A Low, Rather than a High, Total Plasma Homocysteine Is an Indicator of Poor Outcome in Hemodialysis Patients

KAMYAR KALANTAR-ZADEH,*† GLADYS BLOCK,† MICHAEL H. HUMPHREYS,‡ CHARLES J. MCALLISTER,§ and JOEL D. KOPPLE*‖

**Division of Nephrology and Hypertension, Harbor-UCLA Medical Center, Torrance, California, and David Geffen School of Medicine at UCLA, Los Angeles, California; †Division of Public Health Nutrition and Epidemiology, School of Public Health, University of California, Berkeley, California; ‡University of California, San Francisco, San Francisco, California, and Division of Nephrology, San Francisco General Hospital, San Francisco, California; §DaVita, Inc., Torrance, California; and ‖UCLA School of Public Health, Los Angeles, California*

Abstract. An increased level of total plasma homocysteine (tHcy) is a risk factor for poor cardiovascular outcome in the general population. However, a decreased, rather than an increased, tHcy concentration may predict poor outcome in maintenance hemodialysis (MHD) patients, a phenomenon referred to as reverse epidemiology. Associations were examined between tHcy level and markers of malnutrition-inflammation complex syndrome and 12-mo prospective hospitalization and mortality in 367 MHD patients, aged 54.5 ± 14.7 (mean ± SD) years, who included 199 men and 55% individuals with diabetes. tHcy was 24.4 ± 11.8 μmol/L, and 94% of the patients had hyperhomocysteinemia (tHcy >13.5 μmol/L). tHcy had weak to moderate but statistically significant bivariate and multivariate correlations with some laboratory markers of nutrition (serum albumin, prealbumin, creatinine, and urea nitrogen) but no significant correlation with serum C-reactive protein or two proinflammatory cytokines (IL-6 and TNF-α). During 12 mo of follow-up, 191 MHD patients were hospitalized, 37 died, nine underwent renal transplantation, and 38 transferred out. Hospitalization rates were significantly higher in patients with lower tHcy levels. Mortality rate in the lowest tHcy quartile (17.4%) was significantly higher compared with other three quartiles (6.5 to 9.8%; Kaplan-Meier $P = 0.04$). Relative risk of death for the lowest tHcy quartile, even after adjustment for case-mix and serum albumin, was 2.27 (95% confidence interval, 1.14 to 4.53; $P = 0.02$). Hence, tHcy may be a more exclusive nutritional marker in MHD patients with no association with inflammatory measures. Despite a very high prevalence of hyperhomocysteinemia in MHD patients, lower values of tHcy are paradoxically associated with increased hospitalization and mortality. The lowest tHcy quartile confers a twofold increase in risk of death independent of hypoalbuminemia. The nutritional feature of tHcy in MHD patients may explain its reverse association with outcome.

In the general population, an increased level of total plasma homocysteine (tHcy) is a risk factor for increased cardiovascular events and mortality (1–4). In maintenance hemodialysis (MHD) outpatients, however, the association between tHcy and clinical outcome is inconsistent and even paradoxical. Some studies have shown a poor outcome in MHD patients with hyperhomocysteinemia (5–9). However, a few recent studies have suggested that a decreased, not an increased, tHcy concentration is related to a higher prevalence of cardiovascular disease and poor outcome in these individuals (10–12). This seems to be due to the empirical association between a low tHcy and protein-energy malnutrition, which is *per se* a known risk factor for poor clinical outcome in dialysis patients (11, 13, 14). This recently described paradoxical association between tHcy and clinical outcome in dialysis patients has now been referred to as a possible component of the reversal of the cardiovascular risks (15–17). It is believed that both inflammation and protein-energy malnutrition, each independently or together as the "malnutrition-inflammation complex syndrome" (15, 18, 19), are the main contributors of the above-mentioned risk factor reversal phenomenon. Nevertheless, it is not clear whether there is any significant association between tHcy and inflammation in dialysis patients. We studied the hypotheses that a low level of tHcy in dialysis patients indicates protein-energy malnutrition, with or without inflammation, and is associated with poor clinical outcome in maintenance dialysis patients. To verify the above hypotheses, we first examined baseline associations between tHcy and some markers of protein-energy malnutrition and inflammation at the start of the cohort of the Nutritional and Inflammatory Evaluation in Dialysis (NIED) Study (20, 21). We then explored longitudinal associations between the baseline tHcy and the prospective mortality and hospitalization measures in the same group of patients.

Received July 12, 2003. Accepted October 31, 2003.
Correspondence to Dr. Kamyar Kalantar-Zadeh, Departments of Medicine and Pediatrics, Division of Nephrology and Hypertension, Harbor-UCLA Medical Center, Harbor Mailbox 406, 1000 West Carson Street, Torrance, CA 90509-2910. Phone: 310-222-3891; Fax: 310-782-1837; E-mail: kamkal@ucla.edu
1046-6673/1502-0442
Journal of the American Society of Nephrology

DOI: 10.1097/01.ASN.0000107564.60018.51

Materials and Methods

Patients

Participants in the NIED Study originated from a pool of approximately 1200 MHD outpatients in eight DaVita, Inc., dialysis facilities in the South Bay Los Angeles area (see NIED Study web site at www.NIEDStudy.org for more details, as well as previous publications (20, 21)). Inclusion criteria were outpatients who had been undergoing MHD for at least 8 wk, were 18 yr or older, and signed a written consent form. Patients with an anticipated life expectancy of <6 mo (*e.g.*, because of a metastatic malignancy or terminal HIV disease) were excluded. In the initial phase of the NIED Study (October 2001 to March 2002), 385 patients from eight dialysis units signed the written consent form. Subsequently, blood samples were obtained from 367 of these individuals, because 18 patients were not present in the dialysis units at the time of blood drawing. The medical chart of each MHD patient was thoroughly reviewed by a nephrologist (K.K.Z.), and data pertaining to underlying kidney disease, cardiovascular history, and other comorbid conditions were extracted. A modified version of the Charlson comorbidity index, *i.e.*, without the age and kidney disease components, was used to assess the severity of comorbidity (22, 23).

Anthropometric Evaluation

Body weight assessment and anthropometric measurements were performed while patients were undergoing hemodialysis treatment or within 5 to 20 min after termination of the treatment. Triceps skinfold (TSF) thicknesses were measured with a conventional skinfold caliper using standard techniques as described elsewhere (24–26). Mid-arm circumference (MAC) was measured with a plastic tape. Mid-arm muscle circumference (MAMC) was calculated from the formula (24, 27) MAMC = MAC − (3.1416 × TSF). Height was obtained from the patient's chart.

Near Infrared Interactance

To evaluate the percentage of body fat and lean body mass, we performed the near infrared (NIR) interactance (28, 29) at the same time as the anthropometric measurements. A commercial NIR interactance sensor (portable Futrex 6100, Gaithersburg, MD) was used. NIR measurements were performed by placing a Futrex sensor on the non-access upper arm for several seconds, after entering the required data (date of birth, gender, weight, and height) from each patient. NIR measurements of body fat are shown to correlate significantly with subjective global assessment of nutrition (SGA) and other nutritional measures in MHD patients (28, 29).

Laboratory Evaluation

Predialysis blood samples and postdialysis serum urea nitrogen were obtained on a mid-week day and coincided chronologically with the quarterly blood tests of DaVita facilities. Patients had been instructed to fast for at least 4 h before the predialysis blood draw. The single-pool Kt/V was used to represent the weekly dialysis dose, and the normalized protein equivalent of total nitrogen appearance (nPNA), also known as normalized protein catabolic rate (nPCR), was calculated to estimate the daily protein intake (30). Total iron binding capacity (TIBC) represented serum transferrin (31). All routine laboratory measurements were performed by DaVita Laboratories (Deland, FL) using automated methods, and the average values for each laboratory test within the 13-wk study period were calculated and used for data analyses in this study.

Serum C-reactive protein (CRP) and two proinflammatory cytokines—IL-6 and TNF-α—were measured as indices of the degree of inflammation. The high-sensitivity CRP was measured by a turbidometric immunoassay in which a serum sample is mixed with latex beads coated with anti-human CRP antibodies forming an insoluble aggregate (WPCI, Osaka, Japan; normal range, <3.0 mg/L) (32, 33). High-sensitivity IL-6 and TNF-α immunoassay kits based on a solid-phase sandwich ELISA using recombinant human IL-6 and TNF-α were used to measure serum proinflammatory cytokines (R&D Systems, Minneapolis, MN; normal range, IL-6 <9.9 pg/ml, TNF-α <4.7 pg/ml) (34–36). CRP and cytokines were measured in the General Clinical Research Center Laboratories of Harbor-UCLA Medical Center. Serum prealbumin was analyzed by an antigen-antibody complex assay, and total plasma homocysteine (tHcy) concentrations were determined by HPLC at Harbor-UCLA Clinical Laboratories. Normal values of tHcy by this method are 2.8 to 13.5 μmol/L in Harbor-UCLA Core Laboratories.

Hospitalization

Hospitalization was defined as any hospital admission that included at least one overnight stay in the hospital. The admission day was counted as one full hospitalization day, but the discharge day was not. All types of hospitalizations, including those related to dialysis access, were included. Because the vast majority of dialysis access–related hospitalizations did not require overnight admission, essentially only those that were associated with other complications and comorbidities were included. For patients who died and those who left the cohort during the prospective follow-up, the hospitalization rates during the survival time were standardized by using the factor 12/survival-time (in months). The annual hospitalization days and annual hospitalization frequency were calculated as described elsewhere (24, 27).

Statistical Analyses

Conventional *t* test and ANOVA were used to detect significant differences among continuous variables in two or more groups, respectively, when applicable. χ^2 and Kruskal-Wallis rank tests were used for categorical variables. We used Pearson correlation coefficient *r* for analyses of associations between continuous variables and Spearman rank test for categorical variables. Multivariate regression analysis was performed to obtain partial (adjusted) correlations controlled for case-mix features and relevant covariates, including age, gender, race (Black *versus* other), ethnicity (Hispanic *versus* other), insurance status (Medicaid *versus* other), diabetes, Charlson comorbidity score, dialysis center, and dialysis vintage. Hospitalization rate ratios (RR) were calculated using Poisson regression models. We used Cox proportional hazard regression to calculate hazard ratios (HR) of death after controlling for the above-mentioned covariates. Plots of log [−log (survival rate)] against log (survival time) were performed to establish the validity of the proportionality assumption as described elsewhere (27). Kaplan-Meier survival analysis examined the differences in prospective mortality among four quartiles of tHcy. Fiducial limits are given as mean ± SD. Both natural (untransformed) and logarithmic values of tHcy were examined in all models. $P < 0.05$ or a 95% confidence interval (CI) that did not span 1.00 was considered to be statistically significant. *P* value between 0.05 and 0.10 was considered borderline significant. Descriptive and multivariate statistics were carried out with the statistical software Stata version 7.0 (Stata Corp., College Station, TX).

Results

During the 12-mo follow-up period, 191 (52.0%) MHD patients were hospitalized at least once; 37 (10.1%) died, including 26 deaths that were attributed to cardiovascular

events according to physician report; nine underwent renal transplantation; and 38 transferred out of their dialysis units or left the cohort for other reasons. Cardiovascular causes of death included cardiac arrest (13), cardiac arrhythmia (5), myocardial infarction (4), stroke (3), and aortic aneurysm (1). Noncardiac causes of death included septicemia (4), malignancy (2), gastrointestinal bleeding (1), and unknown (4).

Table 1 demonstrates pertinent baseline data at the beginning of the cohort in 367 MHD outpatients. The first column includes the statistical mean and SD for all MHD patients. The second and third columns compare the same values in surviving and deceased patients, respectively. *P* values based on *t* test (fourth column) reflect the degree of statistical significance for the differences between the last two categories. Men composed 54.2% of the study population, which was heavily dominated by Hispanics (46.6%) and blacks (29.4%). More than one half (54.7%) of all patients had diabetes, and diabetic prevalence was even higher among deceased patients (73.5%). Exactly half of all patients had a history of cardiovascular disease, including a myocardial infarction, coronary artery procedures such as angioplasty or surgery, congestive heart failure, and peripheral vascular disease including amputation as documented in their charts and/or obtained by questionnaires. Almost 72% of the patients were taking daily multivitamin supplementation designed specifically for patients with renal failure, which usually includes 1000 μg of folic acid. These

Table 1. Demographic, laboratory, anthropometric, comorbidity, and nutritional values (mean ± SD) in 367 maintenance hemodialysis patients at the start of the NIED Study cohort[a]

Variable	All MHD Patients (n = 367)	Surviving Patients (n = 330)	Deceased Patients (n = 37)	t Test P Value
Gender (% male)	54.2%	53.9%	56.8%	0.7
Race (% black)	29.4%	29.1%	32.4%	0.7
Ethnicity (% Hispanic)	46.6%	46.0%	51.3%	0.5
Diabetes	54.7%	52.8%	73.5%	0.02
History of cardiovascular disease	50.0%	49.7%	52.9%	0.7
Multivitamin intake (1 pill/d or more)	72.2%	73.3%	62.2%	0.15
Folic acid intake (1 mg/d or more)	23.4%	23.0%	27.0%	0.6
Charlson comorbidity score	2.03 ± 1.52	1.95 ± 1.50	2.79 ± 1.45	0.002
Age (y)	54.5 ± 14.7	53.8 ± 14.7	60.9 ± 12.7	0.005
Dialysis vintage (mo)	36.6 ± 34.2	37.5 ± 35.2	28.6 ± 22.6	0.13
Kt/V (single pool)	1.57 ± 0.28	1.57 ± 0.28	1.53 ± 0.27	0.4
nPNA (nPCR)	1.05 ± 0.22	1.05 ± 0.22	1.05 ± 0.25	0.9
Blood hemoglobin (g/dL)	11.93 ± 0.98	11.97 ± 0.96	11.58 ± 1.16	0.02
Total plasma homocysteine (μmole/dL)	24.41 ± 11.75	24.86 ± 12.04	20.44 ± 7.75	0.03
Logarithm of total plasma homocysteine	1.35 ± 0.17	1.36 ± 0.16	1.28 ± 0.19	0.004
Serum albumin (g/dl)	3.86 ± 0.32	3.88 ± 0.31	3.63 ± 0.31	<0.001
prealbumin (mg/dl)	28.1 ± 9.5	28.5 ± 9.5	24.4 ± 8.9	0.01
cholesterol (mg/dl)	143.2 ± 47.0	143.8 ± 46.7	137.2 ± 49.7	0.4
creatinine (mg/dl)	10.8 ± 3.4	11.0 ± 3.4	9.2 ± 2.4	0.002
urea nitrogen (mg/dl)	66.7 ± 17.0	66.6 ± 17.0	68.0 ± 17.7	0.6
phosphorus (mg/dl)	5.9 ± 1.5	5.8 ± 1.4	6.1 ± 2.1	0.22
TIBC (mg/dl)	200 ± 36	201 ± 36	194 ± 40	0.3
ferritin (ng/ml)	655 ± 469	644 ± 455	756 ± 590	0.19
C-reactive protein (mg/L)	6.4 ± 7.8	5.8 ± 6.3	12.3 ± 14.9	<0.001
IL-6 (mg/L)	22.5 ± 57.0	21.2 ± 58.4	33.6 ± 41.7	0.21
TNF-α (mg/dl)	8.4 ± 6.5	8.3 ± 6.5	9.1 ± 6.7	0.5
Postdialysis weight (kg)	73.4 ± 19.6	73.5 ± 19.6	72.1 ± 20.4	0.7
Body mass index (kg/m^2)	26.7 ± 6.2	26.7 ± 6.2	26.1 ± 6.4	0.5
NIR total body fat (%)	26.3 ± 10.8	26.4 ± 10.8	25.3 ± 11.1	0.6
Triceps skin fold (mm)	9.8 ± 7.9	9.8 ± 7.8	10.1 ± 9.6	0.9
Mid-arm muscle circumference (cm)	28.7 ± 5.1	28.8 ± 5.1	28.1 ± 4.9	0.5

[a] Second and third columns compare baseline values between surviving (n = 330) and decreased (n = 37) patients. *P* values are based on *t* test comparing values in surviving and decreased patients. Mortality is over a 12-mo interval.

nPNA, normalized protein nitrogen appearance; nPCR, normalized protein catabolic rate; TIBC, total iron binding capacity; NIR, near infrared interactance; MHD, maintenance hemodialysis.

pills included nephrovite (209), nephrocaps (10), and other brands (46). Moreover, 86 patients were taking folic acid supplement, 1000 μg/d, including 67 who were also taking above-mentioned multivitamins. There was no statistically significant difference in multivitamin or folic acid intake between surviving and deceased patients (Table 1).

Table 1 shows that Charlson comorbidity score was significantly higher among deceased MHD outpatients, who were also on average 7 yr older than their surviving counterparts were. Patients had undergone hemodialysis for 36.6 $\pm$ 34.2 mo (vintage), and their baseline nPNA was 1.05 $\pm$ 0.22. The baseline tHcy was significantly lower in those who died (20.44 $\pm$ 7.75 μmol/dl) compared with those who survived or censored (24.86 $\pm$ 12.04 μmol/dl; $P = 0.03$). Among laboratory tests, baseline values of blood hemoglobin and serum concentrations of albumin, prealbumin, and creatinine were significantly lower and CRP values were higher in deceased patients when compared with surviving ones. There were no statistically significant differences between deceased and surviving patients for body mass index (BMI), total body fat via NIR, TSF, biceps skinfold, and MAMC. Figure 1 shows the distribution of tHcy, which ranged between 5.8 and 113.9 μmol/L (24.4 $\pm$ 11.8 μmol/L; median, 22.3 μmol/L) and was skewed to the right. Only 24 (6.5%) patents had a tHcy in the normal range of 2.8 to 13.5 μmol/L, and the rest (93.5%) had hyperhomocysteinemia. Of note, tHcy in diabetic patients (23.4 $\pm$ 10.3 μmol/L) was slightly but significantly lower than in nondiabetic patients (25.9 $\pm$ 13.4 μmol/L; $P = 0.04$), a finding consistent with previous reports by other investigators (37, 38).

Table 2 shows correlations between tHcy and selected values. Both untransformed and logarithmic values of tHcy were examined. Bivariate (unadjusted) Pearson correlation coefficients were compared with multivariate correlations that controlled for age, gender, race, ethnicity, diabetes, Charlson comorbidity score, dialysis center, and dialysis vintage and are reported. There was a weak but negative correlation between tHcy and the Charlson comorbidity score, indicating that multimorbid patients tended to have a lower tHcy value, but the correlations were not statistically significant after multivariate adjustment. There was a positive correlation between loga-

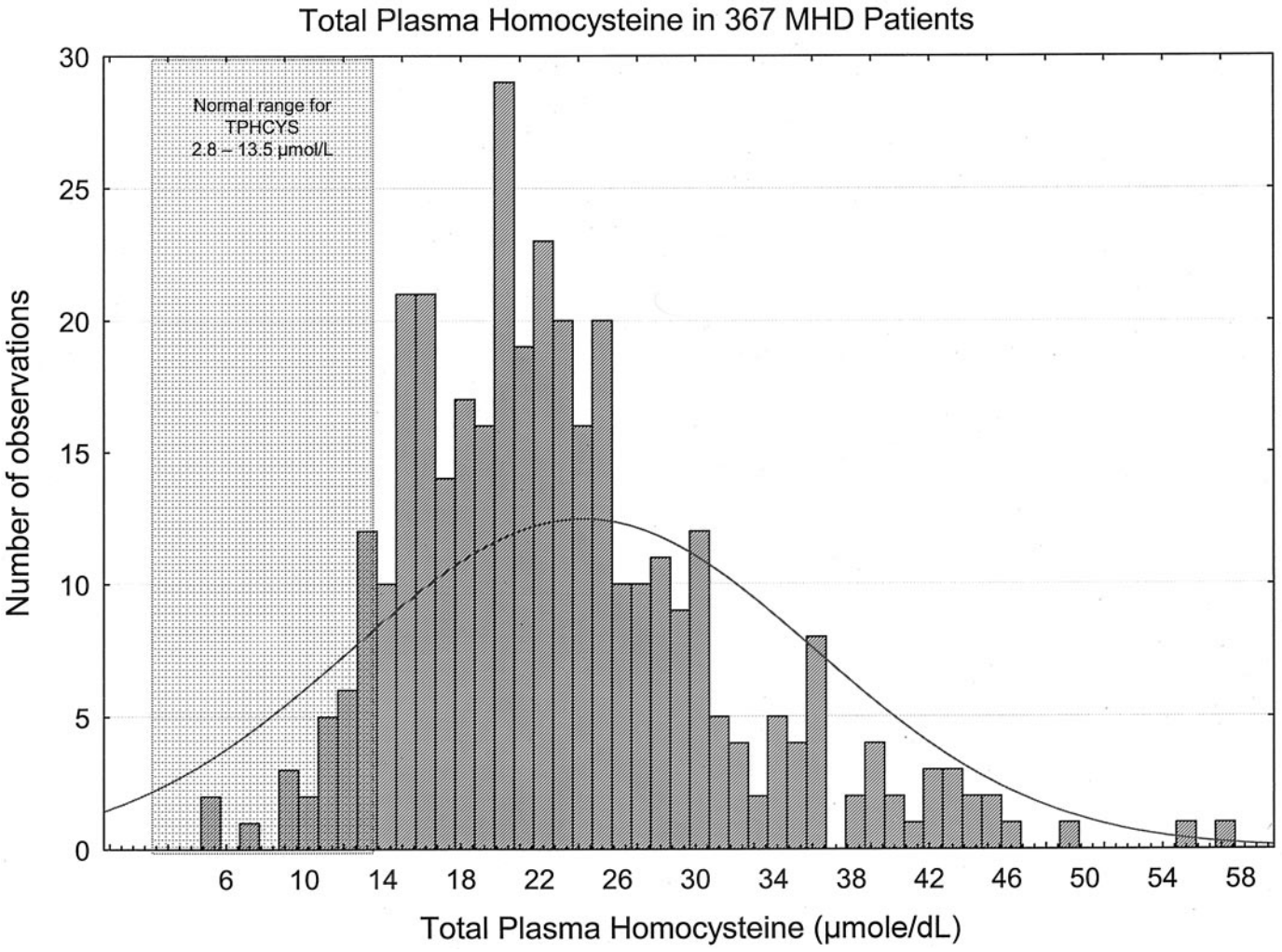

Figure 1. Distribution histogram of total plasma homocysteine in 367 maintenance hemodialysis (MHD) patients. Cases with a total plasma homocysteine (tHcys) >60 μmol/dl are not shown ($n = 7$).

Table 2. Correlation coefficients between total plasma homocysteine (both untransformed values and logarithmic scales) and selected laboratory, comorbidity, and nutritional values[a]

	Total Plasma Homocysteine	Logarithm of tHcy
Charlson comorbidity index	**−0.10** (P = 0.04)/−0.04 (P = 0.04)	**−0.12** (P = 0.03)/−0.05 (P = 0.3)
nPNA (nPCR)	0.07 (P = 0.2)/0.08 (P = 0.12)	**0.10** (P = 0.05)/**0.13** (P = 0.01)
albumin	**0.16** (P = 0.002)/**0.12** (P = 0.02)	**0.19** (P <0.001)/**0.16** (P = 0.002)
prealbumin	**0.16** (P = 0.002)/**0.14** (0.008)	**0.17** (P = 0.001)/**0.15** (0.006)
creatinine	**0.28** (P <0.001)/**0.22** (P <0.001)	**0.29** (P <0.001)/**0.25** (P <0.001)
urea nitrogen	**0.12** (P = 0.02)/**0.12** (P = 0.02)	**0.15** (P = 0.005)/**0.16** (P = 0.003)
TIBC	0.04 (P = 0.4)/0.05 (P = 0.3)	0.07 (P = 0.21)/0.07 (P = 0.17)
Cholesterol	−0.08 (P = 0.13)/−0.04 (P = 0.3)	−0.06 (P = 0.3)/−0.04 (P = 0.4)
C-reactive protein	−0.06 (P = 0.3)/−0.07 (P = 0.17)	−0.06 (P = 0.24)/−0.07 (P = 0.19)
IL-6	−0.03 (P = 0.6)/−0.02 (P = 0.7)	−0.01 (P = 0.8)/−0.01 (P = 0.9)
tumor necrosis factor-alpha	0.02 (P = 0.7)/0.02 (P = 0.7)	0.01 (P = 0.8)/0.01 (P = 0.8)
Body mass index	0.04 (P = 0.4)/0.07 (P = 0.24)	0.06 (P = 0.3)/0.08 (P = 0.14)
Triceps skinfold	0.06 (P = 0.3)/0.10 (P = 0.06)	0.06 (P = 0.24)/**0.11** (P = 0.04)
Mid-arm muscle circumference	0.10 (P = 0.06)/**0.11** (P = 0.04)	0.08 (P = 0.14)/0.09 (P = 0.09)
NIR total body fat (%)	−0.09 (P = 0.08)/−0.01 (P = 0.9)	−0.09 (P = 0.12)/−0.01 (P = 0.9)

[a] Bivariate (Pearson, unadjusted) and multivariate regression adjusted r values, also known as partial correlation, controlled for age, gender, race, diabetes, Charlson score, dialysis center, and dialysis vintage are reported. Statistically significant correlations are in bold.
tHcy, total plasma homocysteine.

rithm of tHcy and nPNA (nPCR), suggesting a higher protein intake in MHD patients whose tHcy was high. Similar but even stronger correlations were found between both untransformed and logarithmic values of tHcy and serum concentrations of such nutritional markers as albumin, prealbumin, creatinine, and urea nitrogen, with serum creatinine showing the strongest

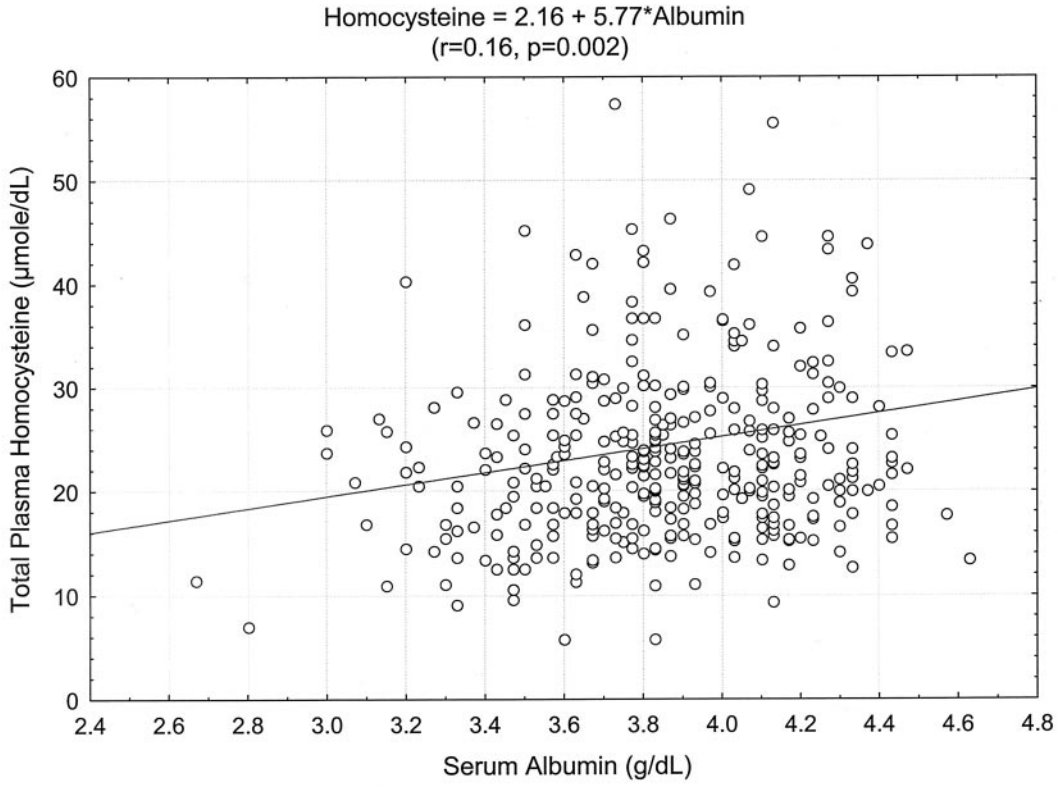

Figure 2. Scatter plot reflecting the correlation between serum albumin and total plasma homocysteine in 367 MHD patients. Cases with a total plasma homocysteine >60 μmol/dl are not shown (n = 7).

correlations (0.22 to 0.29). Figures 2 and 3 show scatter diagrams for correlations between tHcy and serum albumin and creatinine concentrations, respectively. No significant correlations were found between tHcy and other nutritional markers (TIBC and cholesterol) or markers of inflammation (CRP, IL-6, and TNF-α). Among anthropometric measures, only a weak, multivariate adjusted correlation was observed between logarithm of tHcy and TSF and between untransformed tHcy and MAMC, indicating a tendency toward higher tHcy concentrations in patients whose TSF or whose MAMC were larger. Spearman rank correlations were closely similar to Pearson correlations (data not shown).

Table 3 compares four quartiles of tHcy. All-cause mortality rate was significantly higher in the lowest tHcy quartile group (17.4%) compared with the other three groups (6.5 to 9.8%; $P = 0.04$). Cardiovascular death was also higher in the lowest tHcy group (10.9% *versus* 5.4 to 6.5%), but these differences did not achieve statistical significance. Serum concentrations of albumin, creatinine, and urea nitrogen were significantly lower in the lowest tHcy quartile and higher in the highest tHcy quartile, indicating, once again, a positive association between these nutritional markers and tHcy. The values of other nutritional markers or indicators of inflammation were not significantly different across tHcy quartiles. Figure 4 compares the cumulative proportion of the surviving patients in each tHcy quartiles *via* the Kaplan-Meier method. Once again, the lowest tHcy quartile exhibited the lowest survival rate as compared with the other three quartiles (Kaplan-Meier $P = 0.04$).

Tables 4 and 5 show associations between tHcy and RR of hospitalization frequency and total days of hospitalization within 12 mo of prospective follow-up, respectively. In general, there was a consistent trend toward higher hospitalization rates with lower, not higher, tHcy values. Mostly unadjusted, rather than multivariate adjusted, hospitalization frequencies were higher in MHD patients with lower tHcy concentrations (Table 4). However, for total days of hospitalization, all multivariate adjusted RR, which did not include serum albumin, were statistically significant, indicating longer hospital stays for MHD patients with lower tHcy levels (Table 5). For the lowest tHcy quartile, this association was even independent of serum albumin concentration, in that hospitalization RR was 1.15 (95% CI, 1.15 to 1.25; $P = 0.001$) after adjustment for case-mix and hypoalbuminemia. Table 6 shows HR of death for decreasing levels of tHcy as well as across its down-going quartiles. Again, the lowest tHcy quartile, when compared with higher values, was associated with a significantly higher death risk, *i.e.*, a 2.27 time increase in mortality, independent of case-mix variables or hypoalbuminemia (95% CI, 1.14 to 14.53; $P = 0.02$). No major change in the magnitude or direction of the associations was noticed after the inclusion of additional terms for intake of folic acid and multivitamin supplements and history of cardiovascular disease in multivariate models. Finally, mortality models were also examined for cardiovascular death exclusively (26 of 37 deaths). Although the same inverse trends were noticed, the associations did not reach statistical significance. For example, for each 1 μmol/dl

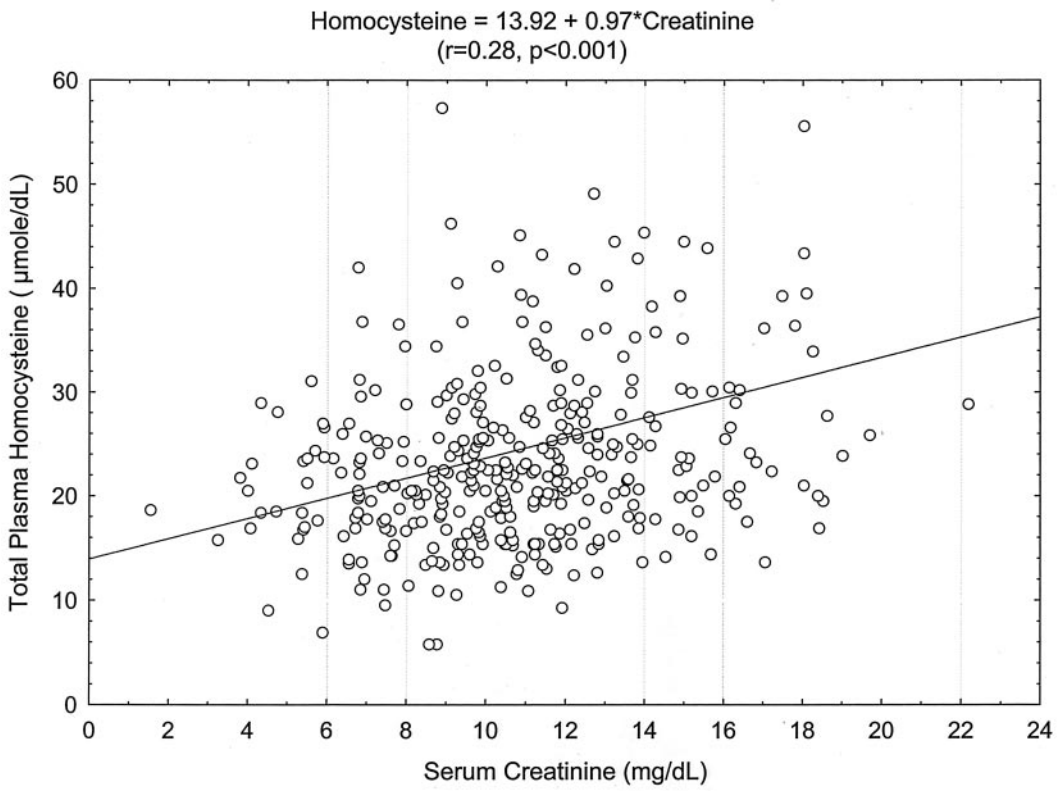

Figure 3. Scatter plot reflecting the correlation between serum creatinine and total plasma homocysteine in 367 MHD patients. Cases with a total plasma homocysteine >60 μmol/dl are not shown ($n = 7$).

Table 3. Relevant demographic, mortality, comorbidity, and nutritional and inflammatory values in quartiles of tHcy[a]

	Quartiles of tHcy				*P* Value
	First (Lowest) Quartile (n = 92)	Second Quartile (n = 92)	Third Quartile (n = 92)	Fourth (Highest) Quartile (n = 91)	
tHcy (μmol/dl)	14.6 ± 2.6	20.1 ± 1.3	24.6 ± 1.5	38.5 ± 15.2	N/A
All-cause mortality (%)	17.4%	6.5%	9.8%	6.6%	**0.04**
Cardiovascular mortality (%)	10.9%	6.5%	5.4%	5.5%	0.4
History of cardiovascular disease (%)	55.6%	46.2%	47.1%	51.1%	0.6
Charlson comorbidity score	2.3 ± 1.7	1.8 ± 1.4	2.1 ± 1.6	1.9 ± 1.4	0.23
Body mass index (kg/m^2)	26.0 ± 5.9	26.9 ± 5.1	26.4 ± 6.3	27.5 ± 7.3	0.4
nPNA (nPCR)	1.03 ± 0.21	1.03 ± 0.23	1.06 ± 0.23	1.09 ± 0.21	0.17
Serum albumin (g/dl)	3.79 ± 0.38	3.87 ± 0.31	3.83 ± 0.31	3.93 ± 0.28	**0.02**
prealbumin (mg/dl)	26.1 ± 10.1	29.1 ± 9.3	28.1 ± 9.4	29.2 ± 9.1	0.10
creatinine (mg/dl)	9.9 ± 3.0	10.4 ± 3.4	10.9 ± 3.2	12.1 ± 3.5	**<0.01**
urea nitrogen (mg/dl)	64.5 ± 14.9	64.5 ± 18.6	66.7 ± 16.8	70.9 ± 17.1	**0.04**
cholesterol (mg/dl)	145.0 ± 48.5	144.6 ± 47.2	148.0 ± 47.6	135.1 ± 44.3	0.3
TIBC (mg/dl)	194 ± 36	203 ± 37	203 ± 35	200 ± 38	0.4
C-reactive protein (mg/l)	6.6 ± 5.9	7.2 ± 9.0	5.5 ± 5.3	6.4 ± 10.1	0.5
IL-6 (mg/L)	20.6 ± 35.2	16.0 ± 26.3	30.4 ± 88.2	22.8 ± 57.1	0.4
TNF-α (mg/L)	8.4 ± 7.8	7.2 ± 3.7	8.6 ± 6.7	9.5 ± 6.9	0.11

[a] *P* value is based on ANOVA or Kruskal-Wallis test for continuous or categorical variables, respectively.

decrease in tHcy, the HR of cardiovascular death was 1.06 (95% CI, 1.00 to 1.12; $P = 0.058$).

Discussion

In this study, we found a high prevalence of hyperhomocysteinemia in a cohort of 367 MHD patients. The baseline values of tHcy exhibited significant correlations with serum creatinine, albumin, prealbumin, and urea nitrogen but did not correlate with inflammatory markers. We also found that lower values of tHcy were associated with increased hospitalization and mortality. Lower tHcy quartile was associated with a twofold increase in death independent of hypoalbuminemia. These findings all are in sharp contrast to the findings in the general population, in whom higher, rather than lower, values of tHcy are associated with poor outcome, including higher risks of cardiovascular events and death. Our findings, however, are consistent with the recently described phenomenon of reverse epidemiology in dialysis patients (15).

Hyperhomocysteinemia is present in the vast majority of maintenance dialysis patients according to almost all descriptive and prevalent studies (5–12). In most of such studies, including our current study, >90% of maintenance dialysis patients have hyperhomocysteinemia, and the mean and median values of tHcy are between 20 and 25 μmol/L. This range is substantially higher than the normal range reported for the general population (<13.5 μmol/L; Figure 1). It has been proposed that this high prevalence of hyperhomocysteinemia in dialysis patients is one of the main reasons for the high rate of cardiovascular events and poor clinical outcome in these individuals (5–9). Although some studies reported positive correlations between increased tHcy and higher rate of mortality in dialysis patients (7–9), other studies failed to show such an association (39, 40) or found paradoxically reversed correlations (10–12). Sirrs *et al.* (10) examined the association between tHcy and vascular access complications in 96 MHD patients and did not find any correlation between them. However, they found a significantly higher 9-mo mortality in MHD patients whose tHcy was <27 μmol/L. Suliman *et al.* (11) examined possible relationships among tHcy, nutritional status, and ischemic cardiovascular disease in a cohort of 117 MHD patients and found that malnourished MHD patients had significantly lower levels of serum albumin and tHcy. Similar to our results, these investigators also reported positive and significant correlations between tHcy levels and serum concentrations of albumin and creatinine (11). They also found that nPNA, an indicator of protein intake, was independently associated with tHcy concentrations. Finally, Wrone *et al.* (12) explored cross-sectional associations between tHcy and history of cardiovascular disease in 459 MHD patients and found that mean tHcy was higher in patients without a history of cardiovascular disease (35.2 μmol/L *versus* 30.4 μmol/L; $P = 0.02$). In multivariate models, the history of cardiovascular disease was still negatively associated with higher tHcy levels (12).

Because hyperhomocysteinemia is common in dialysis patients and because it is a cardiovascular risk factor in the general population, the intuitive inference is that hyperhomocysteinemia is the cause of poor outcome in dialysis population. This possibly flawed reasoning is known in epidemiology as ecological fallacy, in that inferences are based on comparing populations rather than individuals (41). However, a few stud-

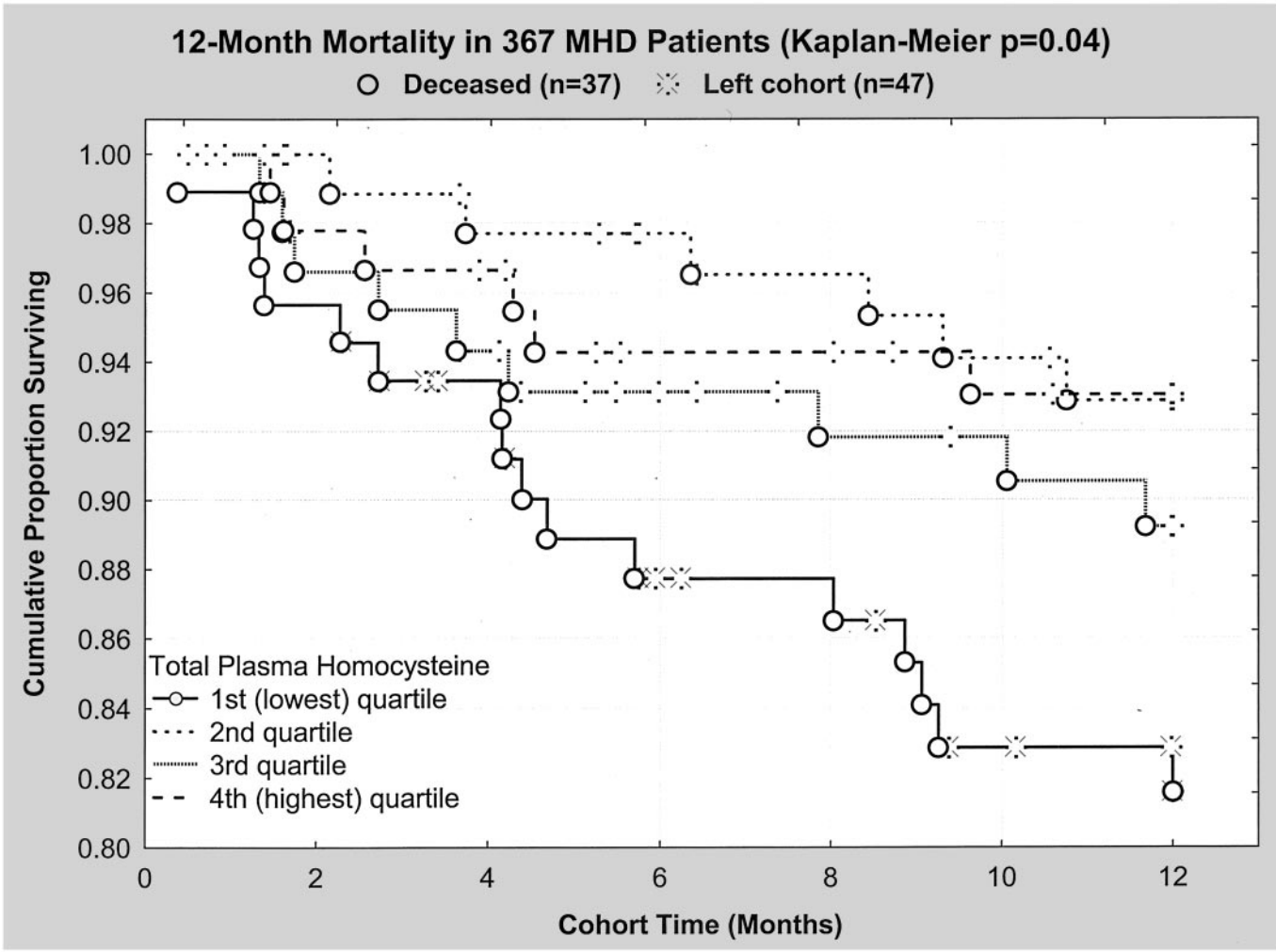

Figure 4. Cumulative proportion of surviving patients according to quartiles of total plasma homocysteine in 367 MHD patients.

Table 4. Association between tHcy and hospitalization frequency, *i.e.*, total number of hospital admissions, over the 12-month follow-up period, as reflected by hospitalization rate ratios (RR) and 95% confidence intervals[a]

	Unadjusted Model	Multivariate Model (without Serum Albumin)	Multivariate Model Including Serum Albumin
tHcy (for each 1 μmol/dl $\downarrow$)	**1.01** (1.01–1.02) $P = 0.007$*	1.01 (1.00–1.02) $P = 0.074$	1.01 (0.99–1.01) $P = 0.4$
Logarithm tHcy (for each 0.1 unit $\downarrow$)	**1.09** (1.04–1.15) $P = 0.001$	**1.07** (1.01–1.13) $P = 0.017$*	1.03 (0.98–1.09) $P = 0.2$
tHcy quartiles (across decreasing four quartiles)	**1.10** (1.02–1.19) $P = 0.016$*	**1.08** (1.01–1.17) $P = 0.048$*	1.04 (0.96–1.13) $P = 0.3$
Lowest tHcy quartile (versus the three highest quartiles)	1.13 (0.94–1.37) $P = 0.2$	1.16 (0.94–1.38) $P = 0.2$	1.06 (0.88–1.29) $P = 0.5$

[a] Multivariate RR values are adjusted for age, gender, race (Black versus other), ethnicity (Hispanic versus other), insurance status (Medicaid versus other), diabetes, Charlson comorbidity score, dialysis center, and dialysis vintage. The extended multivariate model also includes serum albumin concentration. All models are based on Poisson regression analyses. Note that RR for tHcy is based on each 1 μmol/dl *decrease* in tHcy or each 0.1 unit *decrease* in logarithm of tHcy. RR, rate ratio.

ies indeed have shown a positive correlation between tHcy and poor outcome (7, 9, 42), but they are subject to restrictions. For instance, in the study by Mallamaci *et al.* (7), although tHcy was found to be positively associated with cardiovascular outcome in 175 MHD patients who were followed for an average of 29 mo, it failed to independently predict survival.

Table 5. Association between tHcy and total days of hospitalization over the 12-month follow-up period, as reflected by hospitalization RR and 95% confidence intervals[a]

	Unadjusted Model	Multivariate Model (without Serum Albumin)	Multivariate Model Including Serum Albumin
tHcy (for each 1 μmol/dl ↓)	**1.01** (1.01–1.02) $P = 0.001$*	**1.01** (1.01–1.02) $P = 0.001$*	1.00 (0.99–1.01) $P = 0.3$
Logarithm tHcy (for each 0.1 unit ↓)	**1.08** (1.06–1.11) $P < 0.001$	**1.05** (1.03–1.08) $P < 0.001$*	1.02 (0.99–1.04) $P = 0.2$
tHcy quartiles (across decreasing four quartiles)	**1.09** (1.06–1.13) $P < 0.001$	**1.06** (1.03–1.10) $P < 0.001$*	1.02 (0.98–1.05) $P = 0.3$
Lowest tHcy quartile (versus the three highest quartiles)	**1.34** (1.23–1.45) $P < 0.001$*	**1.25** (1.16–1.36) $P < 0.001$*	**1.15** (1.06–1.25) $P = 0.001$*

[a] Multivariate RR values are adjusted for age, gender, race (Black versus other), ethnicity (Hispanic versus other), insurance status (Medicaid versus other) diabetes, Charlson comorbidity score, dialysis center, and dialysis vintage. The extended multivariate model also includes serum albumin concentration. All models are based on Poisson regression analyses. Note that RR for tHcy is based on each 1 μmol/dl *decrease* in tHcy or each 0.1 unit *decrease* in logarithm of tHcy.

Table 6. Association between tHcy and the relative risk of death over a prospective 12-month interval represented by mortality hazard ratios and 95% confidence intervals[a]

	Unadjusted Model	Multivariate Model (without Serum Albumin)	Multivariate Model Including Serum Albumin
tHcy (for each 1 μmol/dl ↓)	**1.06** (1.01–1.09) $P = 0.019$*	**1.06** (1.01–1.11) $P = 0.025$*	1.04 (0.99–1.09) $P = 0.088$
Logarithm tHcy (for each 0.1 unit ↓)	**1.34** (1.11–1.63) $P = 0.003$*	**1.32** (1.09–1.61) $P = 0.005$*	**1.23** (1.01–1.51) $P = 0.048$*
tHcy quartiles (across decreasing four quartiles)	**1.36** (1.01–1.84) $P = 0.046$*	**1.38** (1.01–1.89) $P = 0.042$*	1.30 (0.95–1.79) $P = 0.107$
Lowest tHcy quartile (versus the three highest quartiles)	**2.37** (1.24–4.54) $P = 0.009$*	**2.57** (1.31–5.05) $P = 0.006$*	**2.27** (1.14–4.53) $P = 0.020$*

[a] Multivariate HR values are adjusted for age, gender, race (Black versus other), ethnicity (Hispanic versus other), insurance status (Medicaid versus other), diabetes, Charlson comorbidity score, dialysis center, and dialysis vintage. The extended multivariate model also includes serum albumin concentration. All models are based on Cox proportional hazard regression analyses. Note that RR for tHcy is based on each 1 μmol/dl *decrease* in tHcy or each 0.1 unit *decrease* in logarithm of tHcy.

Moreover, in the aforementioned study, neither serum albumin nor CRP predicted mortality, whereas our study did. Hence, the reason that our current study and three other recent studies (10–12) found the opposite association as compared with a few others (7, 9, 42) could be due to differences in race or ethnicity, comorbidities, the prevalence of diabetes, and/or study designs. Moreover, publication bias may have handicapped or delayed reporting such paradoxical findings in dialysis patients, because the investigators' first impression upon encountering results with inverse association between tHcy and mortality may be to consider them erroneous or flawed and hence to avoid reporting them (15, 43, 44).

The positive correlation between tHcy and markers of protein-energy nutritional status in maintenance dialysis patients may partially, but not fully, explain the paradoxical association between tHcy and mortality in these individuals. Diminished nutritional status, with or without inflammation, is a strong predictor of poor outcome in these individuals (24, 45–48). Similar to our results, both Suliman *et al.* (11) and Wrone *et al.* (12) also reported significant correlations between tHcy and such nutritional markers as nPNA and serum concentrations of albumin, prealbumin, and creatinine. It is important to note that in all three studies, including ours, the association between tHcy and serum creatinine is the strongest as indicted by correlation coefficients, *i.e.*, even stronger than associations with serum albumin concentration (see Figures 1 and 2 and Table 2). Serum creatinine is an indicator not only of dialysis dose and efficiency but also of muscle mass and nutritional state in maintenance dialysis patients (49). Its paradoxical association with improved survival is well known and is described as one of the main components of the reverse epidemiology phenomenon (15, 49–51). Unlike serum albumin, serum creatinine may be less or not at all associated with inflammation. Serum albumin, conversely, is a combined marker of both protein-energy malnutrition and inflammation (52) and, hence, an indicator of malnutrition-inflammation complex syndrome (18). tHcy seems to be a more exclusive nutritional marker, because its association with serum albumin is weaker than that with serum creatinine. Indeed, consistent with this exclusive nutritional hypothesis, we found no association between tHcy and such inflammatory markers as serum CRP, IL-6, and TNF-α concentration (Table 2). The correla-

tion between tHcy and serum creatinine concentration could also be the result of the metabolic association between creatinine and homocysteine. The formation of creatine, the precursor of creatinine, depends on methyl donation by S-adenosylmethionine to become S-adenosylhomocysteine, leading to the formation of homocysteine (53). According to Oishi *et al.* (38), the demethylation pathway of methionine may be disturbed in diabetic MHD patients. This may be the reason for slightly lower serum tHcy levels in diabetic MHD patients when compared with their nondiabetic counterparts as found in the current and other studies (37, 38).

Our current study should be qualified by the possibility of selection bias. During the initial recruitment in eight dialysis units (with >1000 patients), it is possible that only MHD patients who were generally healthier and more health-conscious and had better nutritional status agreed to participate (385 patients). Hence, the final 367 MHD patients of this study whose tHcy was measured might include disproportionately less sick and better nourished individuals. Indeed, the annual mortality rate among all patients of the study dialysis units was 15%, whereas it was as low as 10% among selected patients for the NIED study. However, a selection bias with such a direction generally would lead to a bias toward the null, so without this bias, our positive results probably would have been even stronger and the associations indeed more prominent. Another possible limitation can be related to the relatively short period of longitudinal follow-up (only 12 mo as compared with 4 yr in the study by Suliman *et al.* (11)). Moreover, some associations existed only when logarithmic values of tHcy were analyzed. As depicted in Figure 1, there was a right-sided skewness in tHcy values. Hence, logarithmic transformation was appropriate and the proper method. Finally, another limitation of our study is the lack of blood folate measurements. However, studying the effect of folate deficiency on tHcy is beyond the scope of this epidemiologic study, which focuses on the direction of the association between tHcy and clinical outcome in MHD patients. Some strengths of our study include its large sample size with inclusion of many individuals with diabetes, its longitudinal design, its inclusion of hospitalization data, and the use of extensive markers of inflammation and nutrition at the same time. Our study is unique because, unlike previous studies in which only serum CRP was measured as the marker of inflammation and only mortality was examined, we measured a multiple number of inflammatory makers (CRP, IL-6, and TNF-α) along with a number of anthropometric indicators (TSF and MAMC) and analyzed hospitalization measures.

In our study, we did not find any major association between tHcy and anthropometric values such as BMI or body fat. Wrone *et al.* (12) showed a bivariate correlation coefficient of 0.19 between tHcy and BMI in MHD patients. Our data showed the same trend without adequate statistical significance. This may be due to the selection bias at recruitment level as described above, especially because >55% of our patients were diabetic. However, we found a weak but statistically significant, positive correlation between tHcy and such anthropometric values as MAMC and TSF. Moreover, consistent with two previous studies (11, 12), we, too, found a statistically significant and positive association between tHcy and nPNA, suggesting a positive role of protein intake in determining the tHcy level in MHD patients.

In summary, our data show that in MHD patients, there is a paradoxically inverse association between poor clinical outcome and levels of tHcy, a known risk factor for cardiovascular disease in the general population. Maintenance dialysis patients are a group of highly selected individuals whose high rate of cardiovascular disease and mortality does not seem to be due to such traditional risk factors as hypertension, obesity, hypercholesterolemia, or hyperhomocysteinemia. Such paradoxical associations, in the setting of the unique phenomenon of risk factor reversal, may have major clinical implications in our patients. To that end, the extrapolation of data of traditional risk factors to dialysis patients without first establishing that these factors constitute significant risks in dialysis patients as well may be flawed. The mortality rate of dialysis patients in the United States has not changed significantly in the past 10 to 15 yr (54) despite ongoing efforts to target such traditional risk factors as hypertension, obesity, hypercholesterolemia, and hyperhomocysteinemia. Increasing dialysis dose or use of high-flux dialysis membranes also failed to show any improvement in survival according to the HEMO Study (55). Thus, it may be time to consider carrying out clinical trials that assess the effect of such nontraditional risk factors as malnutrition on survival in maintenance dialysis patients.

Acknowledgment

The NIED Study is supported by the grant DK61162 from the National Institute of Diabetes and Digestive and Kidney Diseases to K.K.-Z., a research grant from Amgen, Inc., to K.K.Z. and J.D.K., and General Clinical Research Center (GCRC) Grant M01-RR00425 from the National Centers for Research Resources, National Institutes of Health.

Presented in part to the annual meeting of the American Society of Nephrology, November 4–6, 2003.

We are indebted to DaVita, Inc., for authorizing and supporting the NIED Study in their facilities. Special thanks go to collaborating renal dietitians in DaVita dialysis facilities in Los Angeles area (Vina Agarwal, Tina Trudnowski, Elizabeth Tekuchi, Agnes Galves, Leslie Martinez, Delma Baylon, Joan Chow, Jennifer Dennis, and Jenia Arzaghi). We are also thankful to Stephanie Griffith (Harbor-UCLA GCRC Core Laboratories) for the management of blood samples and measuring inflammatory markers and to Robert S. Lehn (DaVita Laboratories, Deland, FL) for technical support and database management.

References

1. Homocysteine and risk of ischemic heart disease and stroke: A meta-analysis. *JAMA* 288: 2015–2022, 2002
2. Bautista LE, Arenas IA, Penuela A, Martinez LX: Total plasma homocysteine level and risk of cardiovascular disease: A meta-analysis of prospective cohort studies. *J Clin Epidemiol* 55: 882–887, 2002
3. Nurk E, Tell GS, Vollset SE, Nygard O, Refsum H, Ueland PM: Plasma total homocysteine and hospitalizations for cardiovascular disease: The Hordaland Homocysteine Study. *Arch Intern Med* 162: 1374–1381, 2002

4. Nygard O, Nordrehaug JE, Refsum H, Ueland PM, Farstad M, Vollset SE: Plasma homocysteine levels and mortality in patients with coronary artery disease. *N Engl J Med* 337: 230–236, 1997
5. Mezzano D, Pais EO, Aranda E, Panes O, Downey P, Ortiz M, Tagle R, Gonzalez F, Quiroga T, Caceres MS, Leighton F, Pereira J: Inflammation, not hyperhomocysteinemia, is related to oxidative stress and hemostatic and endothelial dysfunction in uremia. *Kidney Int* 60: 1844–1850, 2001
6. Robinson K, Gupta A, Dennis V, Arheart K, Chaudhary D, Green R, Vigo P, Mayer EL, Selhub J, Kutner M, Jacobsen DW: Hyperhomocysteinemia confers an independent increased risk of atherosclerosis in end-stage renal disease and is closely linked to plasma folate and pyridoxine concentrations. *Circulation* 94: 2743–2748, 1996
7. Mallamaci F, Zoccali C, Tripepi G, Fermo I, Benedetto FA, Cataliotti A, Bellanuova I, Malatino LS, Soldarini A: Hyperhomocysteinemia predicts cardiovascular outcomes in hemodialysis patients. *Kidney Int* 61: 609–614, 2002
8. Bachmann J, Tepel M, Raidt H, Riezler R, Graefe U, Langer K, Zidek W: Hyperhomocysteinemia and the risk for vascular disease in hemodialysis patients. *J Am Soc Nephrol* 6: 121–125, 1995
9. Moustapha A, Naso A, Nahlawi M, Gupta A, Arheart KL, Jacobsen DW, Robinson K, Dennis VW: Prospective study of hyperhomocysteinemia as an adverse cardiovascular risk factor in end-stage renal disease. *Circulation* 97: 138–141, 1998
10. Sirrs S, Duncan L, Djurdjev O, Nussbaumer G, Ganz G, Frohlich J, Levin A: Homocyst(e)ine and vascular access complications in haemodialysis patients: Insights into a complex metabolic relationship. *Nephrol Dial Transplant* 14: 738–743, 1999
11. Suliman ME, Qureshi AR, Barany P, Stenvinkel P, Filho JC, Anderstam B, Heimburger O, Lindholm B, Bergstrom J: Hyperhomocysteinemia, nutritional status, and cardiovascular disease in hemodialysis patients. *Kidney Int* 57: 1727–1735, 2000
12. Wrone EM, Zehnder JL, Hornberger JM, McCann LM, Coplon NS, Fortmann SP: An MTHFR variant, homocysteine, and cardiovascular comorbidity in renal disease. *Kidney Int* 60: 1106–1113, 2001
13. Suliman ME, Stenvinkel P, Barany P, Heimburger O, Anderstam B, Lindholm B: Hyperhomocysteinemia and its relationship to cardiovascular disease in ESRD: Influence of hypoalbuminemia, malnutrition, inflammation, and diabetes mellitus. *Am J Kidney Dis* 41: S89–S95, 2003
14. Suliman ME, Lindholm B, Barany P, Bergstrom J: Hyperhomocysteinemia in chronic renal failure patients: Relation to nutritional status and cardiovascular disease. *Clin Chem Lab Med* 39: 734–738, 2001
15. Kalantar-Zadeh K, Block G, Humphreys MH, Kopple JD: Reverse epidemiology of cardiovascular risk factors in maintenance dialysis patients. *Kidney Int* 63: 793–808, 2003
16. Fleischmann EH, Bower JD, Salahudeen AK: Risk factor paradox in hemodialysis: Better nutrition as a partial explanation. *ASAIO J* 47: 74–81, 2001
17. Nishizawa Y, Shoji T, Ishimura E, Inaba M, Morii H: Paradox of risk factors for cardiovascular mortality in uremia: Is a higher cholesterol level better for atherosclerosis in uremia? *Am J Kidney Dis* 38: S4–S7, 2001
18. Kalantar-Zadeh K, Ikizler A, Block G, Avram M, Kopple J: Malnutrition-inflammation complex syndrome in dialysis patients: Causes and consequences. *Am J Kidney Dis* 42: 864–881, 2003
19. Ifudu O, Uribarri J, Rajwani I, Vlacich V, Reydel K, Delosreyes G, Friedman E: Low hematocrit may connote a malnutrition-inflammation syndrome in hemodialysis patients. *Dial Transplant* 31: 845–878, 2002
20. Kalantar-Zadeh K, McAllister C, Lehn R, Lee G, Nissenson A, Kopple J: Effect of malnutrition-inflammation complex syndrome on erythropoietin hyporesponsiveness in maintenance hemodialysis patients. *Am J Kidney Dis* 42: 761–773, 2003
21. Kalantar-Zadeh K, Block G, McAllister C, Humphreys MH, Kopple M: Association between self-reported appetite and markers of inflammation, nutrition, anemia and quality of life in hemodialysis patients. *Am J Clin Dis* 2004, in press
22. Fried L, Bernardini J, Piraino B: Charlson comorbidity index as a predictor of outcomes in incident peritoneal dialysis patients. *Am J Kidney Dis* 37: 337–342, 2001
23. Beddhu S, Bruns FJ, Saul M, Seddon P, Zeidel ML: A simple comorbidity scale predicts clinical outcomes and costs in dialysis patients. *Am J Med* 108: 609–613, 2000
24. Kalantar-Zadeh K, Kopple JD, Block G, Humphreys MH: A malnutrition-inflammation score is correlated with morbidity and mortality in maintenance hemodialysis patients. *Am J Kidney Dis* 38: 1251–1263, 2001
25. Nelson EE, Hong CD, Pesce AL, Singh S, Pollak VE: Anthropometric norms for the dialysis population. *Am J Kidney Dis* 16: 32–37, 1990
26. Williams AJ, McArley A: Body composition, treatment time, and outcome in hemodialysis patients. *J Ren Nutr* 9: 157–162, 1999
27. Kalantar-Zadeh K, Kopple JD, Block G, Humphreys MH: Association among SF36 quality of life measures and nutrition, hospitalization, and mortality in hemodialysis. *J Am Soc Nephrol* 12: 2797–2806, 2001
28. Kalantar-Zadeh K, Block G, Kelly MP, Schroepfer C, Rodriguez RA, Humphreys MH: Near infra-red interactance for longitudinal assessment of nutrition in dialysis patients. *J Ren Nutr* 11: 23–31, 2001
29. Kalantar-Zadeh K, Dunne E, Nixon K, Kahn K, Lee GH, Kleiner M, Luft FC: Near infra-red interactance for nutritional assessment of dialysis patients. *Nephrol Dial Transplant* 14: 169–175, 1999
30. Kalantar-Zadeh K, Supasyndh O, Lehn RS, McAllister CJ, Kopple JD: Normalized protein nitrogen appearance is correlated with hospitalization and mortality in hemodialysis patients with Kt/V greater than 1.20. *J Ren Nutr* 13: 15–25, 2003
31. Kalantar-Zadeh K, Kleiner M, Dunne E, Ahern K, Nelson M, Koslowe R, Luft FC: Total iron-binding capacity-estimated transferrin correlates with the nutritional subjective global assessment in hemodialysis patients. *Am J Kidney Dis* 31: 263–272, 1998
32. Ridker PM, Rifai N, Rose L, Buring JE, Cook NR: Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. *N Engl J Med* 347: 1557–1565, 2002
33. Erbagci AB, Tarakcioglu M, Aksoy M, Kocabas R, Nacak M, Aynacioglu AS, Sivrikoz C: Diagnostic value of CRP and Lp(a) in coronary heart disease. *Acta Cardiol* 57: 197–204, 2002
34. Pecoits-Filho R, Barany P, Lindholm B, Heimburger O, Stenvinkel P: Interleukin-6 is an independent predictor of mortality in patients starting dialysis treatment. *Nephrol Dial Transplant* 17: 1684–1688, 2002
35. Stenvinkel P, Heimburger O, Jogestrand T: Elevated interleukin-6 predicts progressive carotid artery atherosclerosis in dial-

ysis patients: Association with Chlamydia pneumoniae seropositivity. *Am J Kidney Dis* 39: 274–282, 2002
36. Beutler B, Cerami A: The biology of cachectin/TNF—a primary mediator of host response. *Ann Rev Immunol* 7: 625–655, 1989
37. Suliman ME, Stenvinkel P, Heimburger O, Barany P, Lindholm B, Bergstrom J: Plasma sulfur amino acids in relation to cardiovascular disease, nutritional status, and diabetes mellitus in patients with chronic renal failure at start of dialysis therapy. *Am J Kidney Dis* 40: 480–488, 2002
38. Oishi K, Nagake Y, Yamasaki H, Fukuda S, Ichikawa H, Ota K, Makino H: The significance of serum homocysteine levels in diabetic patients on haemodialysis. *Nephrol Dial Transplant* 15: 851–855, 2000
39. Kunz K, Petitjean P, Lisri M, Chantrel F, Koehl C, Wiesel ML, Cazenave JP, Moulin B, Hannedouche TP: Cardiovascular morbidity and endothelial dysfunction in chronic haemodialysis patients: Is homocyst(e)ine the missing link? *Nephrol Dial Transplant* 14: 1934–1942, 1999
40. van Guldener C, Lambert J, ter Wee PM, Donker AJ, Stehouwer CD: Carotid artery stiffness in patients with end-stage renal disease: No effect of long-term homocysteine-lowering therapy. *Clin Nephrol* 53: 33–41, 2000
41. Morgenstern H: Ecologic studies. In: *Modern Epidemiology*, edited by Rothman K, Greenland S, 2nd Ed., Philadelphia, Lippincott-Raven, 1988, pp 459–480
42. Bostom AG, Shemin D, Verhoef P, Nadeau MR, Jacques PF, Selhub J, Dworkin L, Rosenberg IH: Elevated fasting total plasma homocysteine levels and cardiovascular disease outcomes in maintenance dialysis patients. A prospective study. *Arterioscler Thromb Vasc Biol* 17: 2554–2558, 1997
43. Montori VM, Smieja M, Guyatt GH: Publication bias: A brief review for clinicians. *Mayo Clin Proc* 75: 1284–1288, 2000
44. Olson CM, Rennie D, Cook D, Dickersin K, Flanagin A, Hogan JW, Zhu Q, Reiling J, Pace B: Publication bias in editorial decision making. *JAMA* 287: 2825–2828, 2002
45. Marcen R, Teruel JL, de la Cal MA, Gamez C: The impact of malnutrition in morbidity and mortality in stable haemodialysis patients. Spanish Cooperative Study of Nutrition in Hemodialysis. *Nephrol Dial Transplant* 12: 2324–2331, 1997
46. Acchiardo SR, Moore LW, Latour PA: Malnutrition as the main factor in morbidity and mortality of hemodialysis patients. *Kidney Int Suppl* 16: S199–S203, 1983
47. Bergstrom J, Lindholm B: Malnutrition, cardiac disease, and mortality: An integrated point of view. *Am J Kidney Dis* 32: 834–841, 1998
48. Kalantar-Zadeh K, Kopple J: Malnutrition as a cause of morbidity and mortality in dialysis patients. In: *Nutritional Management of Renal Disease*, edited by Kopple J, Massry S, 2nd Ed., Philadelphia, Lippincott Williams & Wilkins, 2004
49. Canaud B, Garred LJ, Argiles A, Flavier JL, Bouloux C, Mion C: Creatinine kinetic modelling: A simple and reliable tool for the assessment of protein nutritional status in haemodialysis patients. *Nephrol Dial Transplant* 10: 1405–1410, 1995
50. Lowrie EG, Lew NL: Death risk in hemodialysis patients: The predictive value of commonly measured variables and an evaluation of death rate differences between facilities. *Am J Kidney Dis* 15: 458–482, 1990
51. Perez RA, Blake PG, Spanner E, Patel M, McMurray S, Heidenheim P, Lindsay RM: High creatinine excretion ratio predicts a good outcome in peritoneal dialysis patients. *Am J Kidney Dis* 36: 362–367, 2000
52. Kaysen GA, Chertow GM, Adhikarla R, Young B, Ronco C, Levin NW: Inflammation and dietary protein intake exert competing effects on serum albumin and creatinine in hemodialysis patients. *Kidney Int* 60: 333–340, 2001
53. Matthews D: Proteins and amino acids. In: *Modern Nutrition in Health and Disease*, edited by Shils M, Olson J, Shike M, Ross A, 9th Ed., Baltimore, Williams and Wilkins, 1999, pp 11–48
54. United States Renal Data System. Bethesda, US Department of Public Health and Human Services, Public Health Service, National Institutes of Health, 2004
55. Eknoyan G, Beck GJ, Cheung AK, Daugirdas JT, Greene T, Kusek JW, Allon M, Bailey J, Delmez JA, Depner TA, Dwyer JT, Levey AS, Levin NW, Milford E, Ornt DB, Rocco MV, Schulman G, Schwab SJ, Teehan BP, Toto R: Effect of dialysis dose and membrane flux in maintenance hemodialysis. *N Engl J Med* 347: 2010–2019, 2002