Kidney International, Vol. 54 (1998), pp. 627–636

C-reactive protein as an outcome predictor for maintenance hemodialysis patients

WILLIAM F. OWEN and EDMUND G. LOWRIE

Department of Medicine, Renal Division, Brigham and Women's Hospital, Harvard Medical School, Boston, and Fresenius Medical Care, North America, Lexington, Massachusetts, USA

C-reactive protein as an outcome predictor for maintenance hemodialysis patients.

Background. The possible association between inflammatory processes and other outcome measures in ESRD patients led us to measure the blood C-reactive protein (CRP) concentration in a large sample of hemodialysis patients, and to evaluate its statistical relationship with other common laboratory measures and patient survival. This was performed in a prospective, observational analysis with mortality as the principal outcome measure.

Methods. One thousand fifty-four routine blood samples, collected from as many patients during June and July 1995 (one sample per patient), were randomly selected for measurement of CRP, prealbumin, and other routine laboratory measures. Six months after the initial blood tests, patient survival was determined. Logistic regression analysis was the primary statistical tool used to evaluate laboratory associations with odds of death. Bivariate regression and correlation analyses were performed using all available data.

Results. The distribution of CRP values was skewed; approximately 35% of the values exceeded the upper limit of the laboratory's reference range. Serum albumin and prealbumin concentrations both correlated with the serum creatinine concentration ($r = 0.378$ and $r = 0.347$, respectively; P's < 0.001), and were inversely associated with the CRP ($r = -0.254$ and $r = -0.354$, respectively; P's <0.001). CRP was also inversely associated with blood hemoglobin concentrations ($r = -0.235$; $P < 0.001$). Using multiple regression analysis to further explore these relationships, the serum creatinine concentration was inversely associated with CRP ($r = -0.140$; $P < 0.001$). However, after adjustment for the linkage of the serum creatinine with the serum albumin concentration ($r = -0.378$; $P < 0.001$), no relationship with creatinine was observed. Before and after adjustment for serum albumin and prealbumin concentration, the ferritin concentration correlated directly with CRP ($r = 0.148$; $P < 0.001$). Ferritin was inversely and highly correlated with total iron binding capacity ($r = -0.516$; $P < 0.001$). Independent associations of hemoglobin with albumin ($t = 7.16$; $P < 0.001$), prealbumin ($t = 2.39$; $P = 0.017$), and CRP ($t = -4.27$; $P < 0.001$) were observed. Also, the dose of erythropoietin was directly associated with the CRP concentration, before ($r = 0.081$, $P = 0.009$) and after ($t = 2.03$, $P = 0.042$) adjustment for the serum albumin and iron concentrations. CRP correlated directly with neutrophil ($r = 0.318$; $P < 0.001$) and platelet counts ($r = 0.180$; $P < 0.001$), but was weakly and inversely correlated with the lymphocyte count ($r = -0.071$; $P = 0.04$). A logistic regression analysis performed using the laboratory variables revealed a strong, independent, and inverse relationships between the serum albumin and creatinine concentrations, total lymphocyte count, and the odds risk of death. In this model, no significant relationship was observed between the odds risk of death and CRP.

Conclusions. The data presented herein suggest that: (*1*) strong predictable associations exist among laboratory proxies for malnutrition, anemia, and the acute phase reaction, and (*2*) the pathobiology implied by these laboratory abnormalities influence patients' mortal risk primarily through depletion of vital body proteins, not inflammation.

Key words: C-reactive protein, anemia, malnutrition, albumin, end-stage renal disease, survival.

Received for publication December 4, 1997
and in revised form March 13, 1998
Accepted for publication March 13, 1998

The processes of patient care that effect clinical outcomes for patients with end-stage renal disease (ESRD) have undergone increased scrutiny [1]. Because of its statistical power as a predictor of mortality for peritoneal and hemodialysis patients, particular attention has been placed in the area of nutrition, where the serum albumin concentration is routinely used as a proxy of visceral protein nutrition [1–13]. Malnutrition is a common finding among patients with ESRD [1, 2, 6, 7]. Malnutrition of visceral proteins often occurs during the course of many chronic diseases, such as chronic renal failure [14], protracted infections [15], and cancer [16]. The depletion of vital proteins in such conditions typically exceeds that observed in protein/caloric starvation alone [17]. Therefore, it has been suggested that the malnutrition of chronic diseases is mediated in part by the release of one or more catabolic and/or anti-anabolic cytokines that are usually part of the normal effector limb of adaptive immunity. Such proinflammatory pathways also promote the enhanced synthesis of acute phase proteins, described as part of the acute phase reaction [18–21].

The possible inflammatory association between outcome processes in ESRD patients led us to quantitate the C-reactive protein concentration (CRP) and obtain other

Table 1. Distribution of data elements[a]

Variable	Units	*N*	Mean	SD	Reference Range
Primary laboratory tests					
Albumin	gm/dl	1051	3.89	0.43	3.5–5.2
Alkaline phosphatase	U/liter	1051	140.53	194.46	39–117
CO_2	mEq/liter	1051	20.47	3.30	23–29
Creatinine	mg/dl	1051	10.80	3.50	0.6–1.6
C-reactive protein	mg/dl	1054	1.29	2.10	<0.8
Ferritin	ng/ml	1042	322.84	430.05	5–179
Hemoglobin	gm/dl	946	10.16	1.38	11.4–17.0
Iron	mcg/dl	1045	53.51	30.57	37–145
Lactate dehydrogenase	U/liter	1051	210.35	59.04	118–273
Phosphorus	mg/dl	1051	6.00	1.90	2.7–4.5
Potassium	mEq/liter	1051	4.84	0.81	3.5–5.3
Pre-albumin	mg/dl	1053	29.16	7.04	18.0–33.8
Total iron binding capacity	mcg/dl	1015	216.29	49.98	259–388
Iron binding capacity saturation	%	1015	25.22	13.60	20–55
Extended laboratory tests					
BUN	mg/dl	1051	67.26	18.84	6–19
ALT	U/liter	1051	18.26	14.21	0–37
AST	U/liter	869	16.47	21.4	0–40
Lymphocyte count	$10^3/\mu l$	870	1.37	0.62	0.9–5.2
Neutrophil count	$10^3/\mu l$	870	4.90	2.32	1.9–8.0
Platelet count	$10^3/\mu l$	869	221.94	84.66	150–450
Reticulocyte count	%	968	1.72	0.87	0.3–2.1
Other laboratory variables					
Age	years	1054	59.33	15.49	NA
MCHC	g/dl	945	30.91	1.32	33–37
MCV	μcm^3	945	95.24	7.87	80–99
URR	%	973	67.37	8.52	≥65

[a] *N* is number of patients on whom the test was performed; SD is standard deviation. Nine hundred four patients had complete primary laboratory tests. Six hundred ten patients had complete primary and extended tests.

common laboratory measures in a large sample of hemodialysis patients. The serum concentration of CRP reflects the activity of cytokine-mediated acute phase processes [22–25] and is roughly proportional to the extent of tissue injury [26]. Furthermore, selected cytokines stimulate the hepatic production of CRP and apo-ferritin, while inhibiting synthesis of albumin and prealbumin. The data presented herein suggest that in patients with ESRD: (*1*) strong predictable associations exist among laboratory proxies for malnutrition and the acute phase processes, and (*2*) the pathobiology implied by these laboratory abnormalities influences mortal risk in patients primarily through depletion of vital body proteins.

METHODS

Blood samples from 1,054 patients, that were routinely sent to LifeChem clinical laboratory (Rockleigh, NJ, USA) for processing during June and July 1995 (one blood sample per patient), were randomly selected for measurement of the CRP and prealbumin concentrations. Other routinely measured laboratory tests were performed only as ordered by the nephrologists. Because neither the patients nor their nephrologists were aware of their participation in the observational analysis, routine laboratory tests were ordered based on physician preference and clinical indications alone. Because of variability in nephrologists' practice styles and patients' case mix, the individual tests performed differed from patient to patient. The routine laboratory tests were categorized as "primary" or "extended." Primary laboratory tests were those that had been performed on the largest number of patients; extended laboratory tests were performed on a smaller number of subjects, but provided the greatest number of tests for statistical analysis (Table 1). Both categories of tests composed the initial data set.

Six months after the initial blood sampling (January and February 1996), the patients were classified as alive on dialysis or dead. A total of 988 patients lived for six months after blood sampling or died during that interval. The remaining 66 patients were censored from further analysis because of renal transplantation ($N = 26$), transfer to other facilities ($N = 31$), or miscellaneous reasons ($N = 9$), such as recovery of renal function or loss to follow up. Eight hundred forty-five of these 988 patients had complete primary laboratory data; 570 of these 988 patients had complete primary and extended laboratory data.

The patients' survival status, and their individual demographic characteristics extracted from Fresenius Medical Care's Patient Statistical Profile System (PSP), were merged with the initial data set to create the final data set. The PSP contains information for the individual patient's date of birth, gender, etiology of renal disease, and censor date, if applicable [2, 4, 6]. The final data set was used to evaluate the statistical association of the laboratory test

results and other variables with the patients' mortal outcomes. In the final data set, logistic regression analysis was the primary statistical tool used to evaluate associations with odds of death [27]. A forward stepwise regression process was used for two core analyses, which included patient ages, gender, diabetic status, and either the primary laboratory variables or both the primary and extended laboratory variables. The Null Hypothesis of "no association" was rejected if $P \leq 0.05$, considered indeterminate if $P > 0.05$ and ≤ 0.10, and accepted if $P > 0.10$. Bivariate regression and correlation analyses were performed using all available data.

To evaluate for possible analytical bias from the original database and/or the statistical constraints of the logistic regression analysis, three supplementary data analyses were performed ("sensitivity analysis"). Arguably, error or bias in the original data analysis may be introduced by: (*1*) different test ordering practices of the nephrologists, (*2*) volatility of test results that may not be appreciated by the use of single laboratory values, rather than to average serial tests results, and/or (*3*) selection or statistical bias resulting from the elimination of censored patients (because of renal transplantation, transfer to other facilities, or miscellaneous reasons, such as recovery of renal function or loss to follow up), as required for a logistic regression analysis. Firstly, 275 patients did not have an extended laboratory test measured. So that these patients could be included in the supplementary analysis, an average value for an individual extended laboratory test was calculated from those available. This value was then substituted as the missing value for that test among the 275 patients. To evaluate potential differences in the odds risk of death between them and the patients with complete laboratory data (610 patients had complete data for both primary and extended tests), a dummy variable was also created to identify those patients with substituted data; this was also used in a logistic regression analysis. Secondly, rather than using a single, "point in time" value, as in the principal data analysis, the average laboratory test values were calculated from up to three months prior to June 1995 and was substituted. In this circumstance, the single value from the principal analysis was not included in the average. CRP and serum prealbumin concentrations were exempted from these substitutions because no other values were available for them. Thirdly, we performed two analyses of survival time using a Cox proportional hazard model instead of the odds risk of death [28]. Unlike a logistic regression model, the Cox model permits inclusion of the censored patients, as well as those who lived or died during the period of observation.

Different regression models were evaluated by comparing the R-square statistic (R^2), resulting from each of several models. The R^2 term reports the proportion of variability in the dependent variable that is explained by the independent variable. The trend line, 95% confidence interval, and 95% prediction interval were calculated from the models demonstrating the best fit. Unless otherwise specified, all selected models were linear. For some variables, especially CRP and ferritin, the distributions of values were not normal. Normal distributions, without major discontinuities, were generated for CRP by transforming it using the equation $(\log_{10}[1+\text{CRP}])^{1/2}$. Ferritin was transformed to $\log_{10}$ of its concentration. However, both original and transformed values were used for analysis.

CRP was determined by the rate nelphelometry (Beckman Instruments, Inc., Galway, Ireland) [29]. Prealbumin concentration was determined by immunoprecipitin analysis (Incstar Corporation, Stillwater, MN, USA) [30]. Other blood chemistry values were determined on Hitachi Model 736-50 and 747-200 analyzers using conventional methods and reagents (Boehringer Mannheim Corp., Indianapolis, IN, USA), and the serum albumin concentration was determined on that device by the bromocrysal green method. Hematological values were determined using standard laboratory equipment, methods and reagents (H-System; Bayer diagnostics, Terrytown, NY, USA). The urea reduction ratio (URR) was calculated as the difference between the predialysis and postdialysis blood urea nitrogen (BUN) concentration divided by the predialysis value and multiplying the quotient by 100 [2].

RESULTS

Patient demographics and laboratory test values

Over one half (54.8%) of the patients were white, 41.1% were African American, and 4.1% were of other races. Over one third (35.9%) had diabetes mellitus as a comorbid condition. The gender distribution was 47.5% female. Therefore, this patient subset resembled that of the prevalent American ESRD population [31], except for a modest over-representation of African Americans that may have augmented patient survival based on prior observational outcome analyses [32]. The distributions of primary and extended test values are shown in Table 1.

The serum albumin concentrations for this patient group was 3.9 ± 0.4 g/dl (mean $\pm$ SD). The mean serum creatinine concentration was 10.8 ± 3.5 mg/dl. Most patients were moderately to severely anemic. One half had a hemoglobin concentration ≤ 10.9 g/dl with lower quartile ≤ 9.4 g/dl (Table 1). Approximately 84% of the patients (83.7%) received recombinant human erythropoietin (rhEPO), predominantly intravenously (>95%). Patients not receiving rhEPO had higher hemoglobin concentration than patients receiving the hormone (10.8 ± 1.8 and 10.1 ± 1.6 g/dl, respectively; mean $\pm$ SD; $P < 0.001$), and the relationship between hemoglobin concentration and rhEPO dose was inverse ($r = -0.213$; $P < 0.001$). Putative laboratory measures of iron stores [iron, total iron binding capacity (TIBC), iron binding capacity (IBC) saturation, ferritin] suggested that iron deficiency was not prevalent. Although

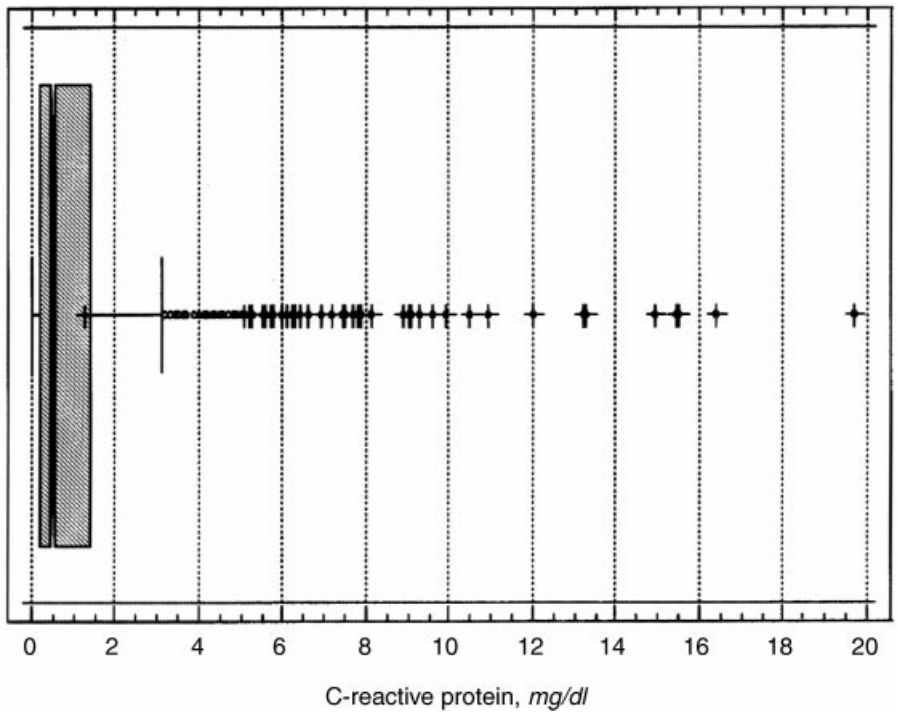

Fig. 1. Box and whisker plot showing the distribution of transformed C-reactive protein (CRP) values for 1,054 ESRD patients. The box defines the upper and lower quartiles, and the mean is shown by the cross. The notch indicates the median. Higher values for CRP are arrayed to the right.

the total iron binding capacity was low, mean iron concentration was normal. IBC saturation was low normal and the ferritin concentration was elevated.

The distribution of CRP is shown in Table 1 and Figure 1. The distribution of CRP values was skewed. Approximately 35% of values from this random patient sample exceeded the upper limit of the laboratory's reference range. The distribution of other data elements is shown in Table 1.

Bivariate and multivariate relationships

Serum albumin and prealbumin concentrations were highly correlated ($r = 0.479$, $P < 0.001$), and both correlated with the serum creatinine concentration ($r = 0.378$ and $r = 0.347$, respectively; P's <0.001). Figure 2 illustrates the relationship of transformed values CRP with serum albumin concentrations ($r = -0.254$; $P < 0.001$), respectively. Both were linear and inverse throughout the ranges of observation. The correlation coefficients were of similar magnitude for transformed and non-transformed CRP values. CRP was also inversely associated with prealbumin concentration ($r = -0.354$; $P < 0.001$).

The bivariate associations between selected variables were examined to more clearly analyze other relationships. Figure 3 illustrates the relationships of transformed values of CRP with blood hemoglobin concentrations ($r = -0.235$; $P < 0.001$). CRP was also inversely associated with IBC ($r = -0.205$; $P < 0.001$). The serum albumin concentration correlated directly with the blood hemoglobin concentration ($r = 0.326$; $P < 0.001$; Fig. 4). Even after adjustment for this interaction, the blood hemoglobin concentration also directly correlated with the prealbumin concentration ($r = 0.259$; $P < 0.001$). Although the blood hemoglobin concentration correlated with the serum creatinine concentration ($r = 0.107$; $P < 0.001$), adjustment for the serum albumin concentration abolished this relationship. Lastly, TIBC was also strongly and inversely correlated with ferritin concentration ($r_{transformed} = -0.516$; $P < 0.001$). Figure 5 illustrates this relationship.

Multiple regression analysis was used to explore the relationship between hemoglobin, visceral protein measures, and CRP. The serum creatinine concentration was inversely associated with CRP ($r = -0.140$; $P < 0.001$). However, after adjustment for its association with the serum albumin concentration ($r = -0.378$; $P < 0.001$), no relationship with creatinine was observed. Before and after adjustment for serum albumin and prealbumin concentration, both transformed and untransformed ferritin concentration were directly correlated with CRP ($r_{transformed} = 0.148$; $P < 0.001$). Independent associations of hemoglobin with albumin ($t = 7.16$; $P < 0.001$), prealbumin ($t = 2.39$; $P = 0.017$), and CRP ($t = -4.27$; $P < 0.001$) were observed, but not with IBC. Also, the dose of rhEPO was directly correlated with the CRP concentration, before ($r = 0.081$, $P = 0.009$) and after ($t = 2.033$, $P = 0.042$) adjustment for the serum albumin and iron concentrations. The dose of rhEPO was not correlated with prealbumin, creatinine, TIBC, IBC Sat, or ferritin concentration. Weak inverse associations between

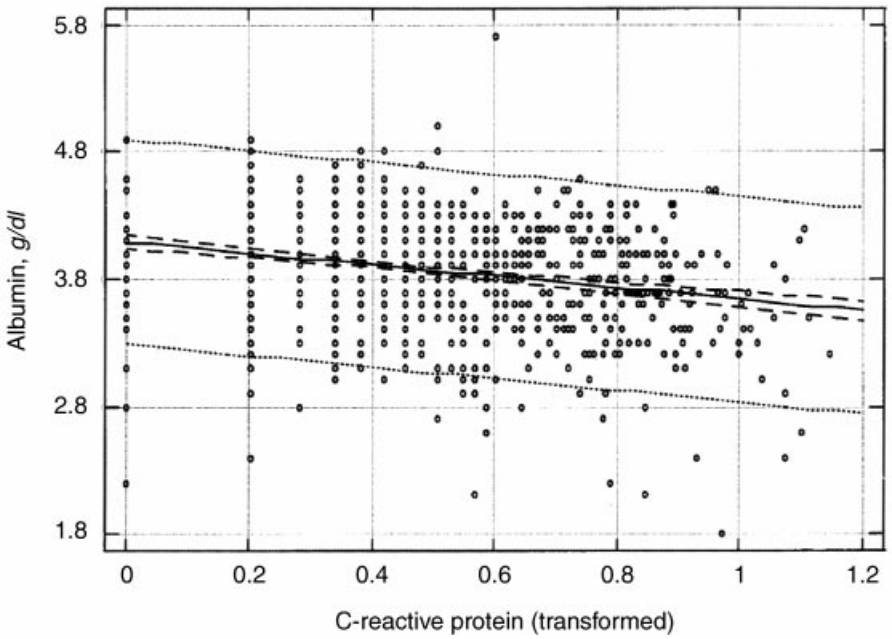

Fig. 2. Regression of serum albumin concentration (g/dl) on transformed C-reactive protein ($r = -0.254$; $P < 0.001$).

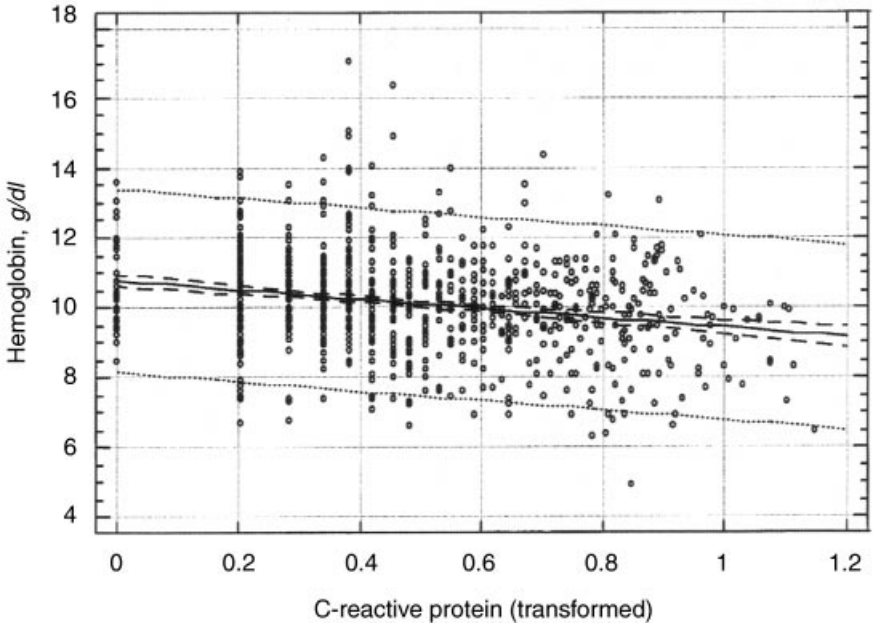

Fig. 3. Regression of blood hemoglobin concentration (g/dl) on transformed C-reactive protein (CRP) values ($r = -0.235$ and $P < 0.001$). Transformed values correspond to real values as follows: 0 = 0; 0.2 = 0.10; 0.4 = 0.45; 0.6 = 1.29; 0.8 = 3.37; 1.0 = 9.00; 1.2 = 26.54. Upper limits of normal for transformed CRP values is <3.37.

rhEPO dose and the concentrations of albumin ($r = -0.060$, $P = 0.051$) and iron ($r = -0.067$, $P = 0.029$) were observed.

CRP correlated directly with neutrophil ($r = 0.318$; $P < 0.001$) and platelet counts ($r = 0.180$; $P < 0.001$), but was weakly and inversely correlated with the lymphocyte count ($r = -0.071$; $P = 0.04$). In turn, the lymphocyte count was directly correlated with serum albumin ($r = 0.104$; $P = 0.002$) and prealbumin concentrations ($r = 0.128$; $P < 0.001$). The neutrophil count was inversely correlated with the serum albumin ($r = -0.126$; $P < 0.001$) and prealbumin concentrations ($r = -0.080$; $P < 0.019$).

Principal mortality analysis

Table 2 shows the results of logistic regression analyses performed using primary plus the extended laboratory variables, before statistical adjustments for other variables in the data set. Higher values of albumin, creatinine, hemoglobin, prealbumin, and TIBC were associated with lower odds risk (OR) of death, whereas older age and having diabetes mellitus were associated with higher odds risk. Higher CRP values were associated with greater death risk in the analysis of primary laboratory variables but not

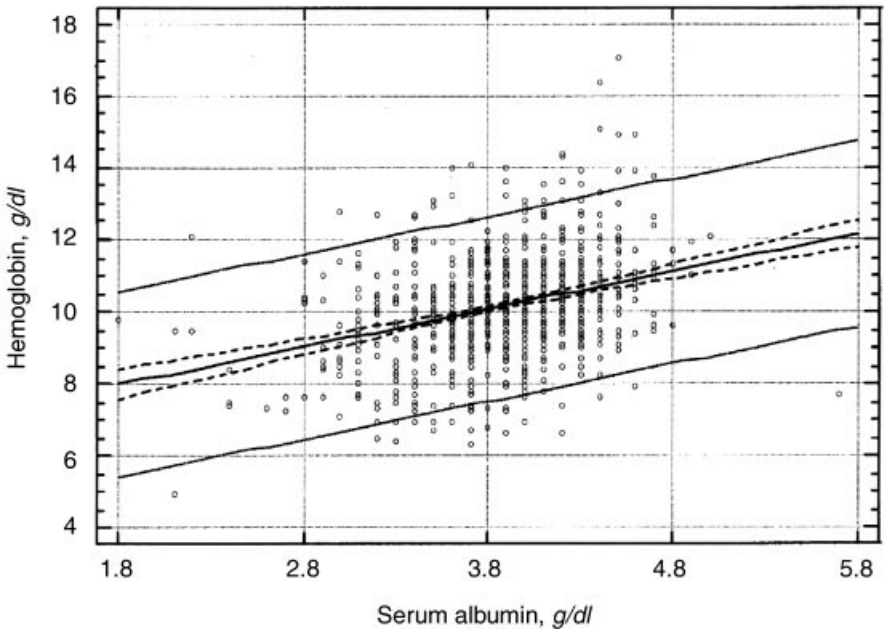

Fig. 4. Regression of blood hemoglobin concentration (g/dl) on serum albumin concentration (g/dl) ($r = 0.326$ and $P < 0.001$).

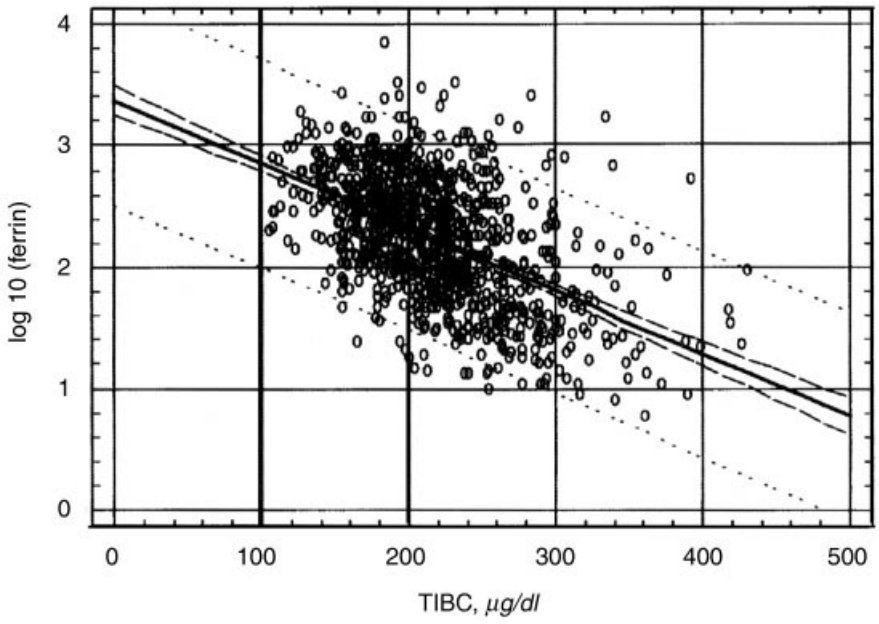

Fig. 5. Regression of total iron binding capacity (TIBC; μg/dl) on transformed ferritin concentration ($r = -0.52$; $P = 0.001$).

primary and extended laboratory variables. Among the extended laboratory variables, higher lymphocyte counts were associated with lower odds risk of death, but higher neutrophil and platelet counts were associated with higher odds risk of death.

A multivariable statistical model was evaluated, adjusting for correlation among the primary laboratory variables (Table 3). Higher values for the serum albumin and creatinine concentrations were associated with lower odds risk of death, whereas higher values of phosphorus were associated with greater odds risk of death. Females enjoyed a survival advantage over males. Age, hemoglobin, TIBC, prealbumin, and CRP were not significantly associated with the odds risk of death after adjustments for other variables in the model. Because age was inversely correlated with the creatinine ($r = -0.483$; $P < 0.001$), the serum creatinine concentrations likely eliminated age as a statistically significant variable from the final model. The final logistic model evaluating the primary plus extended laboratory variables is summarized in Table 4. Again, the serum concentrations of albumin, creatinine, and phosphorus appear as the values most closely associated with odds of death. Even after

Table 2. Logistic regression analysis of demographic and laboratory variables on death

Variable	Units	Primary laboratory variables			Primary and extended laboratory variables		
		χ^2	*P*	OR	χ^2	*P*	OR
Age	years	6.92	0.008	1.026	3.14	0.077	1.021
Gender	Ref = male	0.22	NS		0.38	NS	
Race	Ref = nonwhite	2.10	NS		0.53	NS	
Diabetes mellitus	Ref = nondiabetic	2.99	0.084	1.535	3.66	0.055	1.702
Albumin	g/dl	25.37	<0.001	0.229	14.35	<0.001	0.233
CO_2	mEq/liter	0.75	NS		0.57	NS	
Creatinine	mg/dl	17.31	<0.001	0.825	11.61	<.001	0.827
C-reactive protein	mg/dl	2.80	0.094	1.096	1.84	NS	
Ferritin	mg/dl	0.29	NS		0.03	NS	
Hemoglobin	gm/dl	7.23	0.007	0.759	9.11	0.003	0.679
Iron	mcg/dl	0.60	NS		0.60	NS	
Lactate dehydrogenase	U/liter	2.63	NS		2.98	0.084	1.004
Phosphorus	mg/dl	0.78	NS		0.91	NS	
Potassium	meq/l	1.03	NS		0.68	NS	
Pre-albumin	mg/dl	13.57	<0.001	0.929	9.79	0.002	0.925
Total iron binding capacity	mcg/dl	4.28	0.039	0.994	4.09	0.043	0.992
Iron binding capacity sat.	%	<0.01	NS		<0.01	NS	
Alkaline phosphatase	U/liter				0.03	NS	
ALT	U/liter				1.61	NS	
AST	U/liter				0.61	NS	
Lymphocyte count	$10^3/\mu l$				10.10	0.002	0.926
Neutrophil count	$10^3/\mu l$				8.06	0.005	1.053
Platelet count	$10^3/\mu l$				5.76	0.016	1.004
Reticulocyte count	%				0.03	NS	

Variables are: χ^2, Chi-square statistic; *P*, probability of H_o (NS means not significant to reject @ $P \leq 0.10$); OR, odds ratio of death for the variable if $P \leq 0.10$.

Variables were estimated before the first step of a forward, stepwise, logistic regression process. Death risk was evaluated over the subsequent 6 months from the initial date of data retrieval.

Table 3. Logistic regression model of death risk using adjusted demographic and primary laboratory variables[a]

Variable	χ^2	*P*	OR	95% CL
Albumin *g/dl*	13.2	<0.001	0.29	0.15–0.56
Creatinine *mg/dl*	12.8	<0.001	0.83	0.75–0.92
Phosphorous *mg/dl*	7.5	0.006	1.24	1.06–1.44
Gender (ref = male)	2.7	0.099	0.62	0.35–1.10

[a] See Table 2 for a list of variables evaluated by this model. Variables are listed in order of entry into the model. Death risk was evaluated over the subsequent 6 months from the initial date of data retreival. χ^2 means the Chi-square statistic; *P* means probability of H_o; OR means the odds ratio for death; 95% CL means the 95% confidence limit of the OR.

Table 4. Logistic regression model of death risk using adjusted demographic and primary and extended laboratory variables[a]

Variable	χ^2	*P*	OR	95% CL
Albumin	5.7	.017	0.34	0.14–0.83
Creatinine	6.0	.014	0.85	0.75–0.97
Lymphocyte count	4.3	.038	0.95	0.90–1.00
Phosphorous	5.5	.019	1.26	1.04–1.52

[a] See Table 2 for a list of variables evaluated by this model. Variables are listed in order of entry into the model. Death risk was evaluated over the subsequent 6 months from the initial date of data retreival. χ^2 means the Chi-square statistic; *P* means probability of H_o; OR means the odds ratio for death; 95% CL means the 95% confidence limit of the OR.

adjustment for its correlation with serum albumin concentration, lymphocyte count was also significantly associated with odds of death. Higher lymphocyte counts were associated with more favorable odds,

Sensitivity analyses of mortality

A supplementary analysis was performed that substituted the average value of the extended laboratory tests for the missing value of those tests among the 275 patients with only primary test results. The analysis did not reveal a significant difference between the patient groups ($P = 0.96$ and 0.12 before and after adjustment for other variables in the model, respectively). The second sensitivity analyses that substituted the three-month average value of laboratory tests for the single value did not reveal meaningful differences. Again, the analysis of primary laboratory variables alone revealed the serum albumin (OR = 0.30; $P = 0.003$), creatinine (OR = 0.85; $P = 0.003$), phosphorus concentrations (OR = 1.28; $P = 0.003$) and gender (OR = 0.56; $P = 0.052$) to be significant predictors of the odds of death. The potassium (OR = 1.49; $P = 0.044$) and prealbumin (OR = 0.96; $P = 0.052$) concentrations were also significantly associated with death risks. The analysis of primary plus extended laboratory variables revealed the serum albumin (OR = 0.16; $P < 0.001$), phosphorus concentrations (OR = 1.43; $P = 0.002$), and lymphocyte counts (OR = 0.95; $P = 0.062$) as significant predictors of death risks. However, in this subsidiary analysis, creatinine was not significant, whereas gender (OR = 0.50; $P = 0.095$) and age (OR = 1.03; $P = 0.044$) were significant.

Substituting a Cox model analysis of the primary variables, which included censored patients, yielded results similar to the logistic analysis that did not include censored patients. The concentrations of serum albumin, creatinine, and phosphorus were identified as significantly associated with death risk, but gender was not. A similar analysis of primary plus extended laboratory variables revealed creatinine, hemoglobin, lymphocyte count, phosphorus, and gender to be associated with death risk in the final model. Because the serum albumin concentration strongly correlated with death risk alone, it was selected for inclusion at the first step. However, the combined and direct correlation of death risk with creatinine, lymphocyte count, and hemoglobin contributed to the elimination of the albumin concentration.

DISCUSSION

Detecting the associations that link complicated data elements and then translating the links into meaningful pathobiological dimensions can be a difficult task. This dilemma is particularly true when there is a network of associations between the data elements. The ultimate intent of such analyses is to discover how the clinical and laboratory variables are associated with important outcome measures of clinical care. A fundamental outcome measure that is influenced by the processes of patient care is the odds of survival on hemodialysis. Because it is impossible to internally or externally monitor the processes of clinical care in a direct manner, clinicians and regulators must rely upon laboratory surrogates of these operations. Using a large and representative, national database of hemodialysis patients, we examined the statistical interactions between laboratory variables and dialysis patient mortality. Although some of the *r* values reported herein are relatively low, suggesting that some of the variability in the test being scrutinized was accounted for by factors other than those being analyzed, the very low *P* values make it mathematically unlikely that the associations are random interactions. Considering that this was an uncontrolled, observational analysis of hemodialysis patients, in which uniformity of disease processes and their management was absent, the clinical relationships described by these *r* values are of intellectual significance and clinically relevant.

The logistic regression analysis described a strong, independent, inverse relationships between the serum albumin and creatinine concentrations and the odds risk of death, but no such relationship for CRP. These observations are similar to earlier findings and suggest an inverse association between the body's content of both visceral and somatic proteins and odds of death [2, 5–8, 13, 33]. These laboratory measures are highly correlated, and their independent associations with odds of death suggests that the adequacy of the body's content of protein is strongly associated with the patient's likelihood of long-term survival [6, 7]. The proinflammatory and catabolic cytokines that stimulate the production of CRP also cause muscle catabolism with the liberation of amino acids and inhibition of muscle protein synthesis [22–26]. Furthermore, these cytokines may induce anorexia, so further compromise protein stores through diminished protein/caloric intake. Hence, one nexus between inflammation and visceral/somatic protein content among dialysis patients includes the cytokine-mediated acute phase processes.

In contrast to a previous report [34], the current analysis did not find CRP to be an independent predictor of survival for ESRD patients. Critical differences include the inclusion of a far greater number of subjects, the use of a more extensive laboratory profile, and the analysis of outcomes among ambulatory ESRD patients instead of hospitalized subjects for the present analysis. The current database does not permit extrapolation of these results to patients who are more ill and hospitalized, and vice versa. Because of its more rapid induction and shorter half-life, CRP may be a more dynamic marker of short-term inflammation/injury than the serum albumin or creatinine concentration. An alternative explanation for the observed difference in the predictive power of CRP is that proximate mortality may be better reflected by CRP, whereas longer term patient survival may be predicted by the alternative laboratory surrogates of nutrition. Circumspect extrapolation of CRP as an independent outcome predictor for the hemodialysis patient population is urged.

Similarly, the data presented herein support and extend the hypothesis that a low serum albumin concentration may not simply reflect inadequate intake of dietary protein and/or calories. Depleted stores of vital proteins may result in part from down-regulation of protein synthetic processes and up-regulation of catabolic processes. For example, the *in vitro* treatment of hepatocytes with IL-1β rapidly inhibits albumin synthesis [35]. Furthermore, a dietary and kinetic observational analysis of hypoalbuminemic ESRD patients revealed that some subjects had appropriate protein/caloric intakes, normalized protein catabolic rates, low dialysate albumin losses, and diminished albumin turnover rates [36]. For these patients, the degree of hypoalbuminemia correlated with the elevation of serum levels of laboratory surrogates of inflammation, such as the CRP, α_2-macroglobulin, ferritin, and serum amyloid A concentration [36, 37]. These studies suggest that in some hypoalbuminemic ESRD patients, cytokine-mediated inflammation induces hypoalbuminemia.

Multiple regression analyses revealed independent associations of visceral protein measures and CRP with blood hemoglobin concentration. These findings suggests that anemia is also associated with both cytokine-dependent inflammation and nutritional status. The direct association of CRP with rhEPO dose, and its inverse association with albumin, support this linkage. Further independent support for this hypothesis is provided by the observation that ESRD patients with elevated blood levels of interleukin

(IL)-6 are more anemic, and this anemia is corrected by reducing the cytokine levels [38]. Similarly, nude mice transfected with tumor necrosis factor (TNF) develop severe anemia [39]. The observed iron indices in this patient base also supports the provocative role of proinflammatory cytokines linking erythropoiesis and nutrition. We observed that the serum iron, TIBC, and Sat-IBC tended to be normal to low, but the ferritin was high. This laboratory pattern has been observed for other ESRD patient bases [40, 41]. In a recent analysis, >50% of the patients had IBC saturation <20%, and 44% of the patients had serum ferritin concentration >200 μg/liter [42]. This pattern of alteration for iron indices is commonly seen in the anemia accompanying chronic disease (ACD) [43, 44]. Hematologic indices of ACD have been observed during states of elevated cytokine activity [44].

We also observed an inverse statistical relationship between seemingly disparate processes, like nutritional status and anemia. Similar findings have been reported by others [41, 45]. In those earlier analyses, the serum albumin concentration was the most powerful predictor of anemia among the laboratory measures evaluated. Clinical support for this statistical link is provided by the longitudinal observation of the serum albumin and hemoglobin concentrations in malnourished ESRD patients receiving intradialytic parenteral nutrition (IDPN) [13, 46]. During the six months of observation prior to receiving IDPN, the decline in serum albumin concentration was accompanied by a parallel decrease in hemoglobin concentration, despite increasing rhEPO doses. After the initiation of IDPN, the patients' albumin and hemoglobin concentrations increased, and their odds risk of death declined [13]. Further support for this linkage is provided by the observation that severely malnourished patients without renal disease develop anemia that is reversed by correction of protein/caloric malnutrition alone [47, 48]. Arguably, a cytokine-dependent decline in nutritional status may further impair erythropoiesis in some malnourished patients [19].

In the current analysis, C-reactive protein was inversely associated with laboratory measures of protein stores (albumin, creatinine, prealbumin). CRP is an archetypal acute phase reactant, so called because of increased blood levels with acute inflammatory processes [20, 22–26]. Like ferritin, the hepatic synthesis of CRP is augmented by proinflammatory cytokines, such as IL-1β and TNF-α. The hepatic synthesis of albumin, prealbumin, and transferrin, are reciprocally inhibited by those cytokines [22–24]. Hence, a biochemical explanation exists for the observed pattern of statistical associations and that provides a common pathobiology shared for anorexia, depletion of vital body proteins, laboratory signs of inflammation, and dialysis-associated anemia. The common pathobiologic feature is the presence of proinflammatory cytokines, either alone or in combination with a diminished protein-caloric intake, effecting deleterious changes in multiple physiologic systems.

Lastly, death risk was higher in patients with a low lymphocyte count, an observation also made in other patients during the course of malnutrition [49, 50]. The fundamental role of lymphocytes in the effector limb of adaptive immunity suggests that lymphopenia may increase infectious risk in this population. The observation of a lymphocyte effect on death risk, that is independent of nutritional surrogates like albumin, suggests that deleterious immunologic dysfunction can also occur in this population in the absence of overt laboratory signs of malnutrition. A fundamental unanswered question is what is the proximate cause of the death in these situations: is it the inciting process(es) resulting in the excesses of proinflammatory cytokine(s), and/or is mortality a consequence of the deleterious pathobiologic effects of the cytokines on nutrition, hematopoiesis, etc.? If a particular pathobiologic process links seemingly disparate processes, such as anemia and the depletion of visceral body proteins, the question is raised whether it is the process, its provocateur, or the consequences of the depletion that is most closely associated with mortal risk. Both albumin and creatinine concentrations are closely associated with the odds of death in these and other studies [2, 5, 7, 8, 33,], whereas CRP is not.

Differences in statistical linkages between the logistic regression model and Cox proportional hazard model were observed and are unsurprising. There is no *a priori* reason that identical results should be achieved with the two models. Although both models examine the statistical impact of clinical variables on mortality, their conceptual construct of death risk is quite different. A logistic regression model determines the odds risk of survival, whereas the Cox model assesses variables that effect the length of survival. Because these two models conceptually view patient mortality in different, but complimentary ways, they were used to support the observation about CRP, not to achieve statistical uniformity. Thus, the data presented herein offer additional support for the hypothesis that a major contributor to mortal risk in ESRD is the depletion of body proteins, not inflammation.

Because laboratory surrogates of nutrition, such as the albumin and creatinine concentration are influenced to varying degrees by processes other than protein/caloric intake and nutrient utilization, the statistical linkages described herein should be extended by correlation with more direct measures of patients' nutritional status, such as bioelectrical impedance and dual energy x-ray absorptiometry [51].

Reprint requests to William F. Owen, Jr., M.D., Brigham and Women's Hospital, Dialysis Unit Administrative Office, 75 Francis Street, Boston, Massachusetts 02115, USA.
E-mail: wfowen@bics.bwh.harvard.edu

REFERENCES

1. Department of Health and Human Services: ESRD Core Indicators Project, Health Care Financing Administration. Health Standards and Quality Bureau, April 1996, 1996
2. Owen WF, Lew NL, Liu Y, Lowrie EG, Lazarus JM: The urea reduction ratio and serum albumin concentration as predictors of mortality in patients undergoing hemodialysis. *N Engl J Med* 329: 1001–1006, 1993
3. Lowrie EG, Huang WH, Lew NL, Liu Y: The relative contribution of measured variables to death risk among hemodialysis patients, in *Death on Hemodialysis: Preventable or Inevitable*, edited by Friedman E, Hingham, Kluwer Academic Publishers, 1994, pp 121–141
4. Lowrie EG: Chronic dialysis treatment: Clinical outcome and related processes of care. *Am J Kidney Dis* 24:255–266, 1994
5. Iseki K, Kawazoe N, Fukiyama K: Serum albumin is a strong predictor of death in chronic dialysis patients. *Kidney Int* 44:115–119, 1993
6. 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
7. Lowrie EG, Lew NL: Commonly measured laboratory variables in hemodialysis patients. Relationships among them and to death risk. *Semin Nephrol* 12:276–283, 1992
8. Avram MM, Mittman N, Bonomini L, Chattopadhyay J, Fein P: Markers for survival in dialysis: A seven-year prospective study. *Am J Kidney Dis* 26:209–219, 1995
9. Bergström J: Why are dialysis patients malnourished? *Am J Kidney Dis* 26: 229–241, 1995
10. Chertow G, Bullard A, Lazarus JM: Malnutrition in dialysis patients. *Am J Nephrol* 16:79–89, 1996
11. Ikizler TA, Hakim RM: Nutrition in end-stage renal disease. *Kidney Int* 50:343–357, 1996
12. Avram MM, Fein PA, Antigani A, Mittman N, Mushnick RA, Lustig AR, Lapuz MH, Goldwasser P: Cholesterol and lipid disturbances in renal disease: The natural history of uremic dyslipidemia and the impact of hemodialysis and continuous ambulatory peritoneal dialysis. *Am J Med* 87:55N–60N, 1989
13. Lowrie EG: Conceptual model for a core pathobiology of uremia with special reference to anemia, malnourishment, and mortality among dialysis patients. *Semin Dial* (in press)
14. Kopple J, Berg R, Houser H, Steinman TI, Teschan P: Nutritional status of patients with different levels of chronic renal insufficiency Modification of Diet in Renal Disease Study Group (MDRD). *Kidney Int* 36(Suppl 27):S184–S194, 1989
15. Beisel WR: Herman Award Lecture, 1995: Infection-induced malnutrition-from cholera to cytokines. *Am J Clin Nutr* 62:813–819, 1995
16. Langstein HN, Norton JA: Mechanisms of cancer cachexia. *Hematol/Oncol Clinics N Am* 5:103–122, 1991
17. Espat NJ, Copeland EM, Moldawer LL: Tumor necrosis factor and cachexia: A current perspective. *Surg Oncol* 3:255–262, 1994
18. Klasing KC: Nutritional aspects of leukocytic cytokines. *J Nutr* 118:1436–1446, 1988
19. Grimble RF: Cytokines: Their relevance to nutrition. *Eur J Clin Nutr* 43:217–230, 1989
20. Janeway CA, Travers P: Immunobiology, in *The Immune System in Health and Disease*, London/New York, Current Biology Ltd and Garland Publishing, 1994
21. Henry BJ: *Clinical Diagnosis and Management by Laboratory Methods*. Philadelphia, W.B. Saunders, 1991
22. Steel DM, Whitehead AS: The major acute phase reactants: C-reactive protein, serum amyloid P component and serum amyloid A protein. *Immunol Today* 15:81–88, 1994
23. Dowton SB, Colton HR: Acute phase reactants in inflammation and infection. *Semin Hematol* 25:84–90, 1988
24. Dinarello CA: Mechanisms of disease: Interleukin-1 and the pathogenesis of the acute phase response. *N Engl J Med* 311:1413–1418, 1984
25. Hart WH: C-reactive protein: The best laboratory indicator available for monitoring disease activity. *Cleveland Clin J Med* 56:126–130, 1989
26. Stahl WM: Acute phase protein response to tissue injury. *Crit Care Med* 15:545–550, 1987
27. *Applied Logistic Regression.* Edited by Hosmer DW, Lemeshow S, New York, John Wiley and Sons, 1989
28. Lee ET: *Statistical Methods for Survival Data Analysis*. Belmont, Lifetime Learning, 1980
29. Sternberg JC: A rate nelphelometer for measuring specific proteins by immunoprecipitin reactions. *Clin Chem* 23:1456–1464, 1977
30. Peterson PA Studies on interaction between prealbumin, retinol-binding protein and vitamin A. *J Biol Chem* 246:44–49, 1971
31. US Renal Data System: *USRDS 1996 Annual Data Report*, Bethesda, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, 1996
32. Bloembergen W, Held P, Port FK, Mauger RA, Wolfe RA: Causes of death in dialysis patients: Racial and gender differences. *J Am Soc Nephrol* 5:1231–1242, 1994
33. United States Renal Data System: Excerpts from the 1992 Annual Report: Comorbid conditions and correlations with mortality risk among 3399 incident hemodialysis patients. *Am J Kidney Dis* 20:32–38, 1992
34. Bergström J, Heimbürger O, Lindholm B, Qureshi AR: Elevated serum C-reactive protein is a strong predictor of increased mortality and low serum albumin in hemodialysis patients. (abstract) *J Am Soc Nephrol* 6:573, 1995
35. Ballmer PE, McNurlan MA, Grant I, Garlick PJ: Down-regulation of albumin synthesis in the rat by human recombinant interleukin-1 beta or turpentine and the response to nutrients. *J Parenteral Enteral Nutr* 19:266–271, 1995
36. Kaysen G, Rathore V, Shearer GC, Depner TA: Mechanism of hypoalbuminemia in hemodialysis patients. *Kidney Int* 48:510–516, 1995
37. Kaysen G, Stevenson FT, Depner TA: Determinants of albumin concentration in hemodialysis patients. *Am J Kidney Dis* 29:658–668, 1996
38. Macdougall IC, Allen DA, Tucker B, Baker LRI, Raine AEG: Serum interleukin-6 levels are useful indicators of marrow suppression in patients with resistance to erythropoietin due to inflammatory disease. (abstract) *J Am Soc Nephrol* 4:428, 1993
39. Roodman GD, Johnson RA, Clibon U: Tumor necrosis factor-181 and the anemia of chronic disease: Effects of chronic exposure to TNF on erythropoiesis in vivo. *Adv Exp Med Biol* 271:185–196, 1989
40. Muirhead N, Bargman J, Burgess E, Jindal KK, Levin N, Nolan L, Parfrey P: Evidence-based recommendations for the clinical use of recombinant human erythropoietin. *Am J Kidney Dis* 26(Suppl 1):S1–S24, 1995
41. Madore F, Bridges K, Brugnara C, Lew NL, Lowrie EG, Lazarus JM, Owen WF: Anemia in hemodialysis patients: Variables impacting this novel outcome predictor. *J Am Soc Nephrol* 8:1921–1929, 1997
42. Young EW, Bloembergen WE, Woods JD, Emmert G, Port FK, Wolfe RA, Jones CA, Held PJ: Iron use among erythropoietin treated U. S. hemodialysis patients. (abstract) *Kidney Int* (in press)
43. Cash JM, Sears DA: The anemia of chronic disease: Spectrum of associated diseases in a series of unselected hospitalized patients. *Am J Med* 87:638–644, 1989
44. Means RT, Drantz SB: Progress in understanding the pathogenesis of the anemia of chronic disease. *Blood* 80:1639–1647, 1992
45. Ifudu O, Feldman J, Friedman EA: The intensity of hemodialysis and the response to erythropoietin in patients with end-stage renal disease. *N Engl J Med* 334:420–425, 1996
46. Chertow GM, Ling J, Lew NL, Lazarus JM, Lowrie EG: The association of intradialytic parenteral nutrition administration with survival in hemodialysis patients. *Am J Kidney Dis* 24:912–920, 1994
47. Edozien JC, Rahim-Khan MA: Anemia in protein malnutrition. *Clin Sci* 34:315–326, 1968
48. MacDougall LG, Moodley G, Eyberg C, Quirk M: Mechanisms of anemia in protein-energy malnutrition in Johannesburg. *Am J Clin Nutr* 35:229–235, 1982
49. Wolfson M, Strong CJ, Minturn D, Gray DK, Kopple JD: Nutritional status and lymphocyte function in maintenance hemodialysis patients. *Am J Clin Nutr* 39:547–555, 1984
50. Bansal VK, Popli S, Pickering J, Ing TS, Vertuno LL, Hano JE: Protein-calorie malnutrition and cutaneous anergy in hemodialysis maintained patients. *Am J Clin Nutr* 33:1608–1611, 1980
51. Owen WF: Nutritional status and survival in ESRD patients, in *Mineral and Electrolyte Metabolism* (vol 23), edited by Massry S, Basel, Karger AG, 1997, pp 196–205