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*Original Article*

# Association between serum ferritin and measures of inflammation, nutrition and iron in haemodialysis patients

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**Abstract**

**Background.** Serum ferritin is a frequently used marker of iron status in dialysis patients. Iron administration is to be withheld for ferritin values >800 ng/ml according to K/DOQI guidelines. We hypothesized that such non-iron-related factors as elements of the malnutrition–inflammation complex syndrome (MICS) may increase serum ferritin concentration independently of iron status.

**Methods.** We studied 82 prevalent maintenance haemodialysis (MHD) patients (including 43 men), aged 55.7 ± 15.3 years. The inflammatory and nutritional status was evaluated by serum C-reactive protein (CRP), Subjective Global Assessment (SGA) and its newer, fully quantitative versions, i.e. Dialysis Malnutrition Score (DMS) and Malnutrition–Inflammation Score (MIS).

**Results.** All but six patients had been on maintenance doses of intravenous iron dextran (between 100 and 200 mg/month) during the 10 weeks prior to the measurements. Serum ferritin levels were increased across SGA categories: (ANOVA *P*-value 0.03). Both unadjusted and multivariate adjusted correlation coefficients (*r*) for serum ferritin and CRP *vs* pertinent values were statistically significant for DMS and MIS and some other measures of nutritional status and iron indices. After deleting 10 MHD patients with either iron deficiency (ferritin <200 ng/ml) or iron overload (ferritin >2000 ng/ml), in the remaining 72 MHD patients both bivariate and multivariate correlations were much stronger and statistically significant ($r = -0.33$ and $-0.29$, respectively, $P < 0.01$). A multivariate model showed simultaneous, significant correlations between serum ferritin and both markers of inflammation and iron status independent of each other. After dividing the 72 MHD patients into two groups of serum ferritin based on a K/DOQI recommended serum ferritin cut-off of 800 ng/ml, the MIS and logarithm of serum CRP were significantly higher in the higher ferritin group.

**Conclusions.** Serum ferritin values in the range of 200–2000 ng/ml may be increased due to non-iron-related factors including elements of MICS.

**Keywords:** end-stage renal disease; ferritin; inflammation; iron; malnutrition–inflammation complex syndrome; protein–energy malnutrition

## Introduction

Anaemia of end-stage renal disease can be managed relatively successfully by recombinant human erythropoietin (EPO). Iron administration plays a central role in enhancing anaemia responsiveness to EPO. Serum ferritin concentration and iron saturation ratio are among the two most commonly used markers of iron status in maintenance dialysis patients [1]. According to National Kidney Foundation (NKF) Kidney Disease and Dialysis Outcome Quality Initiative (K/DOQI) guidelines, iron administration should be withheld for a serum ferritin level >800 ng/ml in these patients, since such high ferritin levels may reflect iron overload [2]. However, serum ferritin is also an acute phase reactant and can be increased in inflammation [3–5]. Inflammation is quite common in maintenance dialysis patients, and its prevalence among maintenance haemodialysis (MHD) patients may be as high as 40–60% [6]. Hence, it is quite possible that high levels of serum ferritin are engendered by inflammation independently of iron stores. However, it is not clear whether serum ferritin is indeed significantly increased in inflammation at different levels of body iron in dialysis patients. Moreover, inflammation is closely related to

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protein–energy malnutrition in dialysis patients [6], and the simultaneous combination of these two conditions, also referred to as 'malnutrition–inflammation complex syndrome' (MICS), is observed frequently in dialysis patients [6]. While MICS may play a central role in poor clinical outcome including a high rate of mortality and hospitalization and diminished quality of life, it may also lead to hyperferritinaemia and refractory anaemia including EPO hyporesponsiveness in these individuals [7]. It is not clear whether protein–energy malnutrition alone or combined with inflammation in the form of MICS has a significant effect on serum ferritin in MHD patients. Therefore, we examined the hypothesis that high serum ferritin levels can occur in the setting of inflammation and/or malnutrition, in addition to iron overload, in MHD patients.

## Subjects and methods

### *The patients*

The outpatient chronic dialysis programme at San Francisco General Hospital, the University of California Renal Center, treated 92 adult MHD patients at the time of the study in early 2000. Inclusion criteria were those patients who were undergoing MHD for at least 3 months and who were 18 years or older. One patient was hospitalized in other centres at the time of the study and two patients were not present. Hence, out of 89 eligible MHD patients, 82 individuals agreed to enroll into the study. All but nine patients received intravenous (i.v.) EPO, 1000–36 000 units (7902 ± 7774 units) weekly. Seventy-six patients were receiving a maintenance dose of iron dextran (100–200 mg/month) i.v. during the past 10 weeks prior to measurements, and the others received oral iron preparations. However, according to the billing records of the dialysis unit for i.v. iron, all patients had received on average almost the same range of i.v. iron during the past 6–8 months prior to the study. During this period and prior to it, i.v. iron administration mode was exclusively 'maintenance' (i.e. once or twice monthly), and iron 'repletion' treatment had not been practiced until several months after the initiation of this study. The study was approved by the institutional review board, and written informed consent was obtained from all participants.

### *Conventional Subjective Global Assessment (SGA)*

The SGA of nutritional state is a semi-quantitative scoring system based on nutrition-related history and physical examination [8]. The history consists of: (i) weight loss during the preceding 6 months; (ii) gastrointestinal symptoms; (iii) food intake; (iv) functional capacity; (v) co-morbidities. Each of these features is scored separately as A, B or C reflecting well nourished to severely malnourished categories. The nutritional physical examination includes: (i) loss of subcutaneous fat; (ii) muscle wasting. These two components are classified from 0 to 3 representing normal to severely abnormal. The data are weighted subjectively by the evaluator, who then classifies the patient in one of the three major SGA groups: (i) well nourished, (ii) mildly to moderately malnourished and (iii) severely malnourished. Details on the methods for SGA evaluation in dialysis patients are available as an appendix in a previously published article in the website of the *American Journal of Kidney Diseases* [8].

### *Dialysis Malnutrition Score (DMS)*

Using the components of the conventional, semi-quantitative SGA, one of the authors has developed a fully quantitative scoring system, referred to as the Dialysis Malnutrition Score (DMS) [9], that consists of the seven above-mentioned components of the conventional SGA, in that each component has a score ranging from 1 (normal) to 5 (severely abnormal). Thus, the DMS, i.e. the sum of all seven components, is a number from 7 (normal) to 35 (severely malnourished), a higher DMS representing a greater degree of protein–energy malnutrition. In a recent preliminary report from a cross-sectional study using a different pool of patients, the DMS was significantly correlated with anthropometric values and laboratory measures of nutritional status in MHD patients [9].

### *Malnutrition–Inflammation Score (MIS)*

By combining the seven above-mentioned components of the SGA and DMS with three other nutrition-related items [body mass index (BMI), serum albumin, and total iron binding capacity (TIBC) to represent serum transferrin] in incremental fashion, the so-called MIS with 10 components has recently been created [10]. Each MIS component has four levels of severity from 0 (normal) to 3 (very severe). The sum of all 10 MIS components ranges from 0 to 30 denoting the increasing degree of severity. In a recent prospective study on 83 MHD patients, the MIS was compared with the conventional SGA, the DMS, anthropometry, near infra-red measured body fat percentage, laboratory measures including serum C-reactive protein (CRP), and 12 month prospective hospitalization and mortality rates [10]. The MIS was found to be a comprehensive scoring system with significant associations with prospective hospitalization and mortality as well as measures of nutrition, inflammation and anaemia in MHD patients, and was superior to conventional SGA and to individual laboratory values as a predictor of dialysis outcome and an indicator of MICS [10].

In this study, SGA, DMS and MIS were used by a trained physician (K.K.Z.) to evaluate the nutritional status of each patient within 5–15 min prior to measuring anthropometry. To evaluate the degree of reproducibility, these assessments were repeated after 1 week on a subset of 12 patients by the same physician without reference to the first evaluation. The correlation coefficient ($r$) between the two sets of nutritional assessments was between 0.84 and 0.90 denoting an acceptable degree of reproducibility.

### *Laboratory evaluation*

The laboratory values, except for post-dialysis serum urea nitrogen used to calculate the urea reduction ratio and dialysis dose (Kt/V), were measured immediately prior to the dialysis session at least 16 days after the last i.v. administration of iron. Serum CRP was measured as an indicator of an inflammatory state and was assessed by an

immuno-turbidimetric method (Hitachi 747). The lower limit sensitivity of the CRP assay was 6.9 ng/ml. For patients whose CRP was reported to be < 6.9 ng/ml, an arbitrary average of 3.4 ng/ml was used for statistical analyses. Laboratory values were obtained by automated methods. All blood samples were drawn at least 16 days after the last i.v. iron administration in each MHD patient. Laboratory measurements were performed by Spectra® Laboratories (Fremont, CA).

### *Statistical and epidemiological methods*

We used Pearson's and Spearman's rank order correlation coefficient $r$ for selected analyses where indicated. The Student's $t$-test (two-tailed) or analysis of variance (ANOVA) were used for group mean comparisons between two or more groups of patients, respectively. Multivariate regression analysis was performed to obtain partial (adjusted) correlations ($R^2$) controlled for age, gender, race and presence or absence of diabetes mellitus. Descriptive and multivariate statistics were carried out with the statistical software Stata, version 6.0 (Stata Corporation, College Station, TX). Fiducial limits are given as mean ± SD. A $P$-value of < 0.05 is considered to be statistically significant, a $P$-value between 0.05 and 0.10 is considered marginally significant, and a $P$-value > 0.10 is not significant.

## Results

Table 1 shows relevant demographic, clinical and laboratory values in 82 MHD patients as a whole and in each SGA nutritional category. There were 43 men and 39 women. Almost half of the patients were African–American, and one-third had diabetes mellitus. The age ranged from 22 to 87 years (mean ± SD, 55.7 ± 15.3 years), and patients in SGA group 1 (well nourished) were on average 10–12 years younger than the other two nutritional groups (mildly to severely malnourished). The vintage (duration of maintenance dialysis therapy) varied from 3 months to 12 years (44 ± 33 months) and increased across worsening SGA categories. The BMI was lower and the DMS and MIS were higher as SGA score increased successively from 1 to 3. A similar trend as with the BMI was observed with serum albumin and TIBC concentrations, while serum ferritin and CRP concentrations showed an opposite trend. The mean serum ferritin concentration was 831 ng/ml in all 82 MHD patients. In SGA groups 1–3, serum ferritin was 628, 875 and 994 ng/ml, respectively (ANOVA $P = 0.03$).

Low serum ferritin concentrations (< 200 ng/ml) may be related to depleted iron stores as previously shown in a study in MHD patients based on bone marrow iron store [1]. High serum ferritin values (> 2000 mg/dl) are much more likely to be an indicator of iron overload as shown in several studies including those in thalassemic patients with haemochromatosis [11,12]. Hence, these ranges of serum ferritin values are less likely to correlate with inflammation and/or malnutrition. Therefore, a sub-analysis was performed in that only patients with serum ferritin values between 200 and 2000 ng/ml were analysed. After excluding

**Table 1.** Demographic, laboratory and clinical values in 82 MHD patients as a whole and in three categories of SGA of nutrition: (1) well nourished, (2) mildly to moderately malnourished and (3) severely malnourished

| Variable | All HD patients ($n = 82$) | ANOVA $P$-value | SGA = 1 ($n = 24$) | SGA = 2 ($n = 39$) | SGA = 3 ($n$=19) |
|---|---|---|---|---|---|
| Gender (% male) | 52 | 0.2 | 67 | 49 | 42 |
| Race (% blacks) | 48 | 0.8 | 50 | 44 | 53 |
| Diabetes mellitus (%) | 33 | 0.9 | 29 | 36 | 32 |
| Age (years) | 55.7 ± 15.3 | **0.03** | 47.0 ± 15.1 | 59.4 ± 13.7 | 58.9 ± 15.3 |
| Dialysis vintage (months) | 43.5 ± 32.8 | **0.03** | 31.8 ± 28.7 | 43.7 ± 27.7 | 58.0 ± 42.0 |
| BMI (kg/m$^2$) | 24.9 ± 6.3 | **< 0.001** | 29.3 ± 8.2 | 24.0 ± 4.1 | 21.0 ± 3.9 |
| Dialysis malnutrition score | 12.0 ± 3.2 | **< 0.001** | 8.8 ± 1.2 | 11.8 ± 1.4 | 16.6 ± 2.0 |
| MIS | 7.9 ± 4.0 | **< 0.001** | 4.0 ± 1.2 | 7.7 ± 1.8 | 13.4 ± 3.0 |
| Blood haemoglobin | 10.9 ± 1.5 | 0.4 | 10.9 ± 1.7 | 11.1 ± 1.3 | 10.6 ± 1.8 |
| Serum albumin (g/dl) | 3.80 ± 0.46 | **0.04** | 3.94 ± 0.41 | 3.81 ± 0.37 | 3.58 ± 0.60 |
| Creatinine (mg/dl) | 10.5 ± 3.1 | 0.5 | 11.0 ± 2.7 | 10.5 ± 3.2 | 9.9 ± 3.5 |
| Blood urea nitrogen (mg/dl) | 67.0 ± 17.9 | 0.7 | 64.3 ± 14.6 | 68.2 ± 16.1 | 67.8 ± 24.8 |
| CRP (mg/l) | 14.0 ± 17.8 | 0.09 | 10.0 ± 15.9 | 12.8 ± 12.3 | 21.6 ± 26.5 |
| Ferritin (full range) (ng/ml)[a] | 830.5 ± 473.4 | **0.03** | 628.3 ± 346.6 | 875.2 ± 422.8 | 994.1 ± 624.8 |
| Ferritin (> 200 and < 2000) (ng/ml)[a] | 874.5 ± 378.7 | **0.01** | 703.3 ± 300.8 | 888.9 ± 343.7 | 1,079.7 ± 461.6 |
| TIBC (mg/dl) | 181.0 ± 35.9 | **0.05** | 195.8 ± 28.1 | 175.4 ± 30.3 | 173.7 ± 49.3 |
| Transferrin (mg/dl) | 160.0 ± 35.7 | 0.06 | 174.3 ± 23.3 | 155.1 ± 26.7 | 152.2 ± 56.1 |
| Iron (ng/ml) | 63.7 ± 28.7 | 0.11 | 70.8 ± 23.6 | 56.8 ± 25.7 | 68.9 ± 37.5 |
| Iron saturation ratio (%) | 35.0 ± 13.8 | 0.2 | 35.8 ± 10.0 | 32.3 ± 12.7 | 39.7 ± 18.7 |
| Kt/V | 1.37 ± 0.27 | 0.7 | 1.34 ± 0.28 | 1.39 ± 0.27 | 1.38 ± 0.28 |
| Iron dextran (mg/month) | 177 ± 57 | 0.5 | 188 ± 45 | 174 ± 59 | 168 ± 67 |
| EPO (units/week) | 7902 ± 7774 | 0.6 | 8375 ± 8075 | 7077 ± 6293 | 9000 ± 10 100 |

Continuous variables are reported as mean ± SD and their $P$-values are based on ANOVA test. Non-parametric values (race, gender and diabetes) are in percentage and their $P$-values are based on the chi-square test.

[a]For serum ferritin concentrations, both the full range ($n = 82$) as well as the restricted range ($n = 72$) after deletion of 10 cases with either very low (< 200 ng/ml) or very high (> 2000 ng/ml) ferritin levels are shown. Significant $P$-values (< 0.05) are in bold.

those patients whose serum ferritin level was $<200$ ng/ml ($n=8$) or $>2000$ ng/ml ($n=2$), the remaining 72 patients showed the same increasing ferritin trend across worsening SGA categories with a somewhat improved *P*-value of 0.01 (see Table 1 and Figure 1).

Table 2 compares the bivariate and multivariate correlations of serum ferritin and CRP values with some relevant demographic, clinical and laboratory variables in 82 MHD patients. Bivariate coefficients are based on unadjusted Pearson's correlation, whereas multivariate values are on the basis of multiple regression models and are adjusted for age, gender, race (African–American *vs* others) and diabetes. Serum ferritin had a positive correlation with age, indicating higher ferritin levels in older MHD patients. All three nutritional assessment tools (SGA, DMS and MIS) correlated positively with both serum ferritin and CRP concentrations, i.e. an increased level of these two

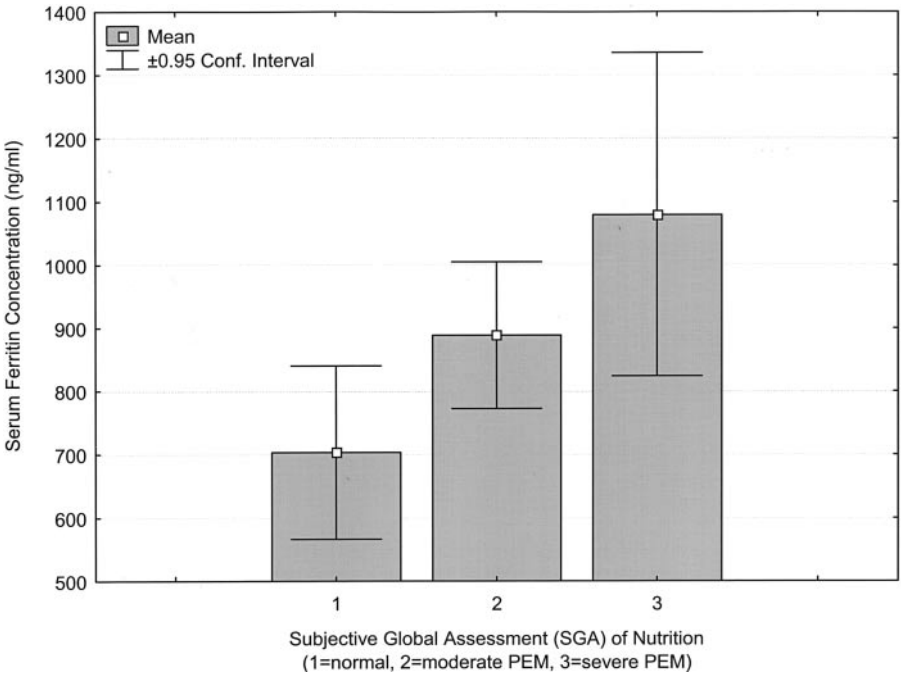


**Fig. 1.** Serum ferritin concentrations in three severity groups of SGA to reflect protein–energy malnutrition (PEM) in 72 MHD patients with a serum ferritin between 200 and 2000 ng/ml. ANOVA *P*-value is 0.01. *Post hoc P*-values: group 1 *vs* group 2, 0.06; group 1 *vs* group 3, 0.003; group 2 *vs* group 3, 0.09.

**Table 2.** Correlation coefficients (*r*) between average serum ferritin and CRP concentrations and relevant demographic, clinical and laboratory values in 82 MHD patients

| | Serum ferritin | Serum CRP |
|---|---|---|
| Age | **0.28** ($P=0.01$)/**0.33** ($P=0.004$) | 0.05 ($P=0.6$)/0.12 ($P=0.4$) |
| BMI | −0.11 ($P=0.3$)/−0.08 ($P=0.5$) | −0.20 ($P=0.08$)/0.18 ($P=0.14$) |
| SGA (1−3) | **0.29** ($P=0.009$)/0.18 ($P=0.09$) | 0.19 ($P=0.08$)/0.23 ($P=0.05$) |
| DMS (7−35) | **0.34** ($P=0.002$)/**0.25** ($P=0.03$) | **0.30** ($P=0.006$)/**0.35** ($P=0.003$) |
| MIS (0−30) | **0.33** ($P=0.002$)/0.24 ($P=0.03$) | **0.43** ($P<0.001$)/**0.51** ($P<0.001$) |
| Blood haemoglobin | **−0.25** ($P=0.02$)/**−0.26** ($P=0.02$) | **−0.31** ($P=0.004$)/**−0.40** ($P<0.001$) |
| Serum albumin | −0.09 ($P=0.4$)/−0.12 ($P=0.2$) | **−0.38** ($P<0.001$)/**0.51** ($P<0.001$) |
| CRP (for full range of ferritin)[a] | 0.13 ($P=0.3$)/0.11 ($P=0.3$) | NA |
| CRP (if 200 < ferritin < 2000 ng/ml)[a] | **0.33** ($P=0.005$)/**0.29** ($P=0.009$) | NA |
| TIBC | −0.20 ($P=0.07$)/−0.11 ($P=0.3$) | **−0.28** ($P=0.01$)/**−0.36** ($P=0.002$) |
| Transferrin | **−0.29** ($P=0.007$)/−0.23 ($P=0.03$) | −0.22 ($P=0.05$)/**−0.31** (p.008) |
| Iron | 0.20 ($P=0.06$)/**0.33** ($P=0.002$) | 0.10 ($P=0.4$)/0.18 ($P=0.13$) |
| Iron saturation | **0.32** ($P=0.003$)/**0.44** ($P<0.001$) | 0.03 ($P=0.8$)/0.05 ($P=0.9$) |
| Iron sat.[b] (if 200 < ferritin < 2000 ng/ml) | **0.29** ($P=0.01$)/**0.42** ($P<0.001$) | 0.04 ($P=0.7$)/0.10 ($P=0.4$) |
| Prescribed EPO dose | 0.11 ($P=0.3$)/0.15 ($P=0.2$) | 0.23 ($P=0.04$)/0.31 ($P=0.009$) |

In each column, the first set of values include the unadjusted (bivariate) *r* with its Pearson *P*-value in parentheses, and the second set includes partial correlation based on a multivariate regression analysis adjusted for case-mix (age, gender, race and diabetes). Correlation coefficient values $\geq 0.25$ are in bold. Sat., saturation.

[a]Correlation between serum CRP and ferritin is calculated for both the full range of ferritin ($n=82$) as well as the restricted range after deleting cases with a ferritin $<200$ or $>2000$ ng/ml ($n=72$).

[b]*P*-values are not adjusted for multiple comparisons.

serum values is associated with a worsened nutritional status. Serum albumin level had a strong and negative correlation with serum CRP but no significant correlation with serum ferritin concentration. Both serum TIBC and transferrin had marginal to moderate, reverse correlation with both serum ferritin and CRP concentrations. Prescribed EPO dose correlated with serum CRP but not ferritin concentrations, denoting that a higher dose of EPO was prescribed to those MHD patients with increasing severity of inflammation (Table 2).

Serum ferritin and CRP concentrations did not initially correlate significantly with each other in 82 MHD patients ($r \leq 0.13$, $P > 0.10$). However, after excluding those patients with ferritin $<200$ or $>2000$ ng/ml, there was a significant correlation in the remaining 72 MHD patients: bivariate $r = 0.33$ ($P = 0.005$) and case-mix adjusted multivariate $r = 0.29$ ($P = 0.009$) (see Table 2). Serum iron saturation ratio correlated strongly and significantly with serum ferritin in all 82 MHD patients (multivariate $r = 0.44$, $P < 0.001$) as well as in 72 patients with a ferritin between 200 and 2000 ng/ml (multivariate $r = 0.42$, $P < 0.001$). These findings suggest that serum ferritin is a marker of iron store independent of its range but correlates with inflammation only in a restricted range of 200–2000 ng/ml in MHD patients. Serum CRP did not significantly correlate with iron markers (see Table 2).

Figure 2 represents multivariate models to examine the effect of combined variation of iron and inflammation on serum ferritin. The following linear model was explored:

$$\text{Ferritin} = \text{iron} + \text{inflammation} + \text{other factors}$$

In the simple linear multivariate model (Figure 2, top), both iron saturation ratio (ISAT) and CRP had simultaneous independent correlations with serum ferritin levels in 72 MHD patients with ferritin ranging between 200 and 2000 ng/ml. The correlation coefficient $r$ for the adjusted association of serum ferritin with serum CRP concentration was 0.31 ($P = 0.005$) and with iron saturation ratio was 0.28 ($P = 0.01$), and the models' multivariate $R^2$ was 0.18 ($P < 0.001$). Quadratic model (Figure 2, bottom) yielded essentially similar results and did not lead to significant improvement (multivariate $R^2 = 0.21$, $P = 0.006$). A more extended linear multivariate model that also included case-mix variables (age, gender, race and diabetes) resulted in similar statistically significant, independent correlations between serum ferritin as the dependent variable and serum CRP ($r = 0.25$, $P = 0.01$) and iron saturation ratio ($r = 0.39$, $P < 0.001$) as predicting variables (multivariate $R^2 = 0.34$, $P < 0.001$).

To further examine the possible association between inflammation and moderately high levels of serum ferritin concentrations, the 72 MHD patients were dichotomized based on a cut-off ferritin value of 800 ng/ml. This value was chosen since it is the upper limit for iron administration to dialysis patients according to K/DOQI guidelines [2]. Table 3 compares

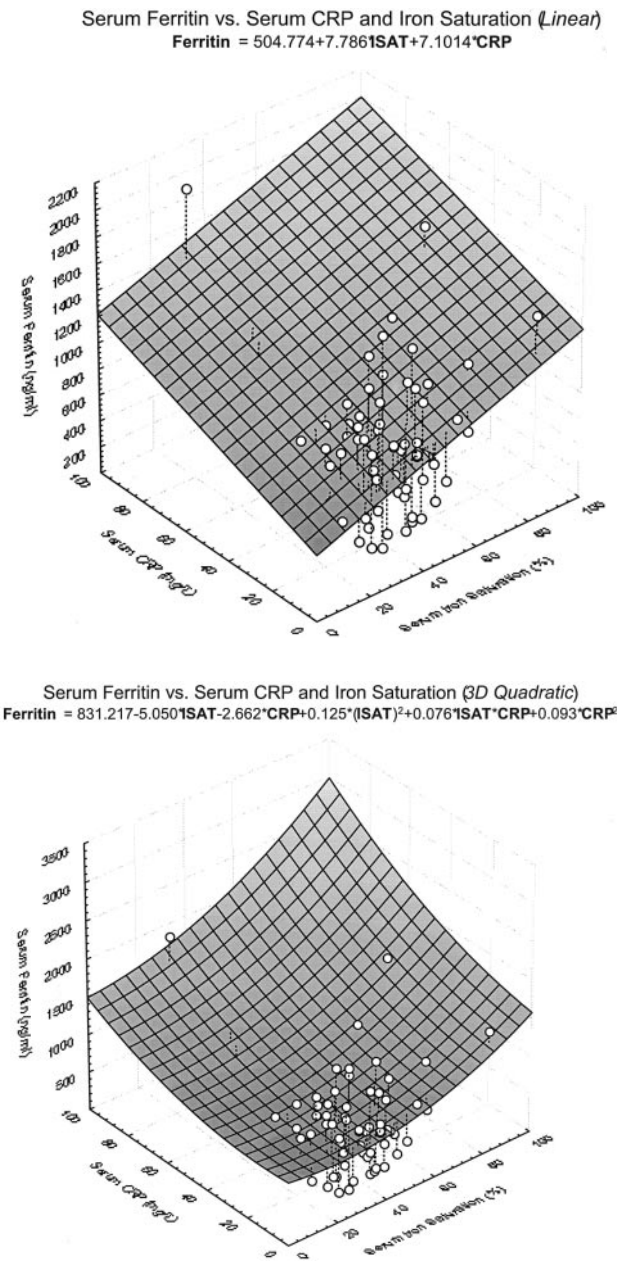


**Fig. 2.** Serum ferritin concentration as reflected by simultaneous contribution of inflammation and iron states, represented by serum CRP concentration and iron saturation ratio, respectively, in 72 MHD patients. (Top) Linear model. (Bottom) Quadratic model. ISAT, iron saturation ratio.

relevant variables in the so-called 'normal range' ferritin (between 200 and 800 ng/ml, $n = 31$) *vs* 'high range' ferritin (between 801 and 2000 ng/ml, $n = 41$). There were more men in the former than in the latter group. The mean MIS was significantly higher in the 'high range' ferritin group ($8.6 \pm 3.9$) compared with the 'normal range' group ($6.9 \pm 3.9$, $P = 0.05$). Similarly, mean serum CRP concentration was 16.4 mg/l in the high range ferritin group, which was 7.0 mg/dl higher than the mean CRP level in the low range ferritin group (the $P$-value was 0.08 for continuous values and 0.05 for the logarithmic scale)

**Table 3.** Comparing pertinent demographic, laboratory and clinical values in patients with a so-called 'normal' serum ferritin concentration, i.e. between 200 and 800 ng/ml, and those with a 'high' serum ferritin concentration, i.e. between 800 and 2000 ng/ml in 72 MHD patients

| | Normal range ferritin: 200 < ferritin ≤ 800 ng/ml ($n = 31$) | High range ferritin: 800 < ferritin < 2000 ng/ml ($n = 41$) | *t*-test *P*-value |
|---|---|---|---|
| Serum ferritin (ng/ml) | 537 ± 169 | 1130 ± 280 | NA |
| Sex (% men) | 65 | 41 | 0.05 |
| Diabetes mellitus (%) | 42 | 29 | 0.3 |
| Race (% black) | 58 | 44 | 0.2 |
| Age (year) | 54.9 ± 14.8 | 58.0 ± 14.7 | 0.4 |
| BMI (kg/m$^2$) | 25.6 ± 6.4 | 25.2 ± 6.7 | 0.8 |
| SGA | 1.8 ± 0.7 | 2.0 ± 0.7 | 0.14 |
| DMS | 11.2 ± 3.1 | 12.5 ± 3.0 | 0.07 |
| MIS | 6.9 ± 3.9 | 8.6 ± 3.7 | 0.05 |
| Blood haemoglobin (g/dl) | 10.97 ± 1.57 | 10.63 ± 1.30 | 0.3 |
| Serum albumin (g/dl) | 3.89 ± 0.44 | 3.73 ± 0.45 | 0.14 |
| Creatinine (mg/dl) | 10.7 ± 3.7 | 10.5 ± 2.3 | 0.9 |
| CRP (mg/l) | 9.40 ± 12.33 | 16.40 ± 18.98 | 0.08 |
| Log of CRP | 0.80 ± 0.35 | 0.99 ± 0.45 | 0.05 |
| TIBC (mg/dl) | 180.5 ± 29.7 | 173.8 ± 29.4 | 0.4 |
| Transferrin (mg/dl) | 161.2 ± 24.3 | 150.2 ± 25.8 | 0.07 |
| Iron (ng/ml) | 58.8 ± 23.0 | 65.6 ± 29.9 | 0.3 |
| Iron saturation (%) | 31.8 ± 9.5 | 37.9 ± 15.6 | 0.06 |
| Kt/V | 1.32 ± 0.28 | 1.42 ± 0.27 | 0.11 |
| Prescribed iron (mg/month) | 174 ± 63 | 173 ± 59 | 0.9 |
| Prescribed EPO (units/week) | 7935 ± 9011 | 6902 ± 5625 | 0.6 |

Calculated *P*-values are based on Student's *t*-test.

(see Figure 3). A higher DMS and serum iron saturation ratio and a lower transferrin concentration were observed in MHD patients with a serum ferritin > 800 ng/ml, but the *P*-values were borderline (0.05 and 0.10) (see Table 3).

## Discussion

We found that in a group of 82 MHD patients serum ferritin concentrations were higher in malnourished patients as assessed by the SGA and other similar nutritional scoring tools. We also found that a moderate correlation existed between serum ferritin and CRP concentrations when the range of serum ferritin was restricted between 200 and 2000 ng/ml. This association was independent of confounding factors such as age, gender, race and diabetes. Multivariate models showed that both CRP and iron saturation ratio, independent of each other, correlated significantly with serum ferritin. Those MHD patients with a serum ferritin value > 800 ng/ml had also higher MIS scores and increased serum CRP levels. These findings imply that serum ferritin is both a marker of iron status and an indicator of inflammation and/or malnutrition in maintenance dialysis patients.

Serum ferritin is a frequently used marker of iron status in dialysis patients [2,4,13]. Serum ferritin concentration results from the leakage of tissue ferritin, an intracellular iron storage protein shell with a molecular weight of ~450 kDa, containing heavy (H) and light (L) subunits [14]. Serum ferritin is slightly different than tissue ferritin and contains little or no iron [14]. While tissue ferritin clearly plays a role in intracellular iron handling, the role of serum ferritin is less clearly understood [14]. The level of ferritin in plasma represents the balance between its secretion, which is directly related to intracellular iron synthesis, and its clearance, mainly in liver and other organs [4]. However, liver dysfunction and inflammatory factors may interfere with the synthesis and clearance of ferritin, thereby increasing serum ferritin levels due to circumstances not related to iron metabolism [14].

During the acute phase response, inflammatory cytokines such as interleukin 1 beta (IL-1$\beta$) and tumour necrosis factor alpha (TNF-$\alpha$) increase the synthesis of both H and L subunits of ferritin [15]. Hence, serum ferritin can be elevated in inflammation. Serum CRP is a marker of inflammation that is a very common condition in dialysis patients [6]. The CRP concentration is shown to be a predictor of cardiovascular disease and mortality in both the general population and dialysis patients. Inflammation and protein–energy malnutrition are closely related to each other in dialysis patients [6]. The simultaneous combination of malnutrition and inflammation has been referred to as 'malnutrition–inflammation complex syndrome' (MICS) [6] or 'malnutrition–inflammation–atherosclerosis' syndrome [16]. The MICS appears to play a central role in poor clinical outcome including the high rate of mortality and hospitalization and diminished quality of life seen in dialysis patients. Moreover, the MICS is also believed to be the underlying condition that leads to 'reverse epidemiology' of cardiovascular risks in these patients, where a low, and not a high, BMI

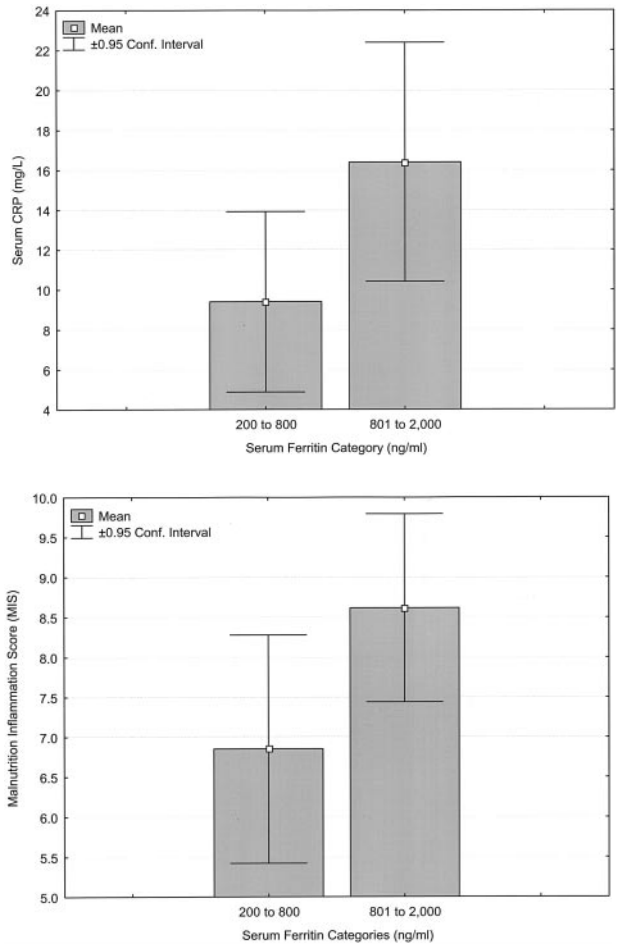


**Fig. 3.** Markers of MICS in serum ferritin categories based on a K/DOQI recommended cut-off level of 800 ng/ml in 72 MHD patients. (Top) Serum CRP concentration (*t*-test *P*-value 0.05 for logarithm of CRP). (Bottom) MIS (*t*-test *P*-value 0.05).

or serum cholesterol is associated with poor dialysis outcome [17]. The MICS may also be a cause of hyperferritinaemia and refractory anaemia including EPO hyporesponsiveness in these individuals. Our current study indicates that measures of protein–energy malnutrition and inflammation, independently or combined in the form of the MICS, have significant associations with serum ferritin in MHD patients. In our current study, serum ferritin did not correlate with serum albumin but did with serum CRP. Moreover, the correlation between nutritional scoring systems, i.e. SGA, DMS and MIS, and serum ferritin, was significant. Serum albumin is a long-term indicator of MICS, whereas CRP may be a short-term marker and has a month-to-month fluctuation [18] as ferritin appears to have. Similarly, we showed that recent changes in serum ferritin may be associated with mortality in dialysis patients [4].

In this study, the association between serum CRP and ferritin was much stronger when serum ferritin values $<200$ or $>2000$ ng/ml were excluded. In our current study, only two MHD patients had a serum ferritin $>2000$ ng/ml, indicating that most of our MHD patients had not received excessive amounts of i.v. iron. As described above, we chose the ferritin threshold of 2000 ng/ml based on several previous studies on iron overload in other individuals such as those with beta-thalassemia [11,12]. Hence, it is highly unlikely, although not impossible, that ferritin values $>2000$ ng/ml are not indicative of some degree of iron overload. In a previous study in a group of renal failure patients, we showed that serum ferritin $<200$ ng/ml was consistent with absolute iron deficiency according to bone marrow iron stores [1]. A low serum ferritin has a high specificity to detect iron deficiency in dialysis patients receiving EPO [1]. Indeed, inflammation may not have an effect on serum ferritin, unless there is enough iron stores in the body so that serum ferritin is somewhat increased [15]. Rogers *et al*. [15] showed that IL-1$\beta$ induces ferritin gene expression by translational control of its mRNA; however, this inflammatory induction of ferritin synthesis is different from iron-dependent ferritin gene expression. They showed that this inflammatory regulation of ferritin requires the background presence of cellular iron [15]. In other words, without adequate iron stores, serum ferritin is low and does not correlate with inflammation, but with enough iron, serum ferritin is a function of both iron and inflammation (see Table 4). This important bench-research finding is consistent with our current and previous clinical and epidemiological findings that, in the setting of absolute iron deficiency, serum ferritin is almost always low [1]. However, once the minimal required iron is available, ferritin regulation also becomes a function of non-iron-dependent factors such as inflammation (Table 4).

According to K/DOQI guidelines, iron administration to dialysis patients should be withheld if serum ferritin is $>800$ ng/ml, since iron overload (haemochromatosis) and its consequences such as poor clinical outcome may ensue [2]. However, an increased serum ferritin is not necessarily a sign of iron overload. Serum ferritin is a marker of malignancy such as in neuroblastoma or renal cell carcinoma [4]. In dialysis patients, hyperferritinaemia is paradoxically associated with EPO resistance and a more severe anaemia [19]. Such findings have been suggested as a component of 'reverse epidemiology' that is described elsewhere in dialysis patients [17].

In the previously reported cases of haemochromatosis among dialysis patients, the observed serum ferritin levels were well above 2000 ng/ml, usually in the 3000–10 000 ng/ml range [13]. Indeed, to our knowledge, with widespread EPO administration to dialysis patients since the early 1990s, there have been much fewer reported cases of haemochromatosis in these patients despite rigorous use of i.v. iron [13]. Hence, moderately increased serum ferritin as high as 2000 ng/ml may not be a sign of iron overload in dialysis patients especially in the post-EPO era. Current guidelines to use such moderately increased serum ferritin ($>200$–$300$ ng/ml) for haemochromatosis screening in the general population are not applicable to the dialysis population. In our current study, there were only two

**Table 4.** Hypothetical, schematic representation of the effect of inflammation on serum ferritin concentration under different iron states in maintenance dialysis patients

| Serum ferritin range | Ferritin $<200$ ng/ml | $200 <$ Ferritin $< 2000$ ng/ml | Ferritin $>2000$ ng/ml |
|---|---|---|---|
| How it may happen | Deficient iron blunts the effect of inflammation on serum ferritin | Iron and inflammation can independently change serum ferritin | Iron overload overwhelms the effect of inflammation on serum ferritin |
| What it means | Serum ferritin = iron | Serum ferritin = inflammation + iron | Serum ferritin= iron |
| What to do | Iron supplementation is indicated | If ferritin $<800$ ng/ml: give iron<br>If ferritin $>800$ ng/ml: check serum CRP, liver enzyme, calculate MIS, rule out malignancies. If EPO resistance persists, iron administration may be indicated | Iron supplementation should be avoided |

patients with a ferritin level $>2000$ ng/ml among 82 MHD patients. When those 72 MHD patients with a serum ferritin ranging between 200 and 2000 ng/ml were divided into two sub-groups based on the serum ferritin cut-off of 800 ng/ml (the K/DOQI recommended ferritin threshold for iron administration), the MIS was significantly higher in the 'high range' ferritin group, and the mean serum CRP level in the 'high range' ferritin group was almost twice as high as that in the 'low-range' ferritin group, while the iron-saturation ratio was only slightly (7%) increased. These findings may indicate that K/DOQI guidelines prohibiting iron administration to those dialysis patients with a moderately increased serum ferritin level, i.e. the 800–2000 ng/ml range, may not be appropriate and may deprive these possibly inflamed but not iron-overloaded patients of required iron supplementation.

To our knowledge, there have been only very few studies indicating a deleterious effect of serum ferritin and iron administration on dialysis outcome. In our own previous study, we showed that a 'recent increase' in serum ferritin from its baseline may be an ominous sign and related to dialysis hospitalization and mortality [4]. Inflammation and/or malnutrition could have led to such abrupt rises in serum ferritin and simultaneous poor outcome in those MHD patients who expired, since their 'pre-death', and not their 'baseline', ferritin values were increased [4]. Indeed, survival analyses in the same study did not show any association between the 'baseline' serum ferritin and mortality [4]. In another study by Feldman *et al.* [20], there was a tendency to increased mortality in those MHD patients who had received higher doses of i.v. iron. However, additional analyses by the same investigators of a large national dataset from 1996 using novel epidemiological and statistical methods have not detected an association between iron administration and survival (personal communication). Several studies in the past had denoted an association between dialysis morbidity, including risk of infection, and iron overload represented by a high serum ferritin [21]. However, such inferences may be flawed since the hyperferritenaemia-associated morbidity could have reflected an independent prognostic factor rather than being due to iron overload. In other words, since serum ferritin is an inflammatory marker, it may be increased in the setting of infection. Infection *per se* may be the primary cause of death and also associated with hyperferritinaemia as a secondary phenomenon. Thus, considering high ferritin levels as the primary cause of increased mortality in the setting of inflammation or infection may be flawed as long as longitudinal studies do not show any temporal relationship between iron administration and poor outcome in dialysis patients.

Another interesting finding of our current study was a significant association between both serum CRP and ferritin levels and the degree of severity of anaemia, similar to previous reports [19,22]. Moreover, those MHD patients with a higher serum CRP level were prescribed higher doses of EPO. Inflammation as a marker of EPO hyporesponsiveness has already been reported [19]. Furthermore, similar to studies by other investigators, we also found elevated serum CRP levels in MHD patients with hypoalbuminaemia and hypotransferrinaemia [23].

Our study should be qualified for its relatively small sample size ($n = 82$) and its cross-sectional design. While causality cannot usually be detected in cross-sectional studies, associations can be examined. Moreover, other markers of inflammation such as IL-1$\beta$, IL-6 or TNF-$\alpha$, were not measured in this study, nor were other measures of malnutrition such as caliper anthropometry or body composition. However, serum CRP and the SGA are valid markers of inflammation and malnutrition in dialysis patients, respectively. Moreover, due to the small sample size, many other covariates such as dialysis vintage and insurance status could not be included in the multivariate model. Despite a small sample size and such limitations, the detected associations found in our study are significant. It should be noted that our study did not examine whether administration of i.v. iron in the setting of inflammation could further intensify such deleterious processes as oxidative stress, which could lead to worsening inflammation and atherosclerosis as recently shown by Drueke *et al.* [24]. Furthermore, our study cannot determine whether more iron was given to MHD patients because they had anaemia due to

inflammation and/or malnutrition or whether iron administration led to MICS and its consequences.

In summary, we found positive associations between serum ferritin and some markers of inflammation and malnutrition in a group of MHD patients. Our findings may have clinical implications, since they may indicate that current restrictions on iron supplementation are inappropriate and not evidence based. Based on the results of our study, we suggest that for serum ferritin values between 800 and 2000 ng/ml, especially if not associated with a high transferrin saturation ratio, non-iron-related factors such as inflammation, liver disease and malignancies are ruled out, e.g. by measuring serum CRP and liver enzymes, calculating MIS, and evaluating the clinical status of such patients. Nevertheless, bone marrow biopsy, as the gold standard, is probably one of the only methods which could more conclusively resolve the problem under such circumstances. More studies are required to confirm our findings in dialysis patients and to re-examine current guidelines on iron administration to dialysis patients.

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*Conflict of interest statement*. K. Kalantar-Zadeh is currently conducting research, sponsored by Amgen Inc., the makers of EPOGEN. He is also a member of the speakers' bureau for Watson Inc., the manufacturer of Infed and Ferrlecit, and for Ortho-Biotech, Inc., manufacturer of Procrit. The remaining authors declared no conflicts of interest.

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