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Association of malnutrition–inflammation complex and responsiveness to erythropoiesis-stimulating agents in long-term hemodialysis patients

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Keywords: erythropoietin-stimulating agent (ESA) therapy, hemoglobin, inflammatory marker, malnutrition–inflammation complex, responsiveness to ESAs

ORIGINAL ARTICLE

ABSTRACT

Background. Protein-energy wasting, inflammation and refractory anemia are common in long-term hemodialysis patients. A decreased responsiveness to erythropoiesis-stimulating agents (ESA) is often the cause of the refractory anemia. We hypothesized that the malnutrition–inflammation complex is an independent predictor of decreased responsiveness to ESAs in hemodialysis patients.

Methods. This cohort study of 754 hemodialysis patients was examined for an association between inflammatory and nutritional markers, including the malnutrition–inflammation score (MIS) and responsiveness to ESA. Cubic spline models were fitted to verify found associations.

Results. The mean (±SD) age of patients was 54 ± 15 years, 53% were diabetic and 32% blacks. MIS was worse in the highest quartile of ESAs responsiveness index (ERI, ESA dose divided by hemoglobin) when compared with 1st quartile (6.5 ± 4.5 versus 4.4 ± 3.4; $P < 0.001$). Both C-reactive protein (log CRP) ($\beta = 0.19$) and interleukin-6 (log IL-6) ($\beta = 0.32$) were strong and independent predictors of ERI using multivariate linear regression. Serum albumin ($\beta = -0.30$) and prealbumin levels ($\beta = -0.14$) were inversely associated with ERI. Each 1 SD higher MIS, higher CRP and lower albumin were

associated with 86, 44 and 97% higher likelihood of having highest versus three lowest ERI quartiles in fully adjusted models [odds ratio (and 95% confidence interval) of 1.86 (1.31–2.85), 1.44 (1.08–1.92) and 1.97 (1.41–2.78)], respectively. Cubic splines confirmed the continuous and incremental nature of these associations.

Conclusions. Malnutrition–inflammation complex is an incremental predictor of poor responsiveness to ESAs in hemodialysis patients. Further studies are needed to assess whether modulating inflammatory or nutritional processes can improve anemia management.

INTRODUCTION

Protein-energy wasting (PEW) and inflammation are closely associated in patients on maintenance hemodialysis (MHD) [1, 2]. Chronic inflammation is common in uremic patients, in part because of genetic predisposition [3, 4], but also because of factors related to the decreased glomerular filtration rate (GFR) and to the dialysis procedure itself [5, 6]. An association between inflammation, nutrition and anemia has been reported in hemodialysis patients [7]. Moreover, in the National Health and Nutrition Examination Survey (NHANES) III population, Eustace *et al.* [8] found an association between the risk of having an increased CRP level with decreasing estimated GFR. Inflammation is an important cause of uremic cachexia, and baseline serum C-reactive protein (CRP) level independently predicts a decrease in fat mass over time in patients on maintenance dialysis [9]. The recognition of the link between inflammation and malnutrition resulted in the description of a syndrome called malnutrition–inflammation complex syndrome (MICS) or PEW [10].

The National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF-KDOQI) defined the hyporesponsiveness to erythropoiesis-stimulating agents (ESA) as the presence of at least one of the following three conditions: (i) a significant decrease in hemoglobin level at a constant ESA dose, (ii) a significant increase in the ESA dose requirement to preserve a certain hemoglobin level or (iii) a failure to raise the hemoglobin level to >11 g/dL despite an ESA dose equivalent to erythropoietin >500 IU/kg/week [11]. Although anemia has been associated with increased rates of death and complications in patients with chronic kidney disease (CKD) who are undergoing dialysis and in those not undergoing dialysis [12, 13], a reduced hematopoietic response to ESAs has also been associated with an increased risk of an adverse outcome [14–18]. Additionally, previous studies have shown an association between erythropoietin resistance and mortality among CKD patients [19, 20]. It has been suggested that the dose of ESA is a frequently neglected confounder for the association between hemoglobin and mortality in randomized trials and that the variable ESA requirements may potentially and plausibly generate confounding by indication [21].

Several studies revealed the association between ESAs responsiveness index (ERI) and nutrition and inflammatory markers in hemodialysis patients [22–26]. Wei *et al.* [27] studied 44 peritoneal dialysis patients and found that patients who need EPO ≥ 150 U/kg/week had higher CRP and lower serum albumin. Another recent large cohort study, which measured CRP in 1754 hemodialysis patients found that patients in the upper CRP quartiles were more likely to be older, recently hospitalized, have a catheter as vascular access, have lower albumin, hemoglobin and transferrin saturation levels and receive higher ESA doses [28]. In our prior smaller study of only 385 patients from the first 12 months of our current cohort [The Nutritional and Inflammatory Evaluation in Dialysis (NIED) study], we found differences in levels of inflammatory markers across increments of ESA dose per kilogram; however, we did not examine systematically ERI over a longer period of time [29]. Larger national cohort studies did examine more elaborate nutritional and inflammatory markers such as pro-inflammatory cytokines and their associations with ESA hyporesponsiveness [30, 31]. Moreover, novel techniques of illustrating trends and incremental associations have not been used previously.

In the current study, we assessed the association between inflammatory and nutritional markers and ERI in 754 patients over a 5-year period (2001–06) using a combination of logistic regressions with cubic spline modeling. We hypothesized that higher levels of inflammatory markers and lower levels of nutritional markers, representing worse malnutrition–inflammation complex, are independent predictors of ERI in patients on MHD.

MATERIALS AND METHODS

Patient population

We studied MHD patients who participated in the NIED study [29]. The original NIED cohort consisted of 754 patients who were recruited from a population base of more than 3000 MHD outpatients treated in eight DaVita maintenance dialysis clinics in Southern California during a period of 6 years. To be included in the study, patients had to be at least 18 years old and receiving outpatient hemodialysis for at least 8 weeks. Patients were excluded if they had an acute infection or had a life expectancy of <6 months. The study was approved by the relevant institutional review committees and all subjects gave informed consent prior to being enrolled in the study. The medical records for each subject were thoroughly reviewed by a collaborating physician in the study. Information such as underlying kidney disease, cardiovascular disease history and other illnesses was abstracted.

Erythropoietin therapy and responsiveness to ESA

In all seven dialysis facilities, precise documentation of the administered doses of recombinant human erythropoietin or epoetin alfa (Epogen) (ESA) and iron was available. The total dose of ESA (U/week) among all 754 MHD patients of this analysis was calculated over the first 13-week (3 month) interval after patients entered the 5-year (7/2001–6/2006) cohort. The average weekly ESA dose then was calculated by dividing the total 3-month dose by 13. For those patients who missed more than 1 week of dialysis treatment or who left the cohort before the end of the third month (because of death,

transplant), the average ESA dose/week was calculated using the actual numbers of weeks they contributed to the cohort. ESA responsiveness (resistance) index was defined as the average weekly ESA dose divided by the average blood hemoglobin as described by Gunnell *et al.* [32] to normalize the amount of required ESA for the degree of severity of anemia. Most nephrologists were not aware of the periods in which this analysis was conducted. We assumed that all nephrologists treated the anemia of their MHD patients according to NKF-DOQI guidelines [33], i.e. to achieve a targeted hemoglobin of 11–12 g/dL (110–120 g/L) and/or a hematocrit of 33–36%.

Malnutrition inflammation score

Using the seven components of the conventional Subjective Global Assessment of Nutrition (SGA), a semiquantitative scale with three severity levels, and combining it with three new elements [body mass index (BMI), serum albumin and total iron binding capacity (TIBC) to represent serum transferrin] in an incremental fashion, the so-called malnutrition–inflammation score (MIS) with 10 components has been created [1]. 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 increasing degrees of severity. In a prospective study in MHD patients, the MIS was compared with the conventional SGA and its refinements, anthropometry, near-infrared measured body fat percentage, laboratory measures including serum CRP, and 12-month prospective hospitalization and mortality rates [1]. 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 anemia 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. In this study, MHD patients were scored by collaborating renal dietitians who were trained adequately for this purpose. To evaluate the degree of reproducibility, the MIS was reassessed randomly by a physician on a subset of 24 patients without reference to the first MIS evaluation. The correlation coefficient (r) between the two MIS assessments was 0.88 denoting a high degree of reproducibility.

Laboratory tests

Pre- and post-dialysis blood samples were obtained on a mid-week day that coincided with the day that the required quarterly blood drawings were obtained for testing at the DaVita dialysis facilities. Single-pooled Kt/V was used to represent the weekly dialysis dose. All laboratory studies were performed by DaVita Laboratories (Deland, FL) using automated methods. Serum high-sensitivity CRP was measured using a turbidometric immunoassay (WPCI, Osaka, Japan; normal range <3.0 mg/L) [34, 35]. Interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) levels were measured with using immunoassay kits (R&D Systems, Minneapolis, MN; units: pg/mL; normal range: IL-6: <9.9 pg/mL, TNF-α: <4.7 pg/mL) [36, 37]. The CRP, TNF-α and IL-6 levels were measured in the General Clinical Research Center Laboratories at Harbor UCLA. Serum transthyretin (prealbumin) was measured by immunoprecipitation and plasma homocysteine concentration was measured by high-performance liquid chromatography in the Harbor-UCLA Clinical Laboratories.

Statistical methods

The NIED study was a prospective study, while our analyses were cross-sectional using baseline data at the inception of the cohort. Data were summarized using proportions, means [±standard deviation (SD)] or medians (inter-quartile range) as appropriate. Categorical variables were compared using χ^2 tests, and continuous variables were compared using t-tests or the Mann–Whitney U-tests, the Kruskal–Wallis H-tests or analyses of variance, as appropriate. We used Pearson's and Spearman's rank-order correlation coefficients for selected analyses where indicated. Multivariate regression analyses including linear and logistic regression were performed to assess the association between inflammatory/nutritional markers and ERI. In our fully adjusted model, we adjusted for age, gender, race, diabetes mellitus, dialysis center, insurance (Medicaid versus others), Kt/V (single pool), blood hemoglobin, serum iron saturation ratio, the Charlson comorbidity score, dialysis vintage and intact parathyroid hormone. The associations were assessed using fractional polynomials and restricted cubic splines. Data analysis was performed using STATA version 11.1 (STATA Corporation, College Station, TX).

RESULTS

Table 1 shows the descriptive analyses of all 754 hemodialysis patients among ERI quartile groups. The mean age was 54 ± 15 years, the proportion of males was 53%, 53% were diabetic and the mean dialysis duration time (vintage) was 30 ± 34 months. Consistent with the Southern California location of the dialysis clinics, this study population had a substantial Hispanic contribution of 49%, and 32% of patients were black. Patients with higher ERI had higher MIS, CRP and IL-6 levels and lower levels of nutritional markers such as serum albumin, prealbumin and percentage of lymphocytes.

Table 2 shows multivariate linear regression between responsiveness to ESA and relevant nutritional and inflammatory values after adjustment for several important confounders in our fully adjusted model. Both CRP (log CRP) ($\beta = 0.19$) and logarithm of IL-6 (log IL-6) ($\beta = 0.32$) were strong and independent predictors of ERI using multivariate linear regression. In addition, serum albumin ($\beta = -0.30$) and prealbumin levels ($\beta = -0.14$) were also found as negative independent predictors of ERI. In addition, a significant, moderate positive correlation was found between ERI and inflammatory markers such as log CRP ($R = 0.18$) (Figure 1, upper panel) and log IL6 ($R = 0.31$) (Figure 1, lower panel).

Table 3 shows the likelihood of ESA the worst hyporesponsiveness using multivariate logistic regression analyses to compare the highest (worse) versus three lowest quartiles (as reference) of responsiveness to ESA. The likelihood of belonging to the highest ERI quartile compared with three lowest quartiles increased by 86% for every 1 SD higher MIS [odds ratio (OR) = 1.86 95% confidence interval (CI): 1.31–2.85], by 44% for every 1 SD higher of CRP (OR = 1.44 95% CI: 1.08–

Table 1. Descriptive analysis of the demographic and laboratory data of all 754 MHD patients and a comparison among ERI) across four quartiles

	Total (n = 754)	ERI (quartile 1) (n = 189); *best responsiveness*	ERI (quartile 2) (n = 188)	ERI (quartile 3) (n = 189)	ERI (quartile 4) (n = 188); *worse responsiveness*	P-value*
Gender (% men)	53	59	49	55	48	0.09
Age (year)	54 ± 15	55 ± 15	55 ± 14	54 ± 14	53 ± 16	0.58
Vintage time (months)	30 ± 34	33 ± 36	27 ± 27	30 ± 37	32 ± 35	0.29
Ethnicity (Hispanics %)	49	48	53	54	43	0.10
Race (% black)	32	29	30	29	42	0.01
Diabetes (%)	53	59	49	55	48	0.10
SLE (%)	2	0	3	2	3	0.17
Glomerulonephritis (%)	9	10	7	11	8	0.57
Polycystic kidney disease (%)	1.5	3	0	2	1	0.08
ESA dose (U/week)	7032 ± 4963	2184 ± 787	4715 ± 832	7837 ± 978	13 415 ± 5113	<0.001
ESA dose per weight (U/kg/week)	102 ± 75	32 ± 14	70 ± 26	114 ± 29	187 ± 82	<0.001
ESA response index (ESA/Hb)	600 ± 459	177 ± 64	387 ± 64	644 ± 77	1,196 ± 499	<0.001
ESA response/wt (ESA/Hb/kg)	8.71 ± 7	2.57 ± 1	5.80 ± 2	9.35 ± 2	16.67 ± 8	<0.001
MIS	5.1 ± 3.6	4.4 ± 3.4	4.5 ± 3.2	4.5 ± 3.1	6.4 ± 4.5	<0.001
Blood Hb (g/dL)	12.1 ± 0.9	12.4 ± 0.7	12.2 ± 0.8	12.2 ± 0.9	11.4 ± 1.2	<0.001
Lymphocyte count (%)	23 ± 8	24 ± 7.5	23 ± 7	23 ± 8	21 ± 8	<0.001
BMI (kg/m^2)	26 ± 6.1	26 ± 5.1	26 ± 5.4	27 ± 6.1	27 ± 7.3	0.07
Kt/V (single pool)	1.61 ± 0.31	1.59 ± 0.28	1.62 ± 0.27	1.63 ± 0.35	1.59 ± 0.33	0.50
nPCR (g/kg/dL)	1.06 ± 0.24	1.03 ± 0.26	1.09 ± 0.24	1.08 ± 0.23	1.04 ± 0.25	0.05
Serum ferritin (ng/mL)	569 ± 412	590 ± 367	552 ± 414	548 ± 426	546 ± 496	0.72
Serum iron (ng/mL)	66 ± 27	75 ± 25	67 ± 27	68 ± 25	55 ± 26	<0.001
TIBC (mg/dL)	208 ± 40	211 ± 35	210 ± 38	208 ± 36	203 ± 49	0.24
Iron saturation (%)	32 ± 11	35 ± 11	35 ± 11	31 ± 11	27 ± 10	<0.001
Albumin (g/dL)	3.88 ± 0.38	3.98 ± 0.33	3.94 ± 0.35	3.94 ± 0.32	3.71 ± 0.46	<0.001
Prealbumin (mg/dL)	28.29 ± 9.6	29.7 ± 9	28.5 ± 10	28.86 ± 9.6	26.14 ± 9.6	0.006
LDL (mg/dL)	80.6 ± 32.3	85.6 ± 32.6	80 ± 36	76.5 ± 27	79.3 ± 32.5	0.23
CRP (mg/L)	5.62 ± 6.70	4.47 ± 6.49	4.98 ± 5.28	5.56 ± 5.79	7.36 ± 8.94	<0.001
IL6 (ng/mL)	17.25 ± 48.29	12.32 ± 46.16	18.77 ± 68.44	12.95 ± 22.33	26.11 ± 55.94	0.03
TNF-α (ng/mL)	8.57 ± 11.50	7.49 ± 9.29	8.99 ± 12.99	9.17 ± 11.26	9.56 ± 13.99	0.24

Continued

Table 1. Continued

	Total (n = 754)	ERI (quartile 1) (n = 189); *best responsiveness*	ERI (quartile 2) (n = 188)	ERI (quartile 3) (n = 189)	ERI (quartile 4) (n = 188); *worse responsiveness*	P-value*
Intact PTH (mcu/L)	319 ± 341	299 ± 322	279 ± 259	319 ± 314	369 ± 441	0.06
Charlson comorbidity score	1.9 ± 1.6	1.8 ± 1.6	2 ± 1.6	2 ± 1.6	2 ± 1.7	0.16

Continuous values are expressed as mean ± SD (range). Count data are expressed as percentages. The cohort includes hemodialysis patients who have been treated with hemodialysis for more than 45 days.
*Categorical variables were compared using χ^2 tests, and continuous variables were compared using t-tests or Mann–Whitney U-tests, the Kruskal–Wallis H-tests or analyses of variance, as appropriate.
ESA, erythropoiesis-stimulating agent; MIS, malnutrition inflammatory score; TIBC, total iron binding capacity; nPCR, normalized protein catabolic rate; TSAT, transferrin saturation; CRP, C-reactive protein; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α.

Table 2. Predictors of ERI using multivariate regression analyses (fully adjusted model)[a]

	β	P-value
Blood lymphocyte percentage	−0.214	<0.001
MIS	+0.029	<0.001
nPCR	+0.020	0.69
Serum albumin	−0.301	<0.001
Serum prealbumin	−0.143	<0.001
Ferritin	+0.103	0.04
TIBC	−0.226	<0.001
Serum iron	−0.215	<0.001
TSAT (%)	−0.084	0.09
Log CRP	+0.193	<0.001
Log IL-6	+0.318	<0.001
Log TNF-α	+0.036	0.48
Intact PTH	+0.151	0.003

[a]Fully adjusted model adjusted for the value for conventional case-mix features (sex, age, race and presence of diabetes), dialysis center, ZIP code, insurance status (fully Medicaid versus others), Kt/V (single pool), blood hematocrit, the Charlson comorbidity score and dialysis vintage.
MIS, malnutrition inflammatory score; TIBC, total iron binding capacity; nPCR, normalized protein catabolic rate; TSAT, transferrin saturation; CRP, C-reactive protein; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α.

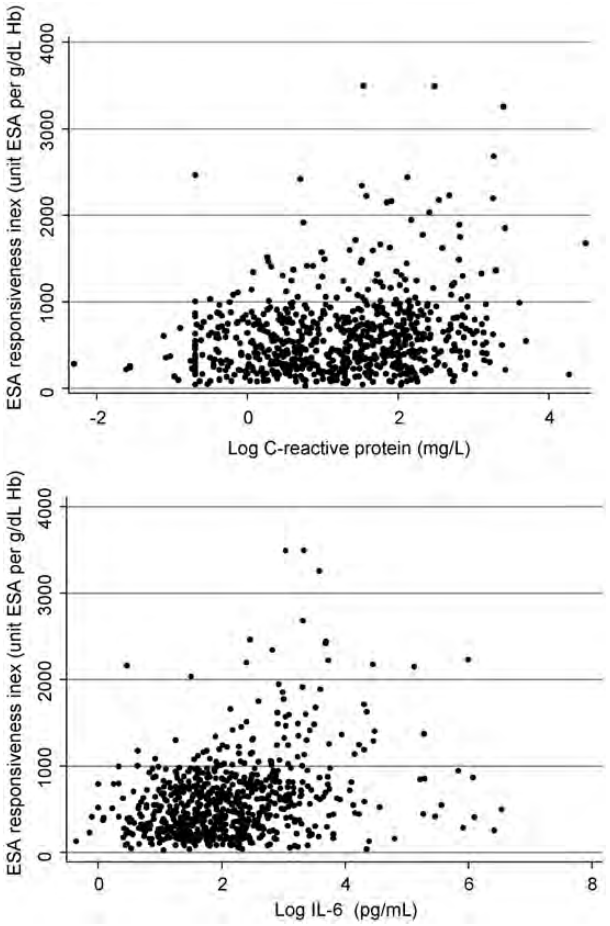

FIGURE 1: Scatter diagram showing the association between two inflammatory markers and ERI in 754 long-term hemodialysis patients. Upper panel: logarithm of CRP; and lower panel: logarithm of IL-6.

1.92) and by 97% for 1 SD lower serum albumin (OR = 1.97 95% CI: 1.41–2.78) in our fully adjusted logistic regression model.

The associations of different inflammatory and nutritional markers with ERI are shown in Figure 2. The association between serum albumin and higher ERI was decremental (Figure 2A). Similar to albumin, lower prealbumin was associated with higher ERI (Figure 2B). In the case of inflammatory

Table 3. Likelihood of the worst ESA hyporesponsiveness using multivariate logistic regression analyzes to compare the highest (worse) versus three lowest quartiles (as reference) of responsiveness to ESA, known here as ERI

	OR	95% CI	P-value
MIS (for 1 SD ↑)	1.86	1.31–2.85	<0.001
Serum CRP (for 1 SD ↑)	1.44	1.08–1.92	0.01
Log of CRP (for 1SD ↑)	1.70	1.24–2.33	0.001
IL-6 (for 1 SD ↑)	4.08	1.96–8.50	<0.001
Log of IL-6 (for 1 SD ↑)	2.18	1.58–3.00	<0.001
TNF-α (for 1 SD ↑)	1.02	0.73–1.44	0.89
Log of TNF-α (for 1 SD ↑)	1.19	0.82–1.73	0.37
Albumin (for 1 SD ↓)	1.97	1.41–2.78	<0.001
Prealbumin (for 1 SD ↓)	1.36	0.98–1.82	0.07
TIBC (for 1 SD ↓)	1.55	1.14–2.12	0.006
%TSAT (for 1 SD ↓)	1.32	0.98–1.79	0.07
Ferritin (for 1 SD ↑)	0.97	0.71–1.31	0.82
Blood lymphocyte count (for 1 SD ↓)	1.13	1.04–1.23	0.005

OR and 95% CI for quartile of ERI comparing the odds of highest ERI quartile group (4th ERI quartile group) versus three lowest ERI quartile groups (1st, 2nd and 3rd ERI quartile groups—as reference), adjusted for case-mix (age, gender, race, diabetes mellitus), dialysis center, insurance (Medicaid versus others), Kt/*V* (single pool), blood hemoglobin, serum iron saturation ratio, the Charlson comorbidity score, dialysis vintage and intact parathyroid hormone using logistic regression analyses.
MIS, malnutrition inflammatory score; TIBC, total iron-binding capacity; TSAT, transferrin saturation; CRP, C-reactive protein; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α.

markers, higher concentrations of log CRP, log IL-6 and MIS were associated with greater odds of the highest ERI quartile (Figure 2C, E and F).

DISCUSSION

In 754 MHD patients with comprehensive data of nutritional and inflammatory indicators, high levels of inflammatory markers and low levels of nutritional markers were independent predictors of hyporesponsiveness to ESA. Assuming that nutritional and anti-inflammatory interventions can correct PEW, our findings may have relevant clinical implications in anemia management in CKD patients to optimize the efficacy of ESA treatment.

In this cross-sectional analysis, all inflammatory factors were associated with hyporesponsiveness to ESAs. Chronic inflammation can inhibit erythropoiesis in part through the effects of IL-1, TNF-α, tumor necrosis factor-β and interferon-γ (IFN-γ). These pro-inflammatory cytokines are powerful inhibitors of erythropoiesis *in vivo* and *in vitro* [38]. The inhibition of erythropoiesis by cytokines, such as TNF-α and IFN-γ, is also important in the development of erythropoietin resistance [39–41]. In a randomized study by Costa *et al.* [42], non-responders to ESA treatment had higher CRP, lower serum albumin levels and lower number of total and CD4+ lymphocytes, compared with responders. A recent study [43] examining lymphocyte characteristics in dialysis patients reported that percentages of circulating CD3 and CD4 T-lymphocytes in peritoneal dialysis and MHD patients were significantly lower than in controls. Uremic toxins may cause defects in cell-mediated immunity, and alteration of cellular nutrients may affect lymphocyte function in MHD patients [44]. Other smaller clinical studies reported associations between ERI indices and inflammatory cytokines [45, 46].

Many studies explored the potential mechanism of how a decrease in inflammatory markers can result in increased erythropoietin responsiveness in MHD patients [47]. Attallah *et al.* performed a randomized, prospective study of MHD patients with unexplained hyperferritinemia and randomly assigned them to receive 300 mg of intravenous vitamin C versus placebo. Hemoglobin levels significantly increased and CRP levels significantly decreased in the treatment group [48]. Statins have been suggested for use in patients with chronic inflammation [47]. Statins are shown to decrease CRP levels irrespective of their effects on lipid levels and may be associated with reduced mortality in patients with ESRD [47, 49]. Chiang *et al.* [50] evaluated the efficacy of low-dose atorvastatin on ESA hyporesponsiveness and found that ERI decreased in the treatment group.

In the Heart Protection Study (HPS), simvastatin reduced the incidence of major vascular events to a similar

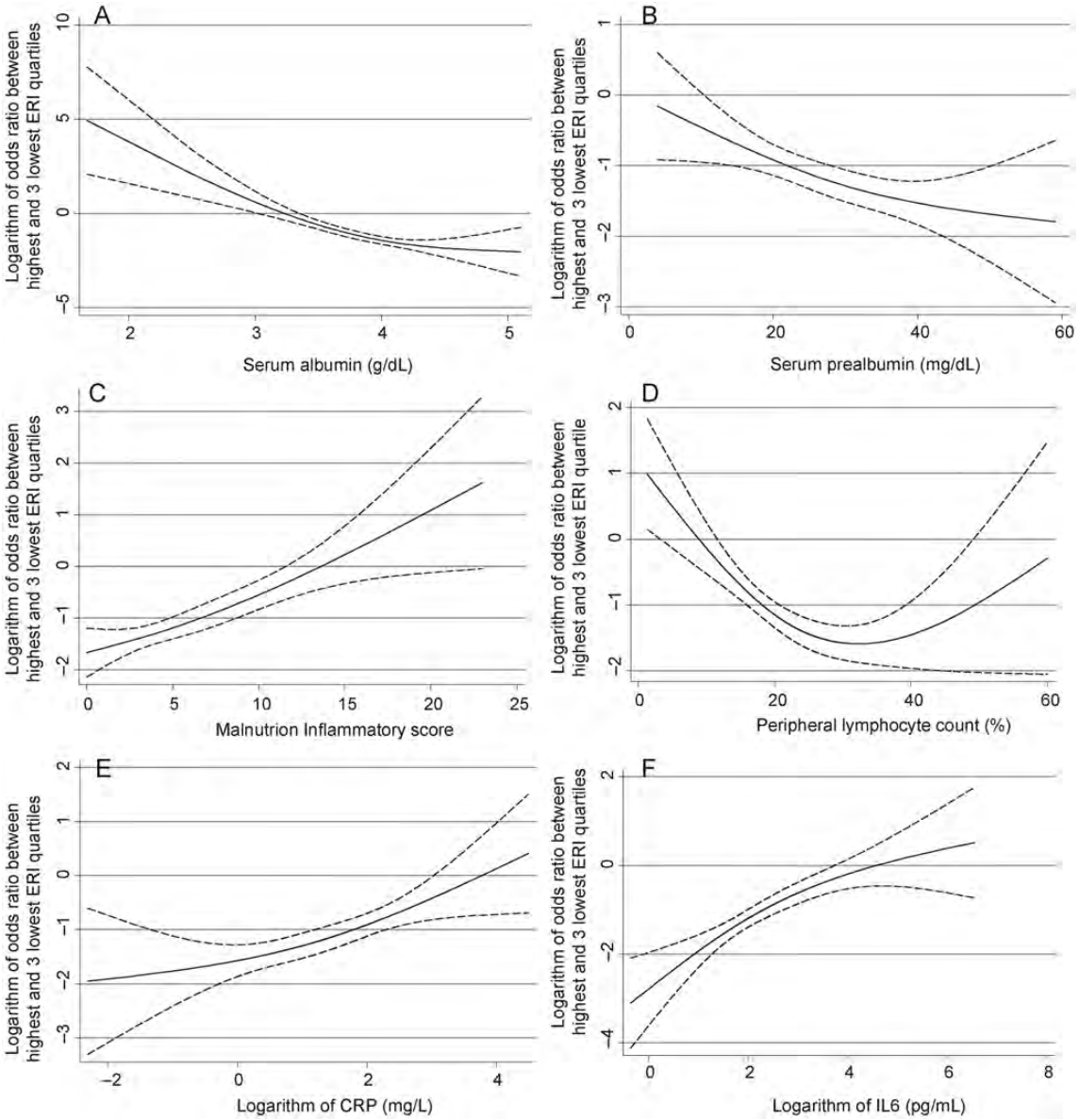

FIGURE 2: Likelihood of the worst ESA hyporesponsiveness using cubic spline analyses to compare the highest (worse) versus three lowest quartiles (as reference) of responsiveness to ESA. Note: adjusted for case-mix (age, gender, race, diabetes mellitus), dialysis center, insurance (Medicaid versus others), Kt/*V* (single pool), blood hemoglobin, serum iron saturation ratio, the Charlson comorbidity score, dialysis vintage and intact parathyroid hormone.

proportion irrespective of presenting CRP, albumin or other circulating inflammatory markers concentrations. There was no evidence that CRP modifies the vascular protective effects of statin therapy. Secondary analysis of the JUPITER study also did not record any evidence that the effect of a statin on vascular events differed according to baseline CRP [51, 52]. CRP is positively associated with smoking, diabetes, physical activity, blood pressure, BMI and might not reflect causality. This is supported by genetic–epidemiological studies that showed no increased cardiovascular mortality among people with genetic high CRP. Therefore, CRP has been excluded as a causal factor on the basis of this [53–55]. CRP is most likely a biomarker that predicts cardiovascular mortality. Most likely, it is more important to treat the underlying (low-grade)-inflammation as evidenced by elevated CRP levels.

Activation of monocyte-enhanced release of cytokines can be caused by membrane-induced complement activation, by direct cell–membrane interaction, and by dialysis fluids containing endotoxin [46]. Panichi *et al.* [56] studied the effects of a vitamin E-coated polysulfone membrane and found that ERI was significantly reduced. Moreover, high-efficiency on-line hemodiafiltration has been shown to improve anemia and to reduce erythropoietin-stimulating agent needs in hemodialysis patients [57]. A significant reduction in plasma CRP and IL-6 levels was also observed. In randomized controlled trials examining the effects of online-produced or filtered ultrapure dialysate on anemia outcomes in MHD patients, ESA doses

were significantly decreased [46, 58, 59]. These studies raise hope that novel therapies to reduce inflammatory processes may decrease ESA dose requirements.

We also found a negative correlation between measured nutritional markers (% of lymphocyte count, serum albumin, prealbumin, low-density lipoprotein and TIBC) and hyporesponsiveness to ESAs. TNF-α, also known as cachectin, is believed to induce anorexia [60]. Although IL-6 and TNF-α have overlapping effects on food intake, the mechanisms of action are not identical. McCarthy [61] showed that the injection of TNF-α reduced food intake in starved rats, but it did not affect gastric emptying; however, the injection of IL-6 reduced both food intake and gastric emptying. Because both protein-energy malnutrition and inflammation are strongly associated with each other and can change many nutritional measures in the same direction, and because the relative contributions of these two conditions on outcomes in dialysis patients are not yet well defined, the term MICS or malnutrition inflammation atherosclerosis has been suggested to denote the important contribution of both of these conditions to poor dialysis outcome [62, 63].

A limitation of our study is that we did not measure other nutritional deficiencies, which can be limiting factors in the production of red blood cells, such as vitamin B_{12} and folate. Gastrointestinal or other bleeding episodes may have occurred. Furthermore, we did not evaluate hemoglobinopathies and other hereditary red blood cell abnormalities, which could cause refractory anemia. We did not record other comorbidities such as malabsorption, which could cause nutritional deficiencies. Additionally, we did not record other diseases, which can cause anemia of chronic disease such as malignancies, hypothyroidism and autoimmune diseases other than systemic lupus erythromatosus (SLE) and we do not have data about smoking status. Another limitation of this study is the possible inclusion of patients who used angiotensin-converting enzyme inhibitors or angiotensin receptor blockers. These medications can cause anemia by defective erythropoiesis [38]. Moreover, inflammatory markers may fluctuate even month to month in dialysis patients [64]. Since only prevalent hemodialysis patients were included in this study, the study may potentially suffer from survivor bias and the results of this study might be not applicable for incident patients [65, 66].

Strengths of our study include (i) its contemporary nature, because data were obtained in the twenty-first century (2001–06); (ii) uniform laboratory measurements, with all laboratory data obtained from a single facility and (iii) large sample size with several measured inflammatory markers.

CONCLUSIONS

High levels of inflammatory markers and low levels of nutritional markers were independent and significant predictors of hyporesponsiveness to ESA. Further studies are needed to assess whether reducing inflammatory processes in hemodialysis patients can increase the responsiveness to ESAs.

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CONFLICT OF INTEREST STATEMENT

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REFERENCES

1. Kalantar-Zadeh K, Kopple JD, Block G *et al.* A malnutrition–inflammation score is correlated with morbidity and mortality in maintenance hemodialysis patients. Am J Kidney Dis 2001; 38: 1251–1263
2. Kaizu Y, Ohkawa S, Odamaki M *et al.* Association between inflammatory mediators and muscle mass in long-term hemodialysis patients. Am J Kidney Dis 2003; 42: 295–302
3. Maruyama Y, Nordfors L, Stenvinkel P *et al.* Interleukin-1 gene cluster polymorphisms are associated with nutritional status and inflammation in patients with end-stage renal disease. Blood Purif 2005; 23: 384–393
4. Balakrishnan VS, Guo D, Rao M *et al.* Cytokine gene polymorphisms in hemodialysis patients: association with comorbidity, functionality, and serum albumin. Kidney Int 2004; 65: 1449–1460
5. Raj DS, Dominic EA, Pai A *et al.* Skeletal muscle, cytokines, and oxidative stress in end-stage renal disease. Kidney Int 2005; 68: 2338–2344
6. Caglar K, Peng Y, Pupim LB *et al.* Inflammatory signals associated with hemodialysis. Kidney Int 2002; 62: 1408–1416
7. Kalantar-Zadeh K, Rodriguez RA, Humphreys MH. Association between serum ferritin and measures of inflammation, nutrition and iron in haemodialysis patients. Nephrol Dial Transplant 2004; 19: 141–149
8. Eustace JA, Astor B, Muntner PM *et al.* Prevalence of acidosis and inflammation and their association with low serum albumin in chronic kidney disease. Kidney Int 2004; 65: 1031–1040
9. Fujino Y, Ishimura E, Okuno S *et al.* C-reactive protein is a significant predictor of decrease in fat mass in hemodialysis patients. Biomed Pharmacother 2005; 59: 264–268

10. Fouque D, Kalantar-Zadeh K, Kopple J *et al.* A proposed nomenclature and diagnostic criteria for protein-energy wasting in acute and chronic kidney disease. Kidney Int 2008; 73: 391–398
11. KDOQI clinical practice guidelines and clinical practice recommendations for anemia in chronic kidney disease. Am J Kidney Dis 2006; 47(5 Suppl 3): S11–S145
12. Locatelli F, Pisoni RL, Combe C *et al.* Anaemia in haemodialysis patients of five European countries: association with morbidity and mortality in the Dialysis Outcomes and Practice Patterns Study (DOPPS). Nephrol Dial Transplant 2004; 19: 121–132
13. Vlagopoulos PT, Tighiouart H, Weiner DE *et al.* Anemia as a risk factor for cardiovascular disease and all-cause mortality in diabetes: the impact of chronic kidney disease. J Am Soc Nephrol 2005; 16: 3403–3410
14. Messana JM, Chuang CC, Turenne M *et al.* Association of quarterly average achieved hematocrit with mortality in dialysis patients: a time-dependent comorbidity-adjusted model. Am J Kidney Dis 2009; 53: 503–512
15. Rossert J, Gassmann-Mayer C, Frei D *et al.* Prevalence and predictors of epoetin hyporesponsiveness in chronic kidney disease patients. Nephrol Dial Transplant 2007; 22: 794–800
16. Lopez-Gomez JM, Portoles JM, Aljama P. Factors that condition the response to erythropoietin in patients on hemodialysis and their relation to mortality. Kidney Int Suppl 2008: S75–S81
17. Kausz AT, Solid C, Pereira BJ *et al.* Intractable anemia among hemodialysis patients: a sign of suboptimal management or a marker of disease? Am J Kidney Dis 2005; 45: 136–147
18. Szczech LA, Barnhart HX, Inrig JK *et al.* Secondary analysis of the CHOIR trial epoetin-alpha dose and achieved hemoglobin outcomes. Kidney Int 2008; 74: 791–798
19. Kaysen GA, Muller HG, Ding J *et al.* Challenging the validity of the EPO index. Am J Kidney Dis 2006; 47: 166
20. Zhang Y, Thamer M, Stefanik K *et al.* Epoetin requirements predict mortality in hemodialysis patients. Am J Kidney Dis 2004; 44: 866–876
21. Bradbury BD, Brookhart MA, Winkelmayer WC *et al.* Evolving statistical methods to facilitate evaluation of the causal association between erythropoiesis-stimulating agent dose and mortality in nonexperimental research: strengths and limitations. Am J Kidney Dis 2009; 54: 554–560
22. Barany P, Divino Filho JC, Bergstrom J. High C-reactive protein is a strong predictor of resistance to erythropoietin in hemodialysis patients. Am J Kidney Dis 1997; 29: 565–568
23. Kalantar-Zadeh K, Kleiner M, Dunne E *et al.* Total iron-binding capacity-estimated transferrin correlates with the nutritional subjective global assessment in hemodialysis patients. Am J Kidney Dis 1998; 31: 263–272
24. Stenvinkel P, Barany P. Anaemia, rHuEPO resistance, and cardiovascular disease in end-stage renal failure; links to inflammation and oxidative stress. Nephrol Dial Transplant 2002; 17(Suppl 5): 32–37
25. Kalantar-Zadeh K, Kopple JD. Relative contributions of nutrition and inflammation to clinical outcome in dialysis patients. Am J Kidney Dis 2001; 38: 1343–1350
26. Kalantar-Zadeh K, Block G, Humphreys MH, Kopple JD. Reverse epidemiology of cardiovascular risk factors in maintenance dialysis patients. Kidney Int 2003; 63: 793–808. Review. PubMed PMID: 12631061
27. Wei M, Bargman JM, Oreopoulos DG. Factors related to erythropoietin hypo-responsiveness in patients on chronic peritoneal dialysis. Int Urol Nephrol 2007; 39: 935–940
28. Bradbury BD, Critchlow CW, Weir MR *et al.* Impact of elevated C-reactive protein levels on erythropoiesis-stimulating agent (ESA) dose and responsiveness in hemodialysis patients. Nephrol Dial Transplant 2009; 24: 919–925
29. Colman S, Bross R, Benner D *et al.* The Nutritional and Inflammatory Evaluation in Dialysis patients (NIED) study: overview of the NIED study and the role of dietitians. J Ren Nutr 2005; 15: 231–243
30. Regidor DL, Kopple JD, Kovesdy CP *et al.* Associations between changes in hemoglobin and administered erythropoiesis-stimulating agent and survival in hemodialysis patients. J Am Soc Nephrol 2006; 17: 1181–1191
31. Kalantar-Zadeh K, Lee GH, Miller JE *et al.* Predictors of hyporesponsiveness to erythropoiesis-stimulating agents in hemodialysis patients. Am J Kidney Dis 2009; 53: 823–834
32. Gunnell J, Yeun JY, Depner TA *et al.* Acute-phase response predicts erythropoietin resistance in hemodialysis and peritoneal dialysis patients. Am J Kidney Dis 1999; 33: 63–72
33. Drüeke TB, Locatelli F, Clyne N *et al.* Normalization of hemoglobin level in patients with chronic kidney disease and anemia. N Engl J Med 2006; 355: 2071–2084
34. Ridker PM, Rifai N, Rose L *et al.* Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. N Engl J Med 2002; 347: 1557–1565
35. Erbagci AB, Tarakcioglu M, Aksoy M *et al.* Diagnostic value of CRP and Lp(a) in coronary heart disease. Acta Cardiol 2002; 57: 197–204
36. Pecoits-Filho R, Barany P, Lindholm B *et al.* Interleukin-6 is an independent predictor of mortality in patients starting dialysis treatment. Nephrol Dial Transplant 2002; 17: 1684–1688
37. Beutler B, Cerami A. The biology of cachectin/TNF—a primary mediator of the host response. Annu Rev Immunol 1989; 7: 625–655
38. Hodges VM, Rainey S, Lappin TR *et al.* Pathophysiology of anemia and erythrocytosis. Crit Rev Oncol Hematol 2007; 64: 139–158
39. Cooper AC, Mikhail A, Lethbridge MW *et al.* Increased expression of erythropoiesis inhibiting cytokines (IFN-gamma, TNF-alpha, IL-10, and IL-13) by T cells in patients exhibiting a poor response to erythropoietin therapy. J Am Soc Nephrol 2003; 14: 1776–1784
40. Pecoits-Filho R, Heimburger O, Barany P *et al.* Associations between circulating inflammatory markers and residual renal function in CRF patients. Am J Kidney Dis 2003; 41: 1212–1218
41. Panichi V, Migliori M, De Pietro S *et al.* Plasma C-reactive protein in hemodialysis patients: a cross-sectional, longitudinal clinical survey. Blood Purif 2000; 18: 30–36
42. Costa E, Lima M, Alves JM *et al.* Inflammation, T-cell phenotype, and inflammatory cytokines in chronic kidney disease patients under hemodialysis and its relationship to resistance to recombinant human erythropoietin therapy. J Clin Immunol 2008; 28: 268–275
43. Guo CH, Wang CL, Chen PC *et al.* Linkage of some trace elements, peripheral blood lymphocytes, inflammation, and

oxidative stress in patients undergoing either hemodialysis or peritoneal dialysis. Perit Dial Int 2011; 31: 583–591
44. Sayarlioglu H, Erkoc R, Demir C *et al.* Nutritional status and immune functions in maintenance hemodialysis patients. Mediators Inflamm 2006; 2006: 20264
45. Goicoechea M, Martin J, de Sequera P *et al.* Role of cytokines in the response to erythropoietin in hemodialysis patients. Kidney Int 1998; 54: 1337–1343
46. Sitter T, Bergner A, Schiffl H. Dialysate related cytokine induction and response to recombinant human erythropoietin in haemodialysis patients. Nephrol Dial Transplant 2000; 15: 1207–1211
47. Kalantar-Zadeh K, Ikizler TA, Block G *et al.* Malnutrition–inflammation complex syndrome in dialysis patients: causes and consequences. Am J Kidney Dis 2003; 42: 864–881
48. Attallah N, Osman-Malik Y, Frinak S *et al.* Effect of intravenous ascorbic acid in hemodialysis patients with EPO-hyporesponsive anemia and hyperferritinemia. Am J Kidney Dis 2006; 47: 644–654
49. Koc M, Dogan C, Arinsoy T *et al.* Statin use is associated with lower inflammation and erythropoietin responsiveness index in hemodialysis patients. Hemodial Int 2011; 15: 366–373
50. Chiang CK, Yang SY, Peng YS *et al.* Atorvastatin increases erythropoietin-stimulating agent hyporesponsiveness in maintenance hemodialysis patients: role of anti-inflammation effects. Am J Nephrol 2009; 29: 392–397
51. Ridker PM, Danielson E, Fonseca FA *et al.* Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. N Engl J Med 2008; 359: 2195–2207
52. Jonathan E, Derrick B, Emma L *et al.* C-reactive protein concentration and the vascular benefits of statin therapy: an analysis of 20,536 patients in the Heart Protection Study. Lancet 2011; 377: 469–476
53. O'Donnell CJ, Nabel EG. Genomics of cardiovascular disease. N Engl J Med 2011; 365: 2098–2109
54. Hingorani AD, Shah T, Kumari M *et al.* Translating genomics into improved healthcare. BMJ 2010; 341: c5945
55. Elliott P, Chambers JC, Zhang W *et al.* Genetic loci associated with C-reactive protein levels and risk of coronary heart disease. JAMA 2009; 302: 37–48
56. Panichi V, Rosati A, Paoletti S *et al.* A vitamin E-coated polysulfone membrane reduces serum levels of inflammatory markers and resistance to erythropoietin-stimulating agents in hemodialysis patients: results of a randomized cross-over multicenter trial. Blood Purif 2011; 32: 7–14
57. Panichi V, Scatena A, Paoletti S *et al.* Impact of dialysis technique on renal anemia. Contrib Nephrol 2011; 171: 261–265
58. Hsu PY, Lin CL, Yu CC *et al.* Ultrapure dialysate improves iron utilization and erythropoietin response in chronic hemodialysis patients—a prospective cross-over study. J Nephrol 2004; 17: 693–700
59. Schiffl H, Lang SM, Bergner A. Ultrapure dialysate reduces dose of recombinant human erythropoietin. Nephron 1999; 83: 278–279
60. Espat NJ, Copeland EM, Moldawer LL. Tumor necrosis factor and cachexia: a current perspective. Surg Oncol 1994; 3: 255–262
61. McCarthy DO. Tumor necrosis factor alpha and interleukin-6 have differential effects on food intake and gastric emptying in fasted rats. Res Nurs Health 2000; 23: 222–228
62. Kalantar-Zadeh K, McAllister CJ, Lehn RS *et al.* Effect of malnutrition–inflammation complex syndrome on EPO hyporesponsiveness in maintenance hemodialysis patients. Am J Kidney Dis 2003; 42: 761–773
63. Stenvinkel P, Heimburger O, Lindholm B *et al.* Are there two types of malnutrition in chronic renal failure? Evidence for relationships between malnutrition, inflammation and atherosclerosis (MIA syndrome). Nephrol Dial Transplant 2000; 15: 953–960
64. Kaysen GA, Dubin JA, Muller HG *et al.* The acute-phase response varies with time and predicts serum albumin levels in hemodialysis patients. The HEMO Study Group. Kidney Int 2000; 58: 346–352
65. Jager KJ, Stel VS, Zoccali C *et al.* The issue of studying the effect of interventions in renal replacement therapy—to what extent may we be deceived by selection and competing risk? Nephrol Dial Transplant 2010; 25: 3836–3839
66. Rothman KJ. Epidemiology: An Introduction. Oxford: Oxford University Press, 2002

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