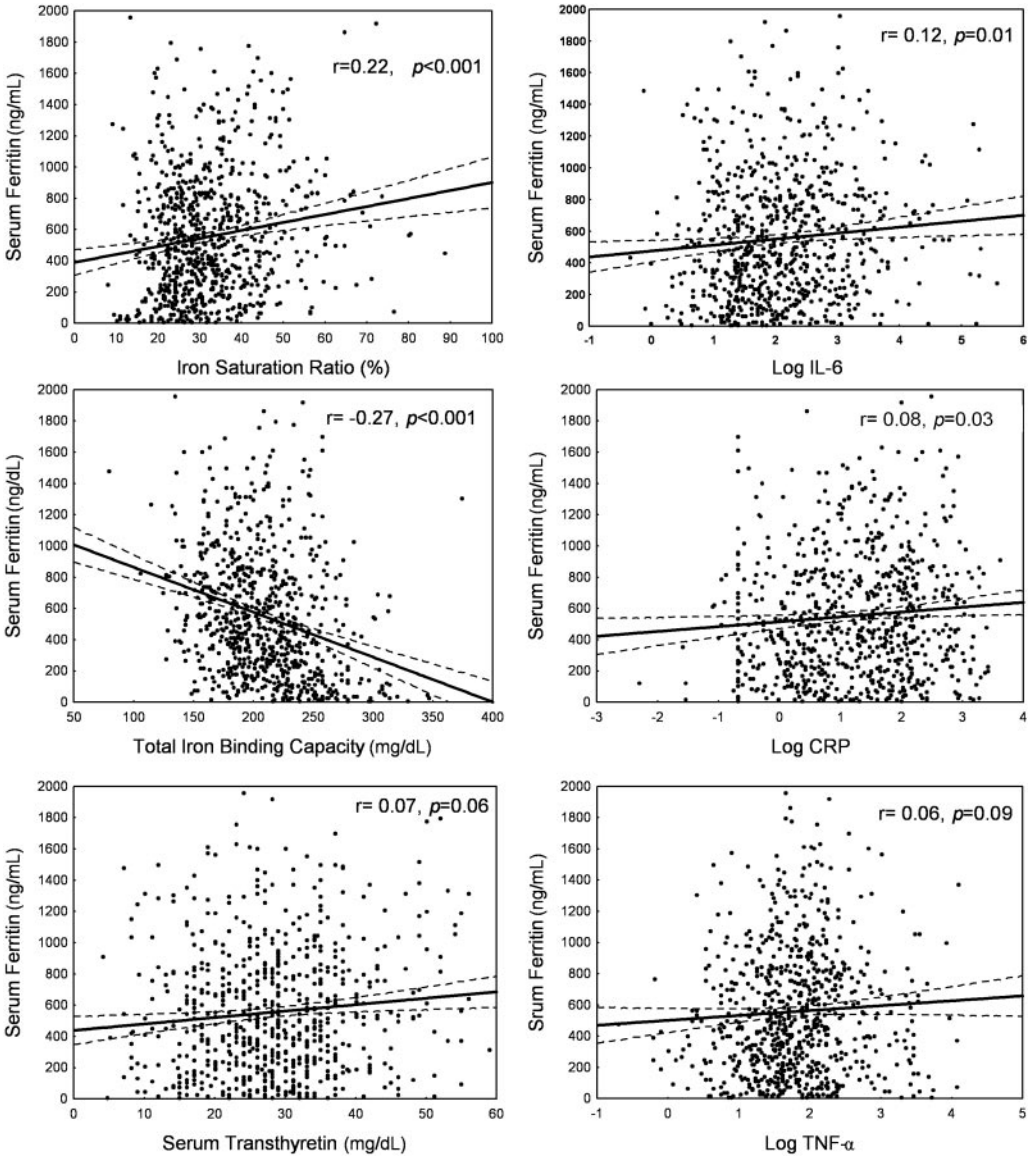

Figure 1. Scatter plots, regression line, and 95% confidence intervals, reflecting the correlation between serum ferritin and baseline serum transthyretin, iron saturation ratio, total binding capacity, log for C-reactive protein (CRP), log for IL-6 (IL-6), and log for TNF alpha (TNF-alpha).

of all 10 MIS components can range from 0 (normal) to 30 (severely malnourished) (22,23).

Laboratory Tests

Predialysis blood samples and postdialysis serum urea nitrogen were obtained on a mid-week day, which coincided chronologically with the drawing of quarterly blood tests in the DaVita dialysis clinics. The single-pool Kt/V was used to represent the weekly dialysis dose. Iron and ferritin were measured via automated radioimmunoassay methods (24,25). TIBC was measured using the summation of a measured serum total iron and a measured unbound iron binding capacity, which is measured spectrophotometrically (26). All routine laboratory measurements were performed by DaVita Laboratories (Deland, FL) using automated methods.

Serum high sensitivity C-reactive protein (CRP) was measured by a turbidometric immunoassay in which a serum sample is mixed with latex beads coated with anti-human CRP antibodies forming an insoluble aggregate (WPCI, Osaka, Japan; unit, mg/L; normal range, <3.0 mg/L) (27,28). IL-6 and TNF-α were measured with immunoassay kits based on a solid phase sandwich ELISA using recombinant human IL-6 and TNF-α (R&D Systems, Minneapolis, MN; units, pg/ml; normal range, IL-6: <9.9 pg/ml; TNF-α, <4.7 pg/ml) (29–31). CRP and the cytokines were measured in the General Clinical Research Center Laboratories of Harbor-UCLA Medical Center. Serum transthyretin (prealbumin) was measured using immunoprecipitin analysis. Plasma total homocysteine concentrations were determined by high performance liquid chromatography in the Harbor-UCLA Clinical Laboratories.

Statistical Methods

χ^2 test and analysis of variance with Bonferroni correction were used to examine the differences between four groups of patients defined based on the ferritin and ISAT levels. Pearson's correlation coefficient (r) was used for analyses of linear associations. Multivariate regression analyses and analysis of covariance were performed to obtain adjusted P values controlled for case-mix and other covariates. Multivariate logistic regression analysis was used to examine the predictive power of IL-6 ≥10 (pg/ml), CRP ≥10 (mg/L), TNF-α ≥5 (pg/ml), and MIS ≥5 in predicting ferritin ≥500 ng/ml. Multivariate logistic regression models were also developed to assess the predictive value of IL-6 ≥10 (pg/ml) and CRP ≥10 (mg/L) according to four categories of serum ferritin and ISAT.

To express the ability of ISAT, IL-6, and their combination to predict moderately high ferritin values, we constructed receiver operating characteristic (ROC) curves each of the aforementioned predictors and serum ferritin ≥500 ng/ml as the reference variable. Then the differences between the areas under ROC curves were examined using *"roccomp"* command in Stata. In a ROC curve, sensitivity (y-axis) is plotted *versus* one minus specificity (x-axis) for each possible cutoff value of ISAT, IL-6, or probability function obtained from the logistic regression model containing both ISAT and IL-6 as independent variables and serum ferritin ≥500 ng/ml as dependent (reference) variable (32). The area under the curve represents the discriminative power of the test. Values are expected to be between 0.5 (indicating no discriminative ability) and 1.0 (indicating highest detection accuracy).

Case-mix and comorbidity covariates included gender, age, race and ethnicity (Hispanics, blacks, Asians and others), diabetes mellitus, the modified Charlson comorbidity scale, dialysis vintage (number of months on MHD treatment), and clinical and laboratory measures of MICS. Nonlinear associations as continuous mortality predictors were also examined using restricted cubic splines as an alternative to inappropriate linearity assumptions (33). Fiducial limits are given as mean ± SD or median and interquartile range; risk ratios include 95% confidence interval levels. A P value <0.05 or a 95% confidence interval that did not span 1.0 was considered to be statistically significant. A P value between 0.05 and 0.20 is also listed with 2 decimals to identify potential type II errors. Descriptive and multivariate statistics were carried out with the statistical software "Stata version 10.0" (Stata Corporation, College Station, TX).

Results

General Characteristics

The 789 MHD patients were 53.5 ± 14.7 yr old (mean ± SD); 47% of patients were women, 50% Hispanic, 32% black, and 55% diabetic. The mean dialysis vintage was 31 ± 34 mo (median, 19 mo; interquartile range, 7 to 44 mo). The average (mean ± SD) baseline serum ferritin in all MHD patients was 571 ± 437 ng/ml (median, 489 ng/ml; minimum, 9 ng/ml;

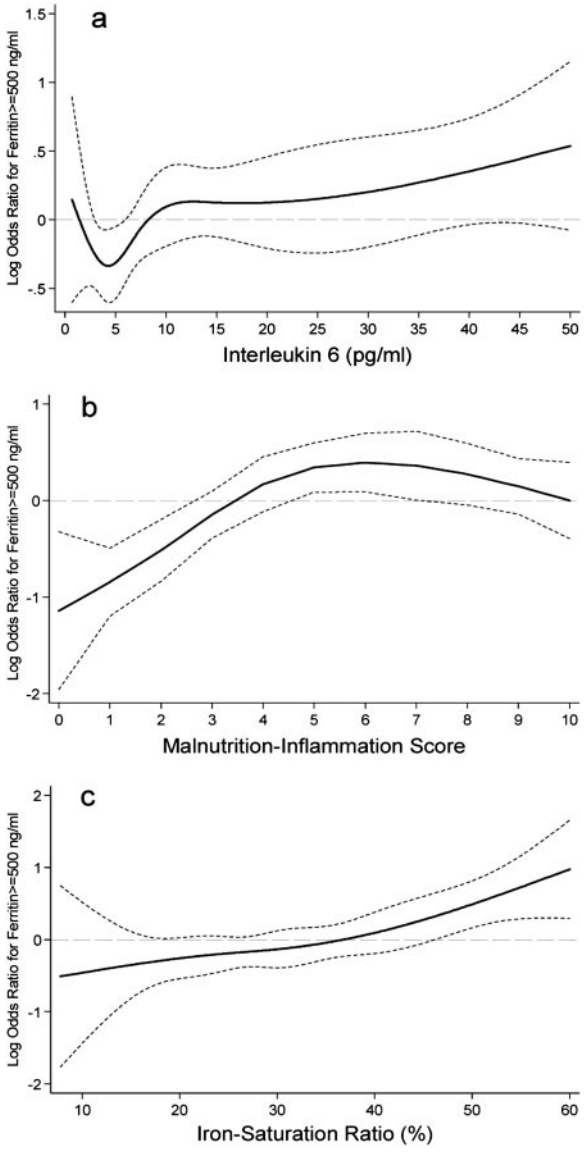

Figure 2. Log odds ratio of having ferritin ≥500 ng/ml in 789 maintenance hemodialysis patients. (A) IL-6. (B) Malnutrition-inflammation score (MIS). (C) Iron saturation ratio (%).

maximum, 2921 ng/ml; interquartile range, 234 to 810 ng/ml). Table 1 shows the values of demographic, clinical, and paraclinical variables in 4 groups of patients classified based on the value of serum ferritin and ISAT. Patient age and dialysis vintage were somewhat higher in patients with ferritin ≥500 ng/ml and ISAT <25%.

Linear Associations

Table 2 shows the correlation coefficients of relevant clinical, nutritional, and inflammatory measures with serum ferritin levels in 789 MHD patients. Serum ferritin was positively correlated with the modified Charlson comorbidity score, MIS, and prealbumin as well as serum iron and ISAT. Its positive correlation with markers of inflammation (*i.e.*, CRP, IL-6, and TNF-α) was attenuated after controlling for case-mix and other confounding variables (Table 2). Figure 1 demonstrates the positive linear association between serum ferritin and ISAT, as well as serum transthyretin and also illustrates that, by increasing the level of inflammatory markers, the level of serum ferritin tends to increase.

Figure 2 shows the unadjusted associations of IL-6, MIS, and ISAT with possibility of having a ferritin ≥500 ng/ml. In general, the higher levels of IL-6, MIS, and ISAT were associated with higher possibility of having a ferritin ≥500 ng/ml. The odds ratios related to these analyses are provided in Table 3, indicating that patients with IL-6 ≥10 pg/ml, MIS ≥5, or ISAT ≥25% had higher chance of having a ferritin ≥500 ng/ml. These associations were robust to adjustment for case-mix and MICS variables. Figure 3 shows the combined (three-dimensional) association of ISAT and IL-6 with odds of a ferritin ≥500 ng/ml, which increased somewhat linearly with rising levels of serum IL-6 or ISAT.

To examine the predictive power of ISAT, IL-6, and their combination in detecting a ferritin ≥500 ng/ml in MHD patients, analysis of area under the ROC curves (AUROC) was used. As shown in Figure 4, the AUROC for the continuum of ISAT and IL-6 were similar (0.57 *versus* 0.56, $P = 0.65$). Combining both IL-6 and ISAT together yielded a significantly higher AUROC (0.61), which was higher than either ISAT or IL-6 alone ($P = 0.025$ and $P = 0.018$, respectively).

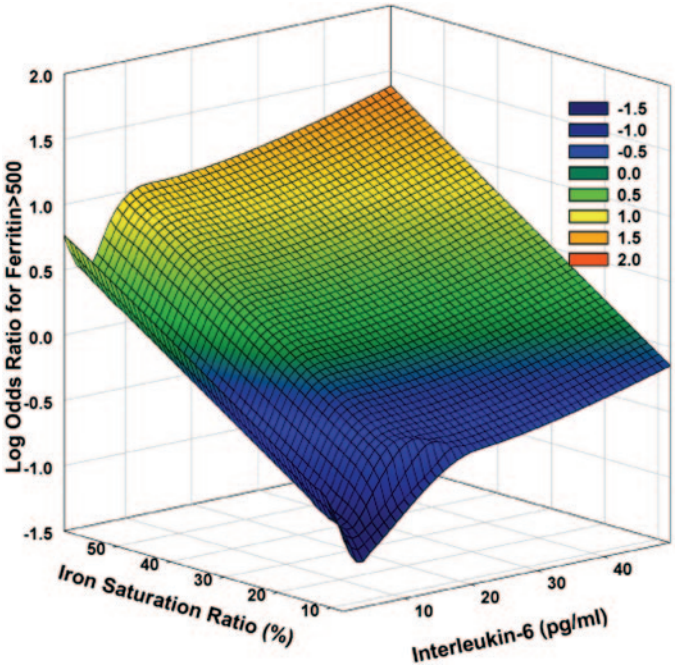

Figure 3. The relationship between the dependent variable, Log odds ratio ferritin ≥500 ng/ml and independent variables (IL-6 and iron saturation ratio).

The unadjusted associations of IL-6 or MIS with the odds of having a paradoxical iron marker constellation, *i.e.*, ferritin ≥500 ng/ml with concomitant ISAT <25%, are shown in Figure 5. These associations showed similar trends as noted in Figure 2.

Multivariate logistic regression analysis was used to examine the odds ratios of combined serum ferritin ≥500 ng/ml and ISAT <25% for three inflammatory measures, *i.e.*, IL-6, CRP, and MIS. As shown in Table 4 and Figure 6, higher odds of a CRP ≥10 mg/L were observed for this group of patients, even after controlling for case-mix and other MICS variables. The

Table 3. Multivariate logistic regression analyses for predicting serum ferritin ≥500 ng/ml ($n = 389$) in 789 maintenance hemodialysis patients

	n	Unadjusted		Case-mix[a] adjusted		Case-mix and MICS[b] adjusted	
		OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
Interleukin-6 ≥ 10 pg/ml	284	1.54 (1.15–2.07)	0.004	1.39 (1.01–1.91)	0.04	1.53 (1.06–2.19)	0.02
MIS[c] ≥5	328	1.68 (1.26–2.25)	<0.001	1.55 (1.12–2.15)	0.009	1.68 (1.18–2.39)	0.004
Iron saturation ratio[d] ≥ 25%	560	1.38 (1.01–1.87)	0.04	1.37 (0.98–1.92)	0.07	1.49 (1.03–2.17)	0.04

MIS, Malnutrition-inflammation score.

[a]Case-mix variables include age, gender, race/ethnicity, diabetes, dialysis vintage, insurance (Medicare), marital status, modified Charlson comorbidity score, dialysis dose (Kt/V), and residual urea clearance (KRU).

[b]Malnutrition-inflammation complex syndrome (MICS) variables include albumin, log erythropoietin dose, creatinine, hemoglobin, phosphorus, total iron binding capacity (TIBC), normalized protein catabolic rate (nPCR), bicarbonate, calcium, white blood count (WBC), lymphocyte percent, body mass index (BMI), and log vitamin D dose.

[c]Albumin, TIBC, and BMI are not included in MICS adjusted model examining MIS.

[d]TIBC is not included in the MICS adjusted model examining iron saturation ratio.

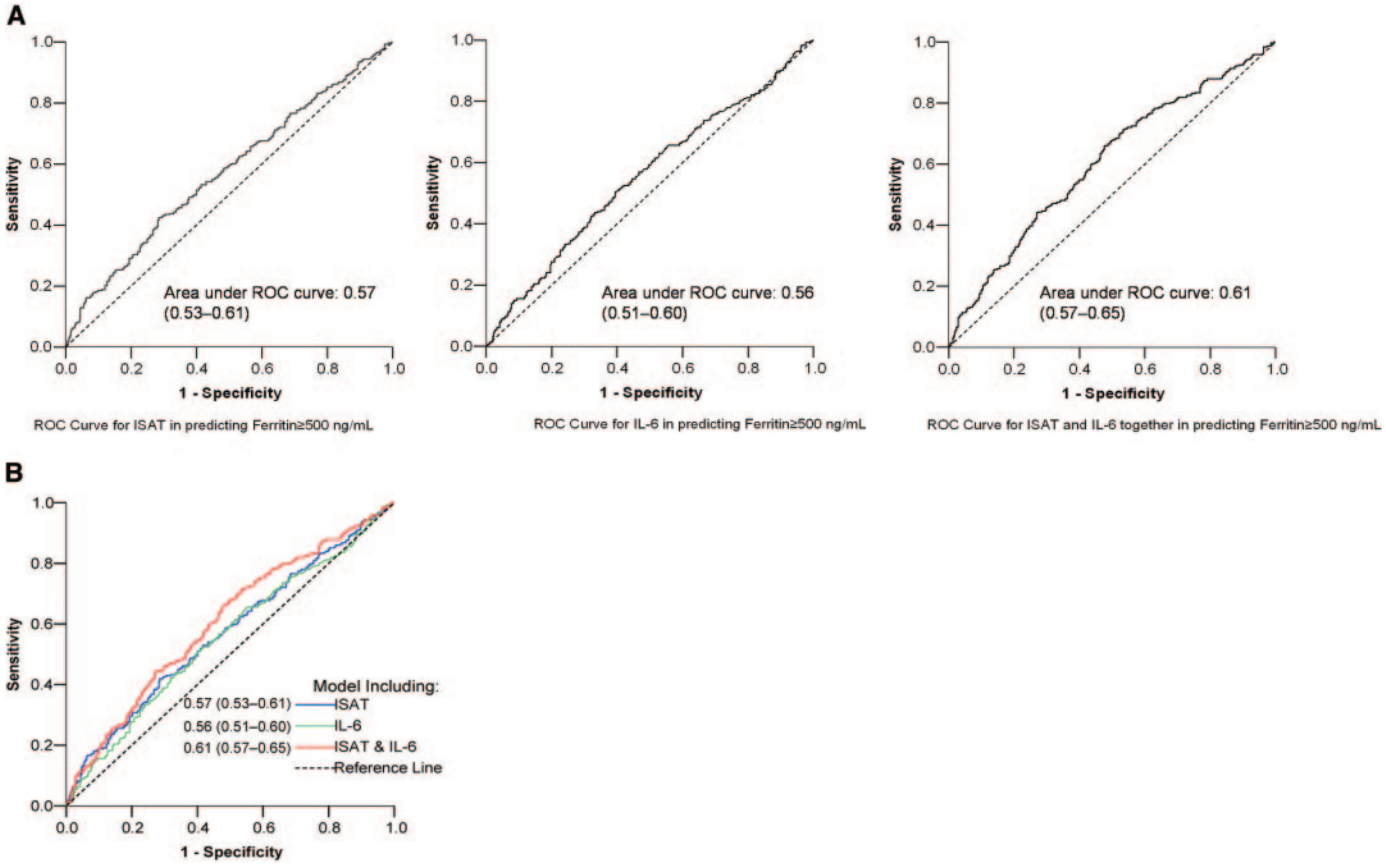

Figure 4. Receiver operating characteristic (ROC) curves of probabilities obtained from logistic regression models including (right) iron saturation ratio (ISAT), (middle) IL-6, and (left) ISAT and IL-6 together as independent variables and serum ferritin ≥500 ng/ml as dependent (reference) variable. Values in parentheses are 95% confidence intervals of the calculated area under the ROC curves.

odds of IL-6 ≥10 pg/ml showed a similar trend, although this association was attenuated after controlling for MICS variables.

Discussion

In this study, in 789 MHD patients in Southern California, we found that patients with the seemingly paradoxical combination of high serum ferritin ≥500 ng/ml but low ISAT <25% had higher inflammatory state compared with other MHD patients. The independent likelihood of inflammation as shown by serum CRP ≥10 mg/L was two times higher in this group compared with those with serum ferritin <500 ng/ml or ISAT ≥25%. This group of MHD patients also had other sins of malnutrition and inflammation, such as higher white blood cell count and lower lymphocyte percentage (34). Both ISAT and IL-6 contributed somewhat equally to high serum ferritin levels >500 ng/ml, indicating that such moderately high ferritin concentrations in MHD patients can happen due to noniron related factors, including inflammation.

Ferritin molecule, with an average molecular weight of 450 kDa, is the main storage molecule for iron and nature's solution to the difficult chemistry of iron and oxygen because it stores iron in a safe and soluble manner that allows for regulated release of iron and mitigates the risk for oxidation *via* free iron atoms (35,36). During the acute-phase response, proinflammatory cytokines increase the synthesis of various subunits of ferritin molecule through an increased translation of preformed ferritin mRNA (37–39). Such alterations are usually parallel to increased hepcidin activity (40,41). Inflammation-induced hyperferritinemia may result in a so-called "functional iron deficiency," which can block iron mobility and, hence, be useful in "acute" inflammation by iron containment in the reticuloendothelial system but harmful under "chronic" inflammation by leading to refractory anemia, such as in CKD or other chronic disease states (7). The results of our study are consistent with the role of inflammation in confounding such iron markers, especially ferritin.

Serum iron, ISAT, and ferritin are the most commonly used laboratory indicators in the diagnosis and management of iron deficiency anemia in CKD patients (7). However, in addition to iron stores, such non–iron-related factors as inflammation and nutritional status may have a bearing on variability of iron markers, especially serum ferritin variability (42). Moderately high serum ferritin concentrations, *e.g.*, in the 500 to 1200 ng/ml range, maybe happened independent of body iron stores (11). A recent report from a large national database of MHD patients in the United States showed that nearly half of all MHD patients in the United States have a serum ferritin ≥500 ng/ml (43). Similarly, historical studied had suggested

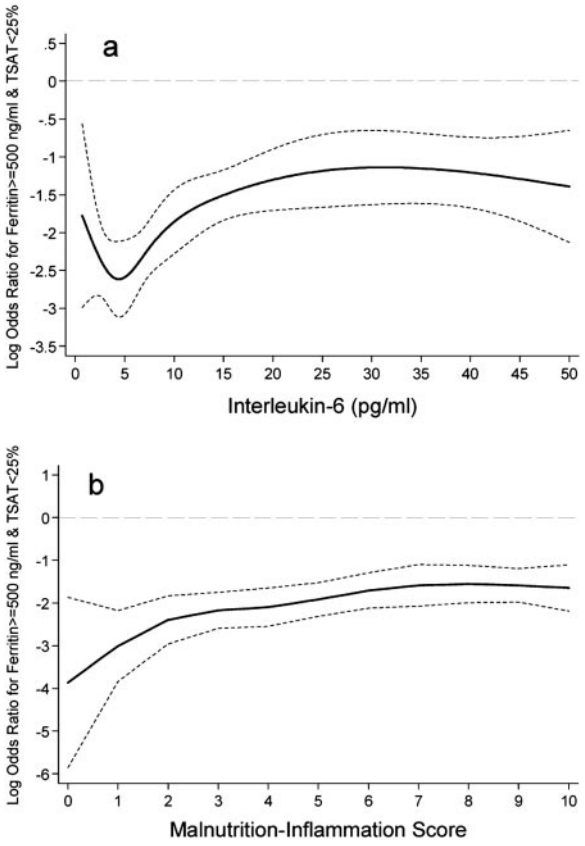

Figure 5. Log odds ratio of having ferritin ≥500 ng/ml and iron saturation <25% together in 789 maintenance hemodialysis patients. (A) IL-6. (B) Malnutrition-inflammation score (MIS).

that such moderately high levels of serum ferritin were not associated with excessive tissue iron store in postmortem autopsies (44).

In our current study, we found that the presence of moderately high serum ferritin is a function of both increased IL-6 and ISAT levels (Figure 3). Moreover, the combination of IL-6 and ISAT had better predicting power than ISAT alone in detecting MHD patients with a ferritin ≥500 ng/ml (Figure 4). These findings indicate that serum ferritin is almost equally both an inflammatory marker and a measure of iron stores. Hence, moderately high levels of serum ferritin should not be assumed to indicate adequate to high iron stores, and patients with ferritin levels >500 ng/ml, especially if combined with a low ISAT of <25%, should not be automatically labeled as iron overload and, hence, deprived of iron supplementation (11,45). Indeed, in a recent randomized clinical trials to assess response to intravenous iron in anemic MHD patients with this seeming paradoxical constellation, administration of 1000 mg of intravenous iron gluconate led to significantly improved anemia and responsiveness to ESA (12,46). Therefore, even though inflammation, especially in the paradoxical high-ferritin/low-ISAT group, may lead to ESA hyporesponsiveness (4), administration of IV iron gluconate still appears to improve anemia management (12,46). This response may be the result of the effect of intravenous iron on decreasing such inflammatory cytokines as TNF-alpha observed in a recent clinical trial (47).

An interesting finding in our study was the positive correlation of serum ferritin with serum prealbumin but negative correlation with TIBC, which is an indirect measurement of

Table 4. Multivariate logistic regression analyses for predicting having IL-6 ≥ 10 (pg/ml), CRP ≥10 (mg/l), and Malnutrition-inflammation score (MIS) ≥5 in the four categories of serum ferritin and iron saturation ratio (ISAT) in 789 maintenance hemodialysis patients

Group	IL-6 ≥ 10 (pg/ml) (n = 284)		CRP ≥10 (mg/l) (n = 111)		MIS ≥5 (n = 328)	
	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
Unadjusted						
Ferritin < 500 and ISAT < 25	2.09 (1.31–3.35)	0.002	1.92 (1.04–3.54)	0.04	1.37 (0.84–2.24)	0.20
Ferritin < 500 and ISAT ≥ 25	1.0 (reference)		1.0 (reference)		1.0 (reference)	
Ferritin ≥ 500 and ISAT < 25	1.60 (1.04–2.46)	0.03	2.82 (1.65–4.83)	<0.001	0.79 (0.51–1.02)	0.27
Ferritin ≥ 500 and ISAT ≥ 25	0.53 (0.37–0.78)	0.001	0.74 (0.42–1.9)	0.29	0.57 (0.40–0.80)	0.001
Case-mix[a] adjusted						
Ferritin < 500 and ISAT < 25	2.01 (1.24–3.26)	0.004	1.81 (0.97–3.38)	0.06	1.26 (0.74–2.13)	0.40
Ferritin < 500 and ISAT ≥ 25	1.0 (reference)		1.0 (reference)		1.0 (reference)	
Ferritin ≥ 500 and ISAT < 25	1.82 (1.15–2.88)	0.01	2.90 (1.36–3.42)	<0.001	0.79 (0.49–1.28)	0.35
Ferritin ≥ 500 and ISAT ≥ 25	0.60 (0.40–0.88)	0.01	0.79 (0.45–1.41)	0.43	0.63 (0.43–0.92)	0.02
Case-mix and MICS[b] adjusted						
Ferritin < 500 and ISAT < 25	1.46 (0.87–2.46)	0.16	1.29 (0.66–2.49)	0.46	1.0 (0.55–1.81)	0.99
Ferritin < 500 and ISAT ≥ 25	1.0 (reference)		1.0 (reference)		1.0 (reference)	
Ferritin ≥ 500 and ISAT < 25	1.18 (0.71–1.87)	0.52	2.00 (1.08–3.73)	0.03	0.51 (0.28–0.89)	0.02
Ferritin ≥ 500 and ISAT ≥ 25	0.55 (0.36–0.83)	0.005	0.73 (0.40–1.35)	0.32	0.58 (0.38–0.90)	0.14

[a]Case-mix variables include age, gender, race/ethnicity, diabetes, dialysis vintage, insurance (Medicare), marital status, modified Charlson comorbidity score, dialysis dose (Kt/V), and residual urea clearance (KRU).

[b]MICS variables include albumin, log erythropoietin dose, creatinine, hemoglobin, phosphorus, normalized protein catabolic rate (nPCR), bicarbonate, calcium, white blood count (WBC), lymphocyte percent, body mass index, and log vitamin D dose.

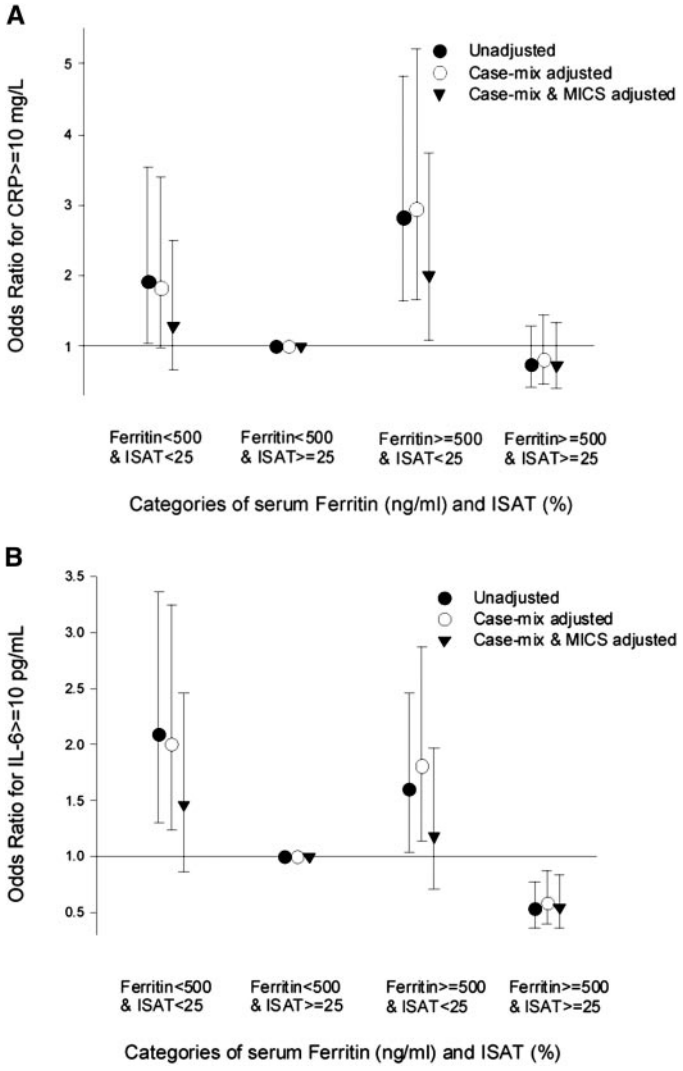

Figure 6. Adjusted odds ratios (ORs) of having IL-6 ≥ 10 (pg/ml) (upper panel) and CRP ≥ 10 (mg/L) (lower panel) according to the four categories of serum ferritin and iron saturation ratio (ISAT) in 789 maintenance hemodialysis patients. Case-mix variables include age, gender, race/ethnicity, diabetes, dialysis vintage, insurance (Medicare), marital status, modified Charlson comorbidity score, dialysis dose (Kt/V), and residual urea clearance (KRU). MICS variables include albumin, log erythropoietin dose, creatinine, hemoglobin, phosphorus, normalized protein catabolic rate (nPCR), bicarbonate, calcium, white blood count (WBC), lymphocyte percent, body mass index (BMI), and log vitamin D dose.

serum transferrin (Table 2; Figure 1) (48). In contrast to albumin, with which ferritin showed no correlation in our study, prealbumin has a shorter half-life and responds faster to nutritional alterations (49). Serum ferritin is known as a positive acute phase reactant, whereas prealbumin, transferrin, and other so-called nutritional markers are considered negative acute phase reactants. The positive association between ferritin and prealbumin suggests that serum ferritin might also have a salutary component that is positively associated with improved nutritional markers. Indeed, a recent epidemiologic study showed that ferritin levels between 500 and 1200 ng/ml, compared with 100 to 200 ng/ml, were associated with greater survival in >56,000 MHD patients across the United States after multivariate adjustment for case-mix and MICS variables (43).

Our study should be qualified for the selection bias upon enrollment and overrepresentation of the Hispanic population in Southern California. However, because the mortality in the original NIED Study cohort was less than the base population (13), it might be argued that a selection bias with such a direction generally would lead to a bias toward the null, so that, without this bias, our positive results might have been even stronger. Another limitation of our study is lack of explicit data on potential contributors of the inflammation in the study population; however, we sought to examine the relative contribution of inflammation to high serum ferritin irrespective of the causes of inflammation. The strengths of our study include the sample size, which was moderately large, the comprehensive clinical and laboratory evaluations, including body composition measures, detailed evaluation of comorbid states by study physicians at baseline, and measuring proinflammatory cytokines and markers.

Conclusion

We found that the probability of having a moderately high serum ferritin ≥500 ng/ml can be explained by both iron stores and inflammation. The seemingly paradoxical combination of serum ferritin ≥500 ng/ml and ISAT <25% in MHD patients is associated with increased level of inflammatory markers. This finding implies that inflammatory states should be taken into account when interpreting a moderately high serum ferritin, especially in the setting of low ISAT. Hence, in dealing with MHD patients with ferritin levels >500 ng/ml, the possibility of high levels of circulating inflammatory cytokines should be considered as an alternative explanation to iron overload. The diagnostic validity and reliability of serum ferritin in diagnosing anemia and iron treatment adequacy need to be revisited in additional studies. Novel methods are needed to decompose different noniron components of the so-called iron markers in CKD and other chronic disease states. Interventional trials are necessary to examine strategies that can modulate the confounding effect of inflammation on iron metabolism, such as hepcidin inhibitors, are needed. Future studies should also aim to answer the question whether therapeutic interventions, including administration of IV iron, are helpful in the setting of moderately high ferritin levels and how the so-called "iron hyporesponsiveness" can be improved in CKD and other similar conditions.

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