ACTA MEDICA LITUANICA. 2004. VOLUME 11 No. 2. P. 7–17
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Malnutrition–inflammation complex syndrome contributes to rHuEPO response in ESRD patients

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Objectives: Anemia is highly prevalent in hemodialysed end-stage renal disease (ESRD) patients. However, various recombinant human erythropoietin (rHuEPO) doses are necessary to reach the same Hb. We evaluated the contribution of malnutrition and systemic inflammation to rHuEPO response in HD patients.

Methods: Forty-four ESRD patients (23 M, 21 F, mean age 52.6 ± ± 13.9 years) were grouped according to the required rHuEPO dose: Group A, n = 27, required rHuEPO dose <100 U/kg/day; Group B, n = 17, required rHuEPO dose ³100 U/kg/week. Presence of malnutrition was evaluated using dietary logs, anthropometric measurements and Subjective Global Assessment (SGA) scores. Presence of inflammation was detected by measuring C-reactive protein (CRP) and serum amyloid A (SAA) levels.

Results: Mean Hb concentration for all 44 patients was 10.4 ± ± 1.7 g/dl and did not differ between the groups. Lower weight ($p < 0.05$), body mass index ($p < 0.02$) and mid-arm circumference ($p < 0.05$) were found in patients taking the higher rHuEPO dose. Mean protein and energy intake for all patients was 1.01 ± 0.23 g/kg/day and 25.4 ± 6.5 kcal/kg/day, respectively, with no difference between the groups. However, protein equivalent of total nitrogen appearance (PNA) was lower in group B ($p < 0.02$). Serum CRP and SAA levels were also higher in group B ($p < 0.02$ and $p < 0.05$, respectively). Multivariate linear regression indicated that the required rHuEPO dose was more likely to be higher in subjects with a worse SGA score ($b = 0.35$; $p < 0.01$) and with a lower daily energy intake ($b = –0.37$; $p < 0.01$) *i.e.* in subjects with malnutrition and in subjects with a higher SAA ($b = 0.32$; $p < 0.02$), *i.e.* patients with inflammation.

Conclusions: The so-called malnutrition–inflammation complex syndrome is supposed to be the main cause of rHuEPO hyporesponsiveness in our study.

Key words: chronic renal failure, chronic inflammation, malnutrition, renal anemia, rHuEPO response

INTRODUCTION

Management of renal anemia seems to be of great importance in ensuring a better quality of life for end-stage renal disease (ESRD) patients undergoing hemodialysis treatment (HD).

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Several multicenter studies have shown that anemia is highly prevalent in ESRD patients. Thirty-four to sixty-seven percent of patients starting renal replacement therapy (RRT) exhibited signs of anemia (1, 2). Lower hematocrit values are commonly associated with a higher hospitalization rate. Lower hematocrit values are also a risk factor for comorbidity and mortality (3).

Since recombinant human erythropoietin (rHuEPO) was introduced into clinical practice more than 15 years ago, there has been a marked improvement in anemia care for ESRD patients.

Nevertheless, data from the Health Care Financing Administration's End-Stage Renal Disease (ESRD) Core Indicators Project showed that despite an improvement in the proportion of dialysis patients reaching adequate hematocrit levels in recent years, the percentage of patients meeting National Kidney Foundation Dialysis Outcomes Quality Initiative (NKF-DOQI) guidelines for anemia was only 42%. Ten percent of patients remained severely anemic (4).

Several factors influence the response to rHuEPO treatment in ESRD patients (5). Some factors have already been determined to influence the rHuEPO response, while there is less confidence in other factors' influence. Chronic inflammation has recently been suggested to be the main cause of morbidity and mortality in ESRD patients (6). Almost two-thirds of ESRD patients on maintenance HD show various signs of chronic inflammation. There is a proof to suggest that chronic inflammation may lead to a poor rHuEPO response (7). Chronic inflammation may also promote malnutrition in ESRD patients. However, it has been proposed by many researchers that an epidemiologic association between indicators of protein-energy malnutrition (PEM) and indicators of inflammation in ESRD does not indicate the direction of causality. The link between elevated levels of acute phase proteins or pro-inflammatory cytokines, anemia, rHuEPO dose and signs of malnutrition is not clearly defined, either. Up to now, the distribution of anemia and PEM among patients as well as individual characteristic rHuEPO responses among ESRD patients on maintenance HD have not been studied in Latvia.

Our aim in this cross-sectional study was to investigate the relationship between some demographic characteristics, laboratory characteristics, nutritional features and malnutrition and systemic inflammation in order to evaluate the effects of malnutrition and systemic inflammatory response on rHuEPO dose requirement in these patients.

MATERIALS AND METHODS

Patients

This study was performed at the largest in-hospital hemodialysis center in Latvia (P. Stradin's University Hospital, Riga, Latvia). Our university hospital-affiliated dialysis program served 51 adult hemodialysis patients at the time of study. We selected patients who had been on dialysis for at least 3 months. The study was approved by the Institutional Review Board and written informed consent was obtained form all participants before their enrollment in the study. We studied 44 patients (23 men, 21 women, mean age 52.6 ± 13.9 years) undergoing maintenance hemodialysis. The causes of chronic renal failure were as follows: chronic glomerulonephritis in 15 patients (34.1%), chronic interstitial nephritis in 8 patients (18.2%), diabetes mellitus in 5 patients (11.4%), polycystic kidney disease in 6 patients (13.6%), secondary amyloidosis in 3 patients (6.8%), Wegener's granulomathosis in 1 patient (2.3%), nodous polyarteriitis in 1 patient (2.3%), prostatic tumor with secondary hydronephrosis in 1 patient (2.3%), rheumathoid polyarthritis in 1 patient (2.3%), hypertensive nephropathy in 1 patient (2.3%) and hereditary aplastic kidneys in 1 patient (2.3%). No concominant illnesses that might be of importance were registered. All patients underwent dialysis 3 times a week for 3.5–4 hours (10.5–12 hours a week) using bicarbonate dialysate with the following ion concentrations: Na^+ – 137.5 mmol/l, K^+ – 2.0 mmol/l, Ca^{++} – 1.75 mmol/l, Mg^{++} – 0.75 mmol/l, Cl^- – 109.5 mmol/l, CH_3COO^- – 2.15 mmol/l, HCO_3^- – 31.6 mmol/l. Hemodialysis was performed using steam sterilized polysulphone membrane dialyzers (manufactured by Fresenius Medical Care AG, Bad Homburg, Germany) with a surface area of 1.3 to 2.4 m^2 and synthetic polyamide S membrane dialyzers (manufactured by Gambro Dialysatoren GmbH, Hechingen, Germany) with a surface area of 1.4 to 2.1 m^2. The purity of the dialysate was checked regularly; neither bacterial nor endotoxic or chemical contamination exceeded European standards.

Twenty-six patients (59.1%) received calcium carbonate as a phosphate-binder (mean daily dose 7.2 ± 2.5 g), and 14 patients (31.8%) were additionally treated with 1.0 to 2.0 μg 1 α-hydroxyvitamin-D_3 on a daily basis. Thirty-three patients (75%) participating in the study received calcium channel blockers and β-blockers as hypotensive medication in accordance with their routine prescription. None of the patients received aspirin or statins (both have anti-inflammatory properties) throughout the study. All patients received additional iron supplementation on a weekly basis, via intravenous injections. Thereafter, some demographic features as well as all anthropometric, biochemical and nutrient data were grouped according to required rHuEPO dose – group A (n = 27) with a rHuEPO dose less than 100 U/kg/week (mean rHuEPO dose 70.5 ± 17.9 U/kg/week; range 32 to 99 U/kg/week); group B (n = 17) with a rHuEPO dose equal to or higher than 100 U/kg/week (mean rHuEPO dose 147.8 ± 49.6 U/kg/week; range, 104 to 303 U/kg/week).

Dietary records

Three-day dietary logs were collected from patients during this study. Patients recorded their food and

beverage intake during an assigned 3-day period, including one dialysis day and two non-dialysis days. Protein and fat intake as well as total dietary energy intake were calculated as a function of the patient's actual body weight per day (g/kg/day and kcal/kg/day, respectively).

Urea kinetic modeling and PCR data

The adequacy of dialysis was calculated according to the method of Daugirdas using a single-pool, two-BUN, variable-volume model and the following formula:

$$Kt/V = -\ln [(R - (0.008 \times t)) + (4 - (3.5 \times \times R))] \times UF/W,$$

where R is the post-dialysis/pre-dialysis BUN ratio, *t* is time of dialysis, UF is the ultrafiltration volume, and W is the "dry" post-dialysis weight (8).

The midweek "protein equivalent of total nitrogen appearance" (PNA) / protein catabolic rate (PCR) was calculated as follows:

$$PNA(PCR) = C_0 / [25.8 + ((1.15) / (spKt/V)) + + (56.4) / (spKt/V)] + 0.168,$$

where C_0 is the pre-dialysis BUN.

Normalization of PNA(PCR) adjusting to a specific body size of patients was performed using the following formula:

$$nPNA(nPCR)\ (g/kg/day) = (PNA) / (V/0.58)),$$

where V is the urea distribution volume or total body water (TBW). TBW was determined according to a population-specific equation for calculating total body water based on TBW measurements using bioelectrical impedance (BEI) offered by Chertow et al (9).

Anthropometric measurements

Anthropometric measurements were conducted immediately after a dialysis session using standard techniques. Pre-dialysis and post-dialysis weight was measured during three consecutive dialysis sessions using an electronic scale. The patients' height was measured without shoes using a wall-mounted stadiometer. Biceps, triceps, subscapular and suprailiac skinfold thickness was measured using the Slim Guide skinfold caliper (Creative Health Products, Plymouth, MI). Skinfold measurements were repeated 3 times on the non-access arm and the average of the three measurements was recorded as the final value. The body mass index (BMI) was calculated as the ratio between the end-of-dialysis body weight (kg) and the height-squared (kg/m^2). The estimates of body fat mass (FM) and fat-free mass (FFM) were made according to the method of Durnin and Wormersley, using the following formula:

$$FM\ (kg) = body\ weight \times [(4.95/D) - 4.5],$$

where D is body density (g/ml) obtained via special equations of age- and sex-adjusted sum of four skinfold measurements.

Fat free mass (FFM) was calculated as follows:

$$FFM\ (kg) = body\ weight - fat\ mass.$$

To estimate skeletal muscle mass, mid upper arm circumference (MAC) was measured using a flexible nonstretchable metal tape.

Subjective Global Assessment

The SGA was used to assess the overall nutritional status of patients participating in the study. It was conducted by collecting information on the patients' medical history and performing a physical examination as described by Detsky et al. (10). A lower SGA score indicated a better nutritional intake.

Biochemical and clinical evaluation

All patients involved in the study were subjected to the following hematological, biochemical and clinical tests: evaluation of pre-dialysis blood hemoglobin concentration, serum creatinine levels, pre-dialysis and post-dialysis urea levels, serum intact PTH concentration, serum albumin, cholesterol, iron, total iron binding capacity, folic acid, vitamin B_{12} and ferritin concentrations. The concentrations were determined from samples collected at the single midweek dialysis session. The serum acute-phase reactant proteins or pro-inflammatory markers – C reactive protein (CRP) and serum amyloid A (SAA) – were also measured.

Hemoglobin values were determined using a Cell-Dyn 1700 hematologic analyser (Abbot Laboratories, Abbot Park, IL) at a laboratory in P. Stradin's University Hospital, Riga, Latvia. For routine biochemical tests, an Abbot Spectrum Series II biochemical analyzer (Abbot Laboratories, Abbot Park, IL) was used at the same laboratory. Transferrin saturation (%) was calculated from serum iron concentration and total iron binding capacity (TIBC) as follows: TS (%) = (Serum Iron/TIBC) × 100.

The serum concentration of folic acid was measured by ion capture assay for quantitative determination of folate using an Abbot Axsym System analyzer (Abbot Laboratories, Abbot Park, IL). Vi-

tamin B_{12} concentration was measured by microparticle enzyme intrinsic factor assay for quantitative determination of vitamin B_{12}. Serum ferritin levels were measured using microparticle enzyme immunoassay and the same analyzer. The level of serum intact parathormone (iPTH) was determined using a DPC IMMULITE immunologic analyser (Diagnostic Products Corporation Los Angeles, CA) by chemiluminescent immunometric iPTH assay (DPC-Immulite kit, Diagnostic Products Corporation Los Angeles, CA). C-reactive protein was measured using latex enhanced immunoturbidimetric immunoassay (Ultra CRP Kit, Polymedco, Inc. Cortlandt Manor, NY for Dimension® Systems, Dade Behring, Inc., Deerfield, IL). Serum amyloid A was measured using the nephelometry method (N Latex SAA Test, Dade Behring Marburg GmbH, Germany) on the Behring Nephelometer Pro Spec (Dade Behring Inc., Deerfield, IL).

Systolic and diastolic arterial blood pressure before and after hemodialysis sessions, treatment time, membrane type, and dry weight were obtained from dialysis records. Information on renal diagnosis, diabetic status, length of time on HD and use of any medications was obtained from patient interviews and medical chart review.

Statistical analysis

All results were expressed as a mean ± SD or median values with their range (for skewed and not normally distributed data). All skewed variables were converted accordingly into log scale. Statistical analysis was performed using the Student's unpaired t test for continuous normally distributed variables. As some of the data were skewed and not normally distributed, differences in those demographic and biochemical parameters between the groups were determined using the Mann–Whitney U test. For categorical response variables, differences between subgroups were assessed by applying the chi-square or Fisher exact test. Unadjusted correlations between two independent variables were assessed using the Pearson correlation model. Multivariate linear forward stepwise regression analysis was performed to determine the independent variables predicting rHuEPO dose. Age, sex, duration of dialysis, BMI, skinfold thickness, SGA score, nPNA, protein and energy intake, iPTH, iron, vitamin B_{12}, folic acid concentrations, CRP and SAA were tested as potential factors to predict the EPO response. P values less than 0.05 were considered significant. All statistical calculations were performed using the Statistica v. 4.5 package (StatSoft Inc., Tulsa, USA).

RESULTS

Demographic and clinical characteristics

Twenty-three men and twenty-one women were observed throughout this study. Their age ranged from 22 to 75 years, mean 52.6 ± 13.9. The mean time on hemodialysis prior to participation was 24.7 ± ± 13.1 months (range, 3 to 148 months). Each hemodialysis session was performed within a mean time of 207 ± 24 minutes. The mean systolic and diastolic arterial blood pressure was 135.0 ± 26.3 and 80.9 ± 13.4 mmHg, respectively. Thirteen patients (29.5%) received angiotensin converting enzyme (ACE) inhibitors as hypotensive medication.

Table 1. **Comparison of selected demographic parameters grouped according to the rHuEPO dose requirement in 44 hemodialysed ESRD patients**

Feature	Group A rHuEPO dose < 100 U/kg/week (n = 27)	Group B rHuEPO dose ≥ 100 U/kg/week (n = 17)	P value
Number of patients (%)	27 (61.4%)	17 (38.6%)	
Gender (M/F)	13/14	8/9	NS
Age (mean ± SD), years	52.4 ± 12.9	53.1 ± 15.5	NS
Duration of HD (mean ± SD), months	19.2 ± 27.3	33.4 ± 37.8	<0.05
Duration of each HD session (mean ± SD), min	208 ± 20	204 ± 27	NS
Systolic blood pressure (mean ± SD), mmHg	141.9 ± 25.7	124.1 ± 23.2	<0.01
Diastolic blood pressure (mean ± SD), mmHg	78.8 ± 8.5	82.9 ± 7.5	NS
Use of ACEI	8 (29.6%)	5 (29.4%)	NS
rHuEPO dose (mean ± SD), U/kg/week	70.5 ± 17.9	147.8 ± 49.6	<0.001
rHuEPO dose (mean ± SD), U/week	5074 ± 1206	9882 ± 4553	<0.001
Iron dose (mean ± SD), mg/week	49.3 ± 12.7	55.2 ± 15.5	NS

Note. All results were expressed as mean ± SD.

Demographic features grouped according to the required rHuEPO dose (group A (n = 27) with an rHuEPO dose less than 100 U/kg/week and group B (n = 17) with an rHuEPO dose equal to or greater than 100 U/kg/week) are listed in Table 1. More than a third (38.6%) of all the HD patients studied had a rHuEPO requirement that was greater than 100 U/kg/week. Patients who spent a shorter time on maintenance HD revealed lower rHuEPO dose values ($p < 0.05$). The systolic arterial blood pressure was found to be lower in patients with an rHuRPO dose greater than 100 U/kg/week ($p < < 0.01$).

Anthropometric measurements and nutrient intake

Anthropometric measurements of 44 maintenance HD patients (who were grouped according to the required rHuEPO dose) and the distribution of nutrient intake obtained from the 3-day dietary logs (expressed as g/kg/day and kcal/kg/day, respectively) are shown in Table 2. The rHuEPO dose-dependent distribution showed significant differences in weight ($p < 0.02$), BMI ($p < 0.05$) and mid-arm circumference ($p < < 0.05$) in patients with an erythropoietin dose less than 100 U/kg/week and patients with a rHuEPO dose greater than that. All variables were of lesser value in cases of a higher required rHuEPO dose. Almost two thirds of all patients (27 patients, 61.4%) showed signs of malnutrition if SGA was applied as a PEM evaluation tool (SGA 2+3 score or moderate + severe malnutrition). We also found the SGA score values as suggesting a more pronounced malnutrition in the group with the higher rHuEPO dose ($p < 0.01$).

Mean protein intake was similar in both groups. Fifteen patients (55.6%) in group A and thirteen patients (52.9%) in group B were found to have a protein intake lower than 1.0 g/kg/day.

Mean energy intake was also similar in both groups. The percentage of patients taking less than 30 kcal/kg/day was significantly higher ($p < 0.01$) in group A (22 patients or 81.5%) when compared to group B (10 patients or 58.2%).

There were no statistically significant differences in fat intake among the patients taking the lower *versus* the higher rHuEPO dose.

Biochemical values

Hematological and biochemical values of the 44 maintenance HD patients grouped by rHuEPO dose are highlighted in Table 3. When comparing the groups, the mean hemoglobin concentration divided by the rHuEPO dose did not differ significantly. Eight patients (29.6%) of group A had a hemoglobin concentration below 10.0 g/dl and 8 patients (47.1%) of group B had the same or a lower hemoglobin concentration. A hemoglobin concentration greater than 12 g/dl was observed in 4 patients (14.8%) of group A and 3 patients (17.6%) of group B. Serum creatinine and urea concentrations were found to be similar in both groups. The iPTH concentration was significantly higher in patients who required greater amounts of erythropoietin ($p < < 0.05$). Serum iron concentration, serum levels of B12 vitamin, folic acid and ferritin did not differ significantly among the groups. Transferrin saturation, reflecting functional iron deficiency, was also the same in both groups. Serum cholesterol was noted to be lower in the group with a rHuEPO dose greater than 100 U/kg/week ($p < 0.02$). Kt/V calculation reflecting the delivered dose of dialysis did not show any differences betwen the groups. The nPNA (measure of net protein degradation and protein intake in maintenance HD patients) values were significantly lower in

Table 2. **Anthropometric variables, SGA score and nutritient intake in 44 hemodialysed ESRD patients grouped by the required rHuEPO dose (U/kg/week)**

Feature	Group A rHuEPO dose < 100 U/kg/week (n = 27)	Group B rHuEPO dose ≥ 100 U/kg/week (n = 17)	P value
Weight (kg)	72.8 ± 10.2	65.4 ± 12.6	<0.02
Height (m)	1.70 ± 0.10	1.71 ± 0.10	NS
BMI (kg/m^2)	26.0 ± 4.6	23.3 ± 3.9	<0.05
FM (kg)	25.5 ± 8.0	21.5 ± 8.1	NS
FFM (kg)	47.3 ± 5.8	43.9 ± 7.9	NS
MAC (cm)	28.3 ± 3.7	25.7 ± 4.6	<0.05
Total 4 skinfold thickness (mm)	62.7 ± 38.2	54.1 ± 33.6	NS
SGA score	1.7 ± 0.8	2.3 ± 0.8	<0.01
Protein intake (g/kg/day)	1.02 ± 0.23	0.99 ± 0.24	NS
Energy intake (kcal/kg/day)	25.3 ± 5.9	25.6 ± 7.6	NS
Fat intake (g/kg/day)	1.03 ± 0.27	1.03 ± 0.41	NS

BMI, body mass index; FM, fat mass; FFM, fat free mass; MAC, mid upper arm circumference; SGA, subjective global assessment.
Note. All results were expressed as mean ± SD.

Table 3. **Biochemical variables of 44 hemodialysed ESRD patients, subdivided by rHuEPO dose (U/kg/week)**

Feature	Group A rHuEPO dose < 100 U/kg/week (n = 27)	Group B rHuEPO dose ≥ 100 U/kg/week (n = 17)	P value
Hemoglobin (g/dl)	10.5 ± 1.7	10.3 ± 1.8	NS
Serum creatinine (mmol/l)	0.88 ± 0.32	0.87 ± 0.27	NS
Serum urea (mmol/l)	22.7 ± 5.7	20.2 ± 7.6	NS
Serum iPTH (pmol/l) §	19.5 (1.3–89.2)	36.1 (6.9–96.4)	<0.05
Serum iron (μg/dl)	35.3 ± 16.0	39.3 ± 16.8	NS
Transferin saturation (%)	28.7 ± 14.3	24.8 ± 17.6	NS
Serum ferritin (ng/ml) §	83 (13–663)	122 (27–588)	NS
Serum folic acid (ng/ml)	5.3 ± 1.8	4.7 ± 1.4	NS
Serum B12 vitamin (pg/ml)	412.7 ± 191.6	453.8 ± 219.8	NS
Serum cholesterol (mmol/l)	4.88 ± 1.17	4.11 ± 1.13	<0.02
Kt/V	1.24 ± 0.26	1.35 ± 0.21	NS
nPNA (g/kg/day)	0.91 ± 0.20	0.81 ± 0.18	<0.05
Serum albumin (g/dl)	3.74 ± 0.41	3.45 ± 0.69	<0.05
Serum CRP (mg/l) §	6.2 (3.7–54.1)	16.5 (3.8–68.9)	<0.02
Serum SAA (mg/l) §	6.5 (1.0–63.5)	12.3 (1.8–255.9)	<0.05

nPNA , protein equivalent of total nitrogen appearance, normalized adjusting to a specific body size; CRP, C-reactive protein; SAA, serum amyloid A.
Note. All results were expressed as mean ± SD, except when indicated otherwise (§), as these data were not normally distributed, median and range were used instead.

patients requiring a higher rHuEPO dose ($p < 0.05$). The nPNA values below 1.0 g/kg/day (recommended lower limit for dialysis patients) were found in 21 patients (77.7%) of group A and in 15 patients (88.2%) of group B. Serum concentrations of albumin were lower, but acute phase proteins CRP and SAA were found to be significantly higher in those patients with a dose of rHuEPO exceeding 100 U/kg/week ($p < 0.05$; $p < < 0.02$ and $p < 0.05$, respectively).

Correlations and multivariate linear regression analysis

The Pearson correlation matrix was utilized to identify a potential relationship between rHuEPO dose (U/kg/week) and clinical, anthropometric, nutrient and biochemical variables. Table 4 depicts those variables showing a significant correlation with erythropoietin dose requirement. The most significant correlation with rHuEPO dose was found to be with daily fat intake ($R = –0.36$; $p < 0.02$) followed by daily energy intake ($R = –0.35$; $p < 0.02$), SGA score ($R = 0.34$; $p < 0.03$), daily protein intake ($R = –0.34$; $p < 0.03$), serum amyloid A ($R = = 0.33$; $p < 0.03$), BMI ($R = –0.33$; $p < 0.03$), serum iPTH level ($R = 0.32$; $p < 0.05$) and serum albumin concentration ($R = –0.32$; $p < 0.05$). Correlations were also found between serum albumin concentrations and CRP ($R = –0.42$; $p < 0.01$) and SAA ($R = –0.44$; $p < < 0.001$). Besides, we noted that the SGA score directly correlated with CRP ($R = 0.38$; $p < 0.02$) and SAA ($R = 0.39$; $p < 0.02$).

Final multivariate linear regression analysis using a forward stepwise elimination technique was performed separately using the rHuEPO dose as a dependent value (expressed both as U/kg/week and U/week). Age, sex, duration of dialysis, BMI, skinfold thickness, SGA score, serum albumin, iron, folic acid, vitamin B12, nPNA, iPTH, protein and energy intake, CRP and SAA were tested as independent variables or potential predicting factors. This model indicates that rHuEPO dose in units per kilogram per week (Table 5) was more likely to be higher in subjects with a higher SGA score, *i.e.* showing a stronger indication of malnutrition ($\beta = = 0.35$; $p < 0.01$), with a lower daily energy intake ($\beta = –0.37$; $p < 0.01$) and with a greater SAA concentration ($\beta = 0.32$;

Table 4. **Correlation (unadjusted data) between the clinical, anthropometric, nutrient and biochemical variables and rHuEPO dose (U/kg/week) of 44 ESRD patients on maintenance hemodialysis**

Feature	Pearson correlation (R)	P value
Serum albumin	–0.32	<0.05
Log serum iPTH	0.32	<0.05
Log serum SAA	0.33	<0.03
Daily protein intake	–0.34	<0.03
Daily energy intake	–0.35	<0.02
Daily fat intake	–0.36	<0.02
BMI	–0.33	<0.03
SGA	0.34	<0.03

BMI, body mass index; SGA, subjective global assessment; CRP, C-reactive protein; SAA, serum amyloid A.

Table 5. **Multivariate linear regression (forward stepwise model) summary for dependent variable rHuEPO dose expressed as U/kg/week in 44 ESRD patients on maintenance hemodialysis**

Feature	Beta (β)	P value
SGA	0.35	<0.01
Daily energy intake	–0.37	<0.01
Log serum SAA	0.32	<0.02

SGA, subjective global assessment; SAA, serum amyloid A.

Table 6. **Multivariate linear regression (forward stepwise model) summary for dependent variable rHuEPO dose expressed as U/week in 44 ESRD patients on maintenance hemodialysis**

Feature	Beta (β)	P value
SGA	0.36	<0.01
Daily energy intake	–0.37	<0.01
Daily protein intake	–0.35	<0.02
Log serum SAA	0.32	<0.02

SGA, subjective global assessment; SAA, serum amyloid A.

$p < < 0.02$). When a similar analysis was applied to the rHuEPO dose expressed as units per week (Table 6), the strongest predictive value was daily energy intake ($\beta = –0.37$; $p < 0.01$) followed by the SGA score ($\beta = 0.36$; $p < 0.01$), daily protein intake ($\beta = = –0.35$; $p < < 0.02$) and SAA concentration ($\beta = = 0.32$; $p < < 0.02$).

DISCUSSION

Anemia in ESRD patients is due predominantly to inadequate red blood cell (RBC) production caused by a low rate of erythropoietin (EPO) synthesis. However, the fact that highly various rHuEPO doses are necessary to reach the target Hb level proves that other factors alongside EPO deficiency determine the rHuEPO response (5). Nowadays, adequate iron stores and its availability to ensure optimum erythropoiesis are considered to be the strongest factor influencing the efficiency of anemia correction in CRF patients. As expected, our study did not find any differences between the groups with regard to such obvious causes of anemia as absolute or functional iron deficiency, blood loss, delivered dose dialysis, or medications which might influence the Hb level.

Another well-studied cause of renal anemia, which at the same time is a factor contributing to impaired rHuEPO response, is chronic inflammation. Other conditions that worsen anemia in CRF patients and influence the rHuEPO amount required to reach the target Hb are secondary hyperparathyroidism and osteitis fibrosa. In the same way, a significant role in the progression of anemia is attributed to the malnutrition and the amount of micronutrients (folic acid, vitamin B12, vitamin C) in food (5).

The number and types of factors vary significantly among the studies, which in turn means that the required rHuEPO dose will differ in order to adjust for anemia correction.

During the last several years, research has confirmed the belief that hemodialysis patients suffer from mild to moderate inflammation associated with elevated levels of so-called positive acute-phase proteins, such as C-reactive protein (CRP) (11), serum amyloid A (SAA) (12), as well as some circulating cytokines, such as interleukin-1, interleukin-6, tumor necrosis factor-α. Some evidence suggests that inflammation could be one of the major risk factors for cardiovascular morbidity and mortality in hemodialysis patients (13). It was found that ESRD patients suffering from coronary heart disease have elevated concentrations of such acute-phase reactants as CRP and SAA. They also frequently show signs of PEM, sometimes even refractory anemia (7). Infection or inflammation may predispose patients to atherosclerosis, catabolic state and hypoalbuminemia (13). Of the various factors that interfere with rHuEPO response, chronic inflammation seems to be of great significance in ESRD patients on maintenance hemodialysis. Proinflammatory cytokines such as TNF-α and IL-1 have been shown to inhibit RBC production by preventing the growth of some erythroid progenitor cells *in vitro* or by stimulating interferon production (14, 15). Inflammation is also a cause of functional iron deficiency. The delivery of iron from reticuloendothelial to hemopoietic cells is blocked when inflammation is present (16). It has been suggested that inflammation reduces absorption of iron in the gut by blocking mucosal uptake and transfer of iron (17).

For quite a while, reduced serum albumin concentration was considered to be the negative marker of inflammation in ESRD patients (11). Serum albumin concentrations under 4 g/dl were found in more than 60% of HD patients participating in our study. Like other authors, we confirmed an inverse correlation between serum albumin concentration and CRP and SAA. With regards to rHuEPO dose requirements, only univariate analysis showed a statistically significant correlation between serum albumin and erythropoietin dose. Thus, another marker – serum amyloid A (SAA) – was applied as a parameter. It served as the best indicator of inflam-

mation processes in our patients. It has been proven that measurement of serum concentration is as effective as measurement of the classical inflammation marker CRP (18). We found that SAA levels were higher in the group of patients who required a greater rHuEPO dose and the univariate correlation and multivariate regression model confirmed SAA (*i.e.* inflammation) as an important feature contributing to the greater rHuEPO dose.

However, reduced serum albumin concentrations are also observed in cases of protein-energy malnutrition (PEM). PEM is rather common among ESRD patients. Its prevalence ranges from 16% to 70% in different studies (19–21). In maintenance hemodialysis such signs of PEM as decreased muscle protein mass (indicated by low pre-dialysis serum creatinine concentration), reduced protein and other nutrient intake (it appears as low protein equivalent of total nitrogen appearance (PNA) /protein catabolic rate (PCR)), low serum concentrations of potassium, phosphorus and cholesterol have been found to be correlated to the mortality rate (22, 23). It has also been noted that adequate nutritional intake of proteins is very important for normal erythropoiesis (24).

The patients participating in our study were examined for malnutrition by several different methods. Serum albumin concentration was determined by calculating protein and energy intake from the three-day-dietary logs and by obtaining some anthropometric measurements and SGA scores. Dietary protein intake (DPI) and dietary energy intake (DEI) in ESRD patients has been measured by researchers before and ranges from 0.94 to 1.02 g/kg/day and from 22.8 to 29.0 kcal/kg/day, respectively (25, 26). Patients enrolled in this study had a mean DPI of 1.01 ± 0.23 g/kg/day and a mean DEI of 25.2 ± ± 6.5 kcal/kg/day. This is consistent with other available data. But does a different amount of ingested nutrients affect erythropoietin requirement? It has been reported that there is a correlation between Ht, rHuEPO dose and serum albumin concentration (24), however, that a correlation exists between other markers specific to malnutrition and rHuEPO dose is not very convincing. Recent studies also report a relationship between BMI and rHuEPO. The BMI closely correlates with body fat content and hyperleptinemia (27), but higher leptin levels in dialysis patients have been described to stimulate erythropoiesis, particularly in an anemic state (28). Stenvinkel et al. reported that serum leptin levels are associated positively with BMI and negatively with rHuEPO requirement (29). It is interesting that (using univariate analysis) the rHuEPO dose in our study was found to be higher in those patients with lower daily protein, energy and fat intake. The multivariate regression also revealed a negative correlation between DEI and weekly erythropoietin dose. This suggests that malnutrition may promote non-homogeneity of rHuEPO response in ESRD patients who are on maintenance hemodialysis.

The SGA score has also been validated as an accurate tool, which could provide reliable information regarding various aspects of PEM. SGA is correlated with the biochemical and other measures of nutritional status and outcomes, such as hospitalization length, comorbidity and mortality rate (30) in the dialysis population. In our survey, moderate and severe malnutrition was found in 64.1% of patients applying the SGA score as a PEM assessment tool. Severe malnutrition (SGA score C) was observed in 29.5% of patients. This is consistent with many multicenter studies reporting prevalence of PEM in dialysis patients. Stenvinkel et al. reported malnutrition in 39% of patients based on the SGA assessment (30). Qureshi et al. found signs of malnutrition in 64% of patients and severe malnutrition was noted in 13% of patients (31). We were able to denote a relationship between the SGA score and rHuEPO dose as well. In a multivariate linear regression analysis the SGA score was able to predict the rHuEPO dose requirement. Although, SGA scores like serum albumin concentration may also be affected by a chronic inflammatory state, as mentioned before. This was confirmed in our study by finding a correlation between SGA score and the SAA level.

Finally, like many other cross-sectional studies, we have to admit the failure to distinguish between malnutrition and inflammation as the two independent causes of rHuEPO hyporesponsiveness. We, like others, observed the rHuEPO dose correlation with the positive and negative markers of systemic inflammatory response such as SAA and serum albumin and with classical signs of PEM such as daily protein, energy and fat intake, BMI and SGA as well. The correlation between markers of inflammation and PEM in our study also reflects a close relationship between those two conditions. This problem – the relative contribution of nutrition and inflammation to various clinical outcome in dialysis patients – has been studied recently. Such studies adress mostly the cardiovascular morbidity and mortality issues. It has been reported that an increased serum levels of CRP and pro-inflammatory cytokines predict mortality in dialysis population. At the same time it was also noted that PEM is not necessarily just the consequence of chronic inflammation.

Thus, it has been recently proposed that malnutrition inflammation complex syndrome exists, which can explain a relationship between PEM, inflammation and cardiovascular disease. Furthermore, Stenvinkel et al. has found a strong relationship between malnutrition, high CRP levels and atherosclerosis in CRF patients (33). It has been referred to as the MIA syndrome. To define the relative contribution of PEM and inflammation to cardiovascular outcome as well as the rHuEPO dose requirement, large interventional trials are necessary. They will help to distinguish between underlying mechanisms, which may contribute to increased cardiovascular morbidity and to poor rHuEPO response.

Hyperparathyroidism (primary as well as secondary) has widely been described as a possible cause of anemia. Several pathways might explain the mode of parathyroid hormone (PTH) action. Firstly, there is enough proof that erythroid progenitor cell proliferation and maturation depend directely on the impact of PTH. It is known that bone marrow erythropoietic cells express calcitriol receptors (32), thus low calcitriol levels observed in secondary hyperparathyroidism might cause anemia. This is supported both by *in vitro* and *in vivo* studies where severe secondary hyperparathyroidism was induced in rats by a low-calcium diet after which parathyreoidectomy was performed (34). Osteitis fibrosa, the most severe manifestation of hyperparathyroidism (35), is assumed to be another cause of erythropoiesis depression in ESRD patients. There are studies to indicate that high doses of alphacalcidol can diminish anemia independently of serum PTH and calcium concentrations (36). However, the mechanism remains unclear. The lifetime of red blood cells is also shortened in the case of secondary hyperparathyroidism, possibly because of increased RBC osmotic fragility (37). It is possible that an increased tendency to suffer from gastrointestinal bleeding, which has been observed in primary hyperparathyroidism, is due to the negative impact of PTH on platelet function (38).

The iPTH concentrations in HD patients participating in our study were significantly higher in the group of patients who required a higher rHuEPO dose. The multivariate regression model also revealed elevated iPTH levels as the feature that might predict a higher rHuEPO dose requirement in ESRD patients during maintenance hemodialysis. In conclusion, this study confirms that erythropoietin requirement differs widely among the ESRD patients on maintenance hemodialysis. A relationship exists between protein-energy malnutrition (assessed by several independent methods) and rHuEPO dose requirement. Malnutrition may worsen renal anemia in dialysis patients and may lead to an attenuated rHuEPO response, thus contributing to higher rHuEPO dose requirements in these patients. However, inadequate protein and energy intake are not the only factors contributing to a higher rHuEPO dose requirement. We noticed that markers of chronic inflammation, such as serum amyloid A, were elevated in patients observed throughout this study and that a correlation with required rHuEPO dose existed. It was impossible to indicate the direction of causality between the PEM and inflammation and to what extent they both influenced the rHuEPO response. Thus, it seems that complex malnutrition–inflammation syndrome (as this condition was recently described by Kalantar Zadeh et al. (39) leeds to hyporesponsiveness to rHuEPO.

Among other variables affecting rHuEPO dose is secondary hyperparathyroidism with an elevated iPTH concentration that may worsen renal anemia in dialysis patients and may lead to an attenuated rHuEPO response, thus contributing to a required higher rHuEPO dose in these patients.

ACKNOWLEDGEMENTS

This study was supported by grant ¹ 01.0432 from the Latvian Council of Science.

Received 12 February 2004
Accepted 16 April 2004

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