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Association of Serum Total Iron-Binding Capacity and Its Changes Over Time with Nutritional and Clinical Outcomes in Hemodialysis Patients

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Key Words

Transferrin · Total iron-binding capacity · Chronic kidney disease · Hemodialysis · Protein-energy wasting

Abstract

Serum transferrin, estimated by total iron-binding capacity (TIBC), may be a marker of protein-energy wasting (PEW) in maintenance hemodialysis (MHD) patients. We hypothesized that low TIBC or its fall over time is associated with poor clinical outcomes. In 807 MHD patients in a prospective 5-year cohort, associations of TIBC and its changes over time with outcomes were examined after adjustment for case-mix and markers of iron stores and malnutrition-inflammation including serum interleukin-6, iron and ferritin. Patients with serum TIBC ≥ 250 mg/dl had higher body mass index, triceps and biceps skinfolds and mid-arm muscle circumference and higher serum levels of iron but lower ferritin and inflammatory markers. Some SF-36 quality of life (QoL) components were worse in the lowest and/or highest TIBC groups. Mortality was incrementally higher in lower TIBC levels (p-trend < 0.001). Adjusted death hazard ratio was 1.75 (95% CI: 1.00–3.05, $p = 0.05$) for TIBC < 150 compared to TIBC of 200–250 mg/dl. A fall in TIBC > 20 mg/dl over 6 months was associated with a death hazard ratio of 1.57 (95% CI: 1.04–

2.36, $p = 0.03$) compared to the stable TIBC group. Hence, low baseline serum TIBC is associated with iron deficiency, PEW, inflammation, poor QoL and mortality, and its decline over time is independently associated with increased death risk.

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Introduction

Many individuals with advanced chronic kidney disease (CKD) including those undergoing maintenance hemodialysis (MHD) treatment suffer from protein-energy wasting (PEW), also referred to as uremic malnutrition [1, 2]. PEW and inflammation, which often occur together in CKD and which are hence referred to collectively as the malnutrition-inflammation complex (or cachexia) syndrome (MICS), are associated with poor quality of life and increased morbidity and mortality [3–6]. Examining the relative contribution of the various components of the MICS to poor outcomes may lead to discovering modifiable mechanisms that underlie poor CKD survival. However, it is not known what nutritional or inflammatory markers are more appropriate or robust for studying the MICS-mortality association.

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The International Society of Renal Nutrition and Metabolism (ISRNM) Expert Panel has recently suggested a series of criteria to assess nutritional status and inflammation in CKD [2]. Among the biochemical markers, a low level of serum transferrin, which is a traditional marker of nutritional status and which can be measured directly via conventional radioimmunoassays (RIA), is suggested as a potential indicator of PEW [2]. In CKD patients, however, serum transferrin concentration is usually assessed indirectly by measuring serum total iron-binding capacity (TIBC) [7–9]. There is a high statistical correlation between RIA-measured serum transferrin and iron-binding-based measured TIBC with a correlation coefficient R^2 of 0.95 or greater [7].

Transferrin is a 90-kDa beta-globulin that binds and transports iron in the plasma [10]. The liver is the primary site of synthesis. Low values are found in many disease states including infection, malignancy, hepatocellular disease, and nephrotic syndrome [9]. In the general population, increased serum transferrin values occur in iron deficiency, pregnancy and other high estrogen and progesterone states including administration of oral contraceptives [11]. However, in CKD patients, iron deficiency may be paradoxically associated with lower TIBC values [7, 12]. TIBC is indeed the denominator of the ‘iron saturation ratio’, i.e. iron divided by TIBC [12]. Despite being suggested as a marker of PEW by the ISRNM consensus committee [2], it is not clear whether the TIBC calculated transferrin is an independent predictor of mortality and other outcomes in CKD patients. Moreover, the association between TIBC and other clinical outcomes such as health-related quality of life (HR-QoL) has not yet been studied.

Given the fact that TIBC is measured monthly in virtually all 400,000 dialysis patients in the United States to calculate the iron saturation ratio, we sought to examine the utility of serum TIBC and its changes over time in assessing the nutritional status and in predicting HR-QoL and mortality after controlling for iron markers and surrogates of nutritional status and inflammation including circulating levels of pro-inflammatory cytokines. We analyzed the data from a well-studied National Institute of Health (NIH) sponsored cohort of 807 MHD patients who were followed for up to 5 years and in whom repeated nutritional and inflammatory measures including cytokines, body composition and HR-QoL were assessed. We hypothesized that TIBC is an independent outcome predictor in MHD patients and that its changes over time have a bearing on relevant clinical outcomes.

Methods

Subjects and Setting

We studied MHD patients who were participating in the Nutritional and Inflammatory Evaluation in Dialysis (NIED) Study [13]. The original patient cohort was derived from a pool of approximately 1,300 MHD outpatients in eight DaVita, Inc., chronic dialysis facilities in the South Bay Los Angeles area (see NIED Study website at www.NIEDStudy.org for more details). Inclusion criteria were outpatients who had been undergoing MHD for at least 8 weeks, were 18 years or older and who signed a local Institutional Review Board approved consent form. Patients with an anticipated life expectancy of less than 6 months (for example, due to a metastatic malignancy or advanced HIV/AIDS disease) were excluded. From October 1, 2001, through December 31, 2006, 893 MHD patients from eight DaVita dialysis facilities in the Los Angeles South Bay area signed the informed consent form and underwent the periodic evaluations of the NIED Study. For these analyses, data including baseline serum TIBC, to reflect serum transferrin, were available in 807 MHD patients.

The medical chart of each MHD patient was thoroughly reviewed by a collaborating physician, 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 comorbidities [14, 15]. The 807 MHD patients were followed for up to 63 months, i.e. until December 31, 2006.

Health-Related Quality of Life Scores

The SF36, a short form HR-QoL scoring system with 36 items, which includes eight independent scales, is a well-documented, self-administered questionnaire and has been widely used and validated in MHD patients [6, 16]. The eight scales of SF36 are summarized into two dimensions: ‘Physical health’ and ‘Mental health’.

Anthropometric Measures

Body weight assessment and anthropometric measurements were performed while patients were undergoing a hemodialysis treatment or within 5–20 min after termination of the treatment. Biceps skinfold and triceps skinfold thicknesses were measured with a conventional skinfold caliper using standard techniques as previously described [17, 18].

Near Infrared Interactance

To estimate the percentage of body fat and fat-free body mass, near infrared (NIR) interactance technology was utilized at the same time as the anthropometric measurements [19, 20]. A commercial near-infrared interactance sensor with a coefficient of variation of 0.5% for total body fat measurement (portable Futrex 6100[®], Gaithersburg, Md., USA, www.futrex.com) was used. NIR measurements were performed by placing a Futrex[®] sensor on the nonaccess upper arm for several seconds, and entering the required data (date of birth, gender, weight and height) of each patient. NIR measurements of body fat have been shown to correlate significantly with other nutritional measures in MHD patients [20].

Laboratory Tests

Predialysis blood samples and postdialysis serum urea nitrogen were obtained on a mid-week day, which coincided chronologically with the drawing of quarterly blood tests in the DaVita facilities. The single-pool Kt/V was used to represent the weekly dialysis dose. All routine laboratory measurements were performed by DaVita® Laboratories (Deland, Fla., USA) using automated methods. TIBC was measured using the summation of a measured serum total iron and a measured unbound iron-binding capacity (UIBC), which is measured spectrophotometrically. The test principle uses a known concentration of iron to saturate the available binding sites on the transferrin [21]. A final reaction occurs using excess unbound divalent iron with a FerroZine chromogen to form a magenta complex that is measured.

Serum high sensitivity C-reactive protein (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 (manufacturer: WPCI, Osaka, Japan, unit: mg/l, normal range: <3.0 mg/l) [22, 23]. Interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF- α) were measured with immunoassay kits based on a solid-phase sandwich ELISA using recombinant human IL-6 and TNF- α (manufacturer: R&D Systems, Minneapolis, Minn., USA; units: pg/ml; normal range: IL-6: <9.9 pg/ml, TNF- α : <4.7 pg/ml) [24, 25]. CRP and the cytokines were measured in the General Clinical Research Center Laboratories of Harbor-UCLA Medical Center. Plasma total homocysteine concentrations were determined by high-performance liquid chromatography in the Harbor-UCLA Clinical Laboratories. Serum transthyretin (prealbumin) was measured using immunoprecipitin analysis [26].

Statistical Methods

Pearson's correlation coefficient (r) was used for analyses of linear associations. Simple linear regression and χ^2 test for trend were used to examine trends of continuous and dichotomous variables, respectively, across the four groups of TIBC. Multivariate regression analyses and analysis of covariance were performed to obtain adjusted p values controlled for case-mix and other covariates. A restricted cubic splines graph was utilized as an exploratory data analysis (EDA) strategy to illustrate systematic relations between serum TIBC and mortality. This method also served to examine the non-linear associations as continuous mortality predictors as an alternative to inappropriate linearity assumptions [27]. Thereafter, to calculate the relative risks of death, hazard ratios (HR) were obtained using Cox proportional hazard models after controlling for the relevant covariates. Plots of $\log[-\log(\text{survival rate})]$ against $\log(\text{survival time})$ were performed to establish the validity of the proportionality assumption. Kaplan-Meier analyses were utilized to assess the differences in surviving proportions between categories of TIBC change. Case-mix and comorbidity covariates included gender, age, race and ethnicity (Hispanics, African-Americans, Asians and others), diabetes mellitus, the modified Charlson comorbidity scale, dialysis vintage (number of months on MHD treatment), and serum iron; and laboratory measures of the MICS in fully adjusted Cox models included serum CRP, IL-6, and albumin concentrations. To mitigate the regression to the mean phenomenon, baseline serum TIBC was included as a covariate in all Cox proportional hazard models utilized to assess the death HRs of TIBC change. Fiducial limits are given as mean \pm SD or median and

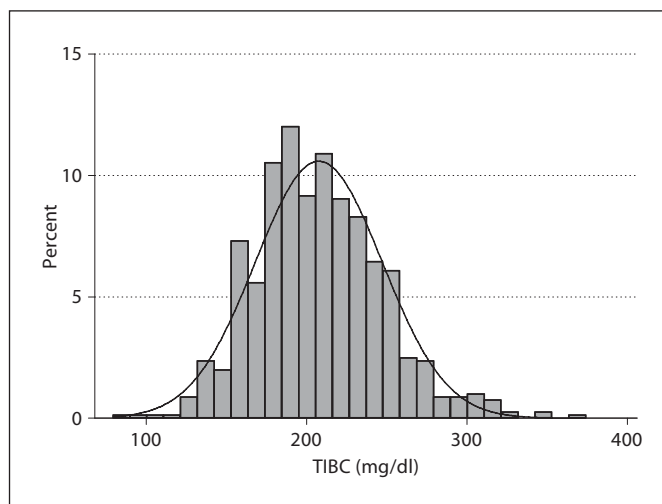


Fig. 1. Distribution of the baseline serum TIBC in 807 maintenance hemodialysis patients.

interquartile range, when appropriate; risk ratios include 95% CI levels. $p < 0.05$ or a 95% CI that did not span 1.0 was considered to be statistically significant. A p value between 0.05 and 0.20 is also listed with 2 decimals to identify potential type II errors. Descriptive and multivariate statistics were carried out with the statistical software 'Stata version 10.0' (Stata Corporation, College Station, Tex., USA).

Results

General and Laboratory Characteristics

Baseline demographic, clinical, and laboratory values in the 807 MHD patients under study are shown in table 1. Patients' mean age (\pm SD) was 53.6 ± 14.8 years; 47% of patients ($n = 379$) were women, 50% ($n = 406$) were Hispanic and 32% ($n = 257$) were African-American. The mean dialysis vintage was 31 ± 34 months (median: 19.2, inter-quartile range: 7–46 months). As depicted in figure 1, the average baseline serum TIBC in the 807 MHD patients was 208 ± 40 mg/dl (median: 205, minimum: 79, maximum: 374, inter-quartile range: 181–232 mg/dl).

We selected four a priori categories of serum TIBC levels with 50-mg/dl increments based on the practical clinical utility of the cutoff levels and as suggested in the malnutrition-inflammation score (MIS) by Kalantar-Zadeh et al. [4] <150 mg/dl ($n = 39$, 5%), 150 to <200 mg/dl ($n = 330$, 41%), 200 to <250 mg/dl ($n = 326$, 40%) and ≥ 250 mg/dl ($n = 112$, 14%). Table 1 shows the relevant demographic,

Table 1. Baseline demographic, clinical, and laboratory values in total and according to the four a priori selected groups of TIBC in 807 maintenance hemodialysis patients

Variable	TIBC, mg/dl					p for trend
	total (n = 807)	<150 (n = 39)	150 to <200 (n = 330)	200 to <250 (n = 326)	≥250 (n = 112)	
<i>Demographic</i>						
Age, years	53.7 ± 14.7	53.7 ± 16.4	54.8 ± 14.7	53.4 ± 14.3	51.5 ± 14.8	0.06
Women, %	47	51	51	45	38	0.01
Marital status: % married	47	35	41	53	51	0.007
Race: % African-American	32	41	34	31	25	0.03
Ethnicity: % Hispanic	50	56	51	48	53	0.71
Primary insurance: % Medicare	52	53	53	51	54	0.91
Diabetes mellitus, %	56	49	59	53	58	0.79
Charlson comorbidity score	1.9 ± 1.6	1.6 ± 1.5	2.0 ± 1.6	1.8 ± 1.7	1.9 ± 1.6	0.77
Mortality, %	30	51	33	27	20	<0.001
<i>Body composition</i>						
Body mass index	26.5 ± 6.1	24.6 ± 5.3	26.2 ± 6.1	26.8 ± 6.1	27.2 ± 6.5	0.03
Triceps skinfold, mm	17.6 ± 10.0	14.0 ± 9.0	17.6 ± 9.8	17.4 ± 10.1	19.7 ± 10.4	0.03
Biceps skinfold, mm	9.9 ± 7.9	8.6 ± 7.6	9.6 ± 7.4	10.0 ± 7.9	11.1 ± 9.4	0.06
Mid-arm muscle circumference, cm	25.9 ± 4.4	25.0 ± 3.8	25.7 ± 4.7	26.3 ± 3.9	26.2 ± 4.9	0.08
Near infrared measured body fat, %	26.6 ± 10.8	24.6 ± 10.8	26.6 ± 10.9	26.6 ± 10.8	27.0 ± 10.9	0.48
<i>Hemodialysis treatment measures</i>						
Dialysis vintage, months	31.1 ± 33.7	51.0 ± 36.7	35.6 ± 33.6	27.8 ± 34.2	20.2 ± 26.3	<0.001
Dialysis dose, Kt/V single pool	1.61 ± 0.31	1.59 ± 0.32	1.61 ± 0.30	1.60 ± 0.31	1.63 ± 0.32	0.69
nPCR, g·kg ⁻¹ ·day ⁻¹	1.06 ± 0.24	0.98 ± 0.22	1.05 ± 0.23	1.09 ± 0.24	1.07 ± 0.26	0.03
Erythropoietin dose, 1,000 U/week	14.5 ± 12.6	22.5 ± 18.5	14.7 ± 12.7	13.5 ± 11.6	14.4 ± 12.2	0.026
<i>Biochemical measurements</i>						
Albumin, g/dl	3.88 ± 0.38	3.55 ± 0.48	3.81 ± 0.39	3.98 ± 0.33	3.97 ± 0.34	<0.001
Transthyretin (prealbumin), mg/dl	28.1 ± 9.7	20.9 ± 8.0	27.4 ± 8.8	29.6 ± 10.3	27.8 ± 9.4	0.001
Creatinine, mg/dl	10.2 ± 3.3	10.0 ± 3.8	10.5 ± 3.3	10.3 ± 3.2	9.5 ± 3.1	0.06
Calcium, mg/dl	9.4 ± 0.7	9.1 ± 0.7	9.4 ± 0.7	9.4 ± 0.7	9.4 ± 0.7	0.07
Iron, mg/dl	66.6 ± 26.9	46.6 ± 18.7	58.6 ± 21.1	72.4 ± 26.4	80.4 ± 33.9	<0.001
Phosphorus, mg/dl	5.8 ± 1.5	6.2 ± 2.2	5.7 ± 1.5	5.8 ± 1.4	5.8 ± 1.3	0.93
Ferritin, ng/ml	571 ± 437	788 ± 420	638 ± 392	556 ± 461	348 ± 411	<0.001
Bicarbonate, mg/dl	22.2 ± 2.9	22.5 ± 3.1	22.4 ± 2.8	22.1 ± 2.8	22.0 ± 3.2	0.11
Total homocysteine, μmol/l	23.8 ± 11.0	21.7 ± 12.2	24.6 ± 11.7	23.6 ± 10.7	22.8 ± 9.5	0.44
C-reactive protein, mg/l	5.7 ± 6.9	7.9 ± 5.5	6.2 ± 8.4	5.5 ± 5.8	4.2 ± 4.1	0.001
IL-6, pg/ml	18.4 ± 51.5	35.4 ± 66.4	18.9 ± 51.2	17.8 ± 55.8	12.6 ± 25.5	0.06
TNF-α, pg/ml	8.9 ± 12.0	9.2 ± 11.2	8.4 ± 10.9	8.6 ± 10.5	11.0 ± 17.8	0.18
Blood hemoglobin, g/dl	12.1 ± 1.0	11.6 ± 1.4	11.9 ± 1.0	12.2 ± 0.8	12.2 ± 1.1	<0.001
WBC, × 1,000 cells/μl	7.2 ± 2.0	7.8 ± 3.2	7.2 ± 2.0	7.1 ± 1.9	7.3 ± 1.9	0.55
Lymphocytes, % of total WBC	22.7 ± 7.8	21.0 ± 8.3	22.2 ± 7.9	23.1 ± 7.7	23.6 ± 7.9	0.02

All values are presented as mean ± SD or percentages. Kt/V = Dialysis dose.

* p values for dialysis dose (vintage), ferritin, erythropoietin dose, CRP, IL-6, and TNF-α are based on the logarithmic values of these measures.

clinical and laboratory measures the four categories of TIBC. The proportion of women and African-American was higher in the groups with lower TIBC levels; however, there was no trend in terms of having diabetes mellitus and

other comorbidities according to the modified Charlson comorbidity score. In addition, the groups of patients with lower serum TIBC had higher dialysis vintage in contrast to their counterparts with higher serum TIBC levels.

Table 2. Baseline SF-36¹ health-related quality of life scores across TIBC increments in 624² maintenance hemodialysis patients

Variable	TIBC, mg/dl				p for trend
	<150 (n = 28)	150 to <200 (n = 254)	200 to <250 (n = 252)	≥250 (n = 90)	
SF-36 overall					
SF-36 total score	48 ± 21	52 ± 21	51 ± 21	49 ± 21	0.50
SF-36 dimensions					
SF-36 mental health	51 ± 22	55 ± 20	53 ± 20	53 ± 20	0.96
SF-36 physical health	45 ± 20	48 ± 23	47 ± 21	44 ± 21	0.31
SF-36 scales					
Body pain	55 ± 29	60 ± 28	59 ± 29	57 ± 30	0.47
General health	44 ± 23	45 ± 23	46 ± 21	42 ± 20	0.81
Mental health	61 ± 23	68 ± 21	66 ± 19	66 ± 21	0.82
Physical function	38 ± 26	47 ± 30	47 ± 30	45 ± 29	0.99
Role emotional	42 ± 45	52 ± 43	45 ± 43	47 ± 42	0.53
Role physical	38 ± 40	40 ± 42	35 ± 42	26 ± 36	0.02
Functionality	60 ± 28	63 ± 27	62 ± 28	62 ± 28	0.71
Vitality	49 ± 24	48 ± 23	49 ± 22	48 ± 22	0.80

¹ Short form quality of life score with 36 questions.

² Of all the 807 patients under study, data of quality of life were available for 624 patients.

Even though MHD patients with lower TIBC levels had lower BMI and triceps and biceps skinfold thicknesses, and, to a lesser extent, had a lower mid-arm muscle circumference, there were no remarkable differences between the four groups of serum TIBC levels with regard to near infrared measured total body fat. Among laboratory measures, serum albumin, transthyretin (pre-albumin), iron, ferritin, and blood hemoglobin levels were lower in the groups with lower TIBC; however, in these same patients, measures of inflammation (i.e. serum CRP and IL-6) were higher.

Health-Related Quality of Life

Table 2 shows the averaged crude (nonstandardized) scores of SF-36 among 624 MHD patients who answered this HR-QoL questionnaire. The scores are grouped according to the selected serum TIBC increments presented in table 1. There were no statistically significant associations in most scales and the two dimensions of the SF-36, but there were some trends towards lower HR-QoL scores in the highest and/or lowest TIBC groups.

Linear Associations

Table 3 shows the correlation coefficients of relevant clinical, nutritional and inflammatory measures with se-

rum TIBC levels in 807 MHD patients. Serum TIBC was correlated with the nPNA (nPCR), a surrogate of dietary protein intake as well as serum albumin, transthyretin, ferritin, iron, and logarithms of CRP and IL-6. However, the linear association between serum TIBC and transthyretin no longer persisted after adding serum albumin to the covariates adjusted. Figure 2 demonstrates the positive linear association between serum TIBC and albumin as well as iron, and also, illustrates that when the level of inflammatory markers in serum is increased, the level of serum TIBC decreases.

TIBC and Survival

Over the 5 years that the cohort was monitored, 241 (30%) patients died, 95 (12%) underwent transplantation, and 153 (20%) left the cohort without further follow-up information. Figure 3 shows the cubic splines graph illustrating the unadjusted and case-mix adjusted associations between baseline serum TIBC and mortality in the 5-year cohort of 807 MHD patients. A somewhat robust trend towards increased risk of death was observed in the MHD patients especially with lower TIBC levels. Figure 4 shows the hazard ratios (HR) of mortality for the four a priori selected groups of serum TIBC levels after stepwise adjustments. The calculated death hazard ratios are shown

Table 3. Unadjusted and multivariate adjusted Pearson's correlation coefficient of baseline TIBC and other relevant variables in 807 maintenance hemodialysis patients

Variable	Unadjusted	Case-mix ¹ adjusted	Case-mix + albumin adjusted	Case-mix + albumin + inflammatory markers (full model) ²
General				
Age	-0.07*	-0.09*	-0.02	-0.01
Charlson comorbidity score	-0.01	-0.01	0.04	0.05 ^o
Nutritional				
Body mass index	0.07 ^o	0.10**	0.10**	0.14***
Creatinine	-0.05 ^o	0.02	-0.05 ^o	-0.07 ^o
Triceps skinfold	0.09*	0.14***	0.14***	0.16***
Biceps skinfold	0.08*	0.13**	0.13***	0.16***
Mid-arm muscle circumference	0.06 ^o	0.08*	0.09*	0.10*
Near infrared measured body fat	0.09	0.11**	0.11**	0.14***
Fat weight	0.05*	0.10**	0.11**	0.15***
Lean weight	0.09*	0.09*	0.11**	0.14***
nPCR	0.11**	0.08*	0.04	0.02
Laboratory				
Albumin	0.28***	0.31***	-	0.23***
Prealbumin (transthyretin)	0.13***	0.11**	-0.01	-0.03
Creatinine	-0.05 ^o	0.02	-0.05 ^o	-0.07 ^o
Erythropoietin dose	-0.09**	-0.08*	-0.02	0.01
Ferritin	-0.25***	-0.20***	-0.21***	-0.22***
Iron	0.35***	0.32***	0.27***	0.25***
Calcium	0.08*	0.13***	0.03	0.04
Phosphorus	0.00	0.02	0.03	0.04
Homocysteine	-0.02	0.03	-0.01	-0.02
Inflammatory				
log C-reactive protein	-0.17***	-0.16***	-0.12**	-0.11*
log IL-6	-0.17***	-0.14***	-0.06 ^o	-0.03
log TNF-α	-0.02	0.00	0.00	0.01

¹ Case-mix variables include age, gender, race/ethnicity, diabetes, dialysis vintage, insurance (medicare), marital status, modified Charlson comorbidity score, dialysis dose (Kt/V), and kidney residual urine (KRU).

² Full model consist of case-mix variables, albumin, and three inflammatory markers: C-reactive protein, interleukin-6, tumor necrosis factor-α.

^o p value 0.20 to 0.05; * p value 0.05 to 0.01; ** p value 0.01 to 0.001; *** p value <0.001.

Bold = Correlation coefficient >0.10.

in table 4. A serum TIBC below 150 mg/dl (5% of patients) was associated with significantly increased risk of death in the unadjusted and case mix and case mix plus MICS adjusted analyses ($p < 0.001$ to $p < 0.05$), although the p value increased to 0.24 after full multivariate adjustment that included all nutritional and inflammatory markers including serum albumin, CRP, TNF-α and IL-6.

Changes in TIBC and Survival

In order to examine whether longitudinal changes in serum TIBC over time affected survival in MHD patients

whose serum TIBC was remeasured after 6 months ($n = 625$), the magnitude and direction of change in TIBC were calculated. Patients with drop in serum TIBC greater than 20 mg/dl over the first 6 months of observation had a 57% increased risk of death compared to those with almost stable TIBC values (table 5). As shown in figure 5, the Kaplan-Meier survival curves for the three groups of changes in serum TIBC levels show a lower survival in patients with a fall in serum TIBC greater than 20 mg/dl over 6 months.

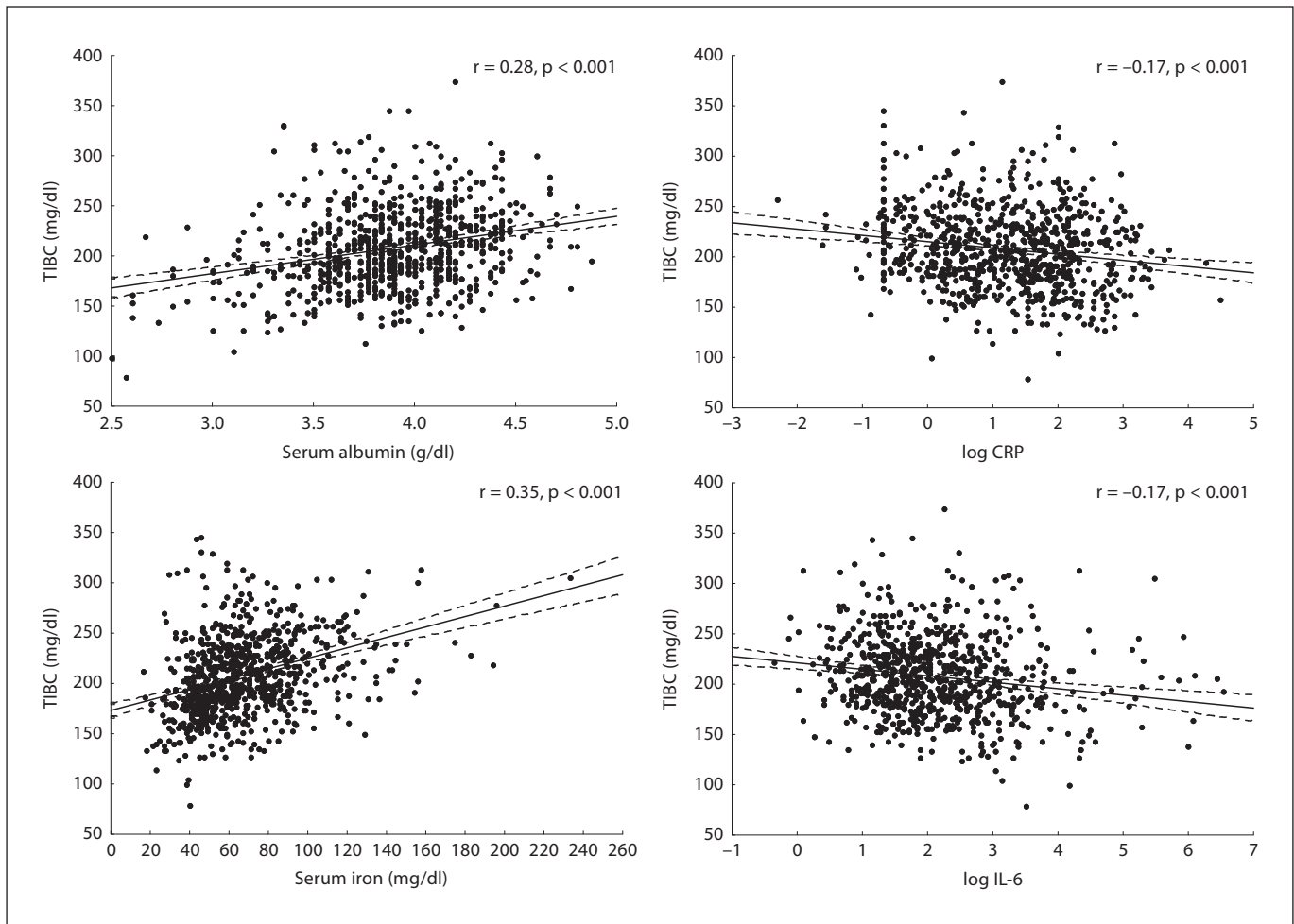


Fig. 2. Scatter plots, regression line and 95% CIs, reflecting the correlation between TIBC and baseline serum albumin, serum iron, log for CRP, and log for IL-6.

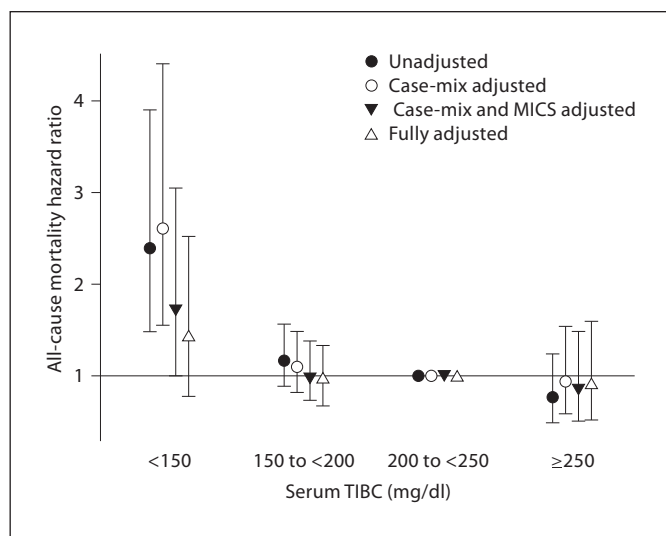
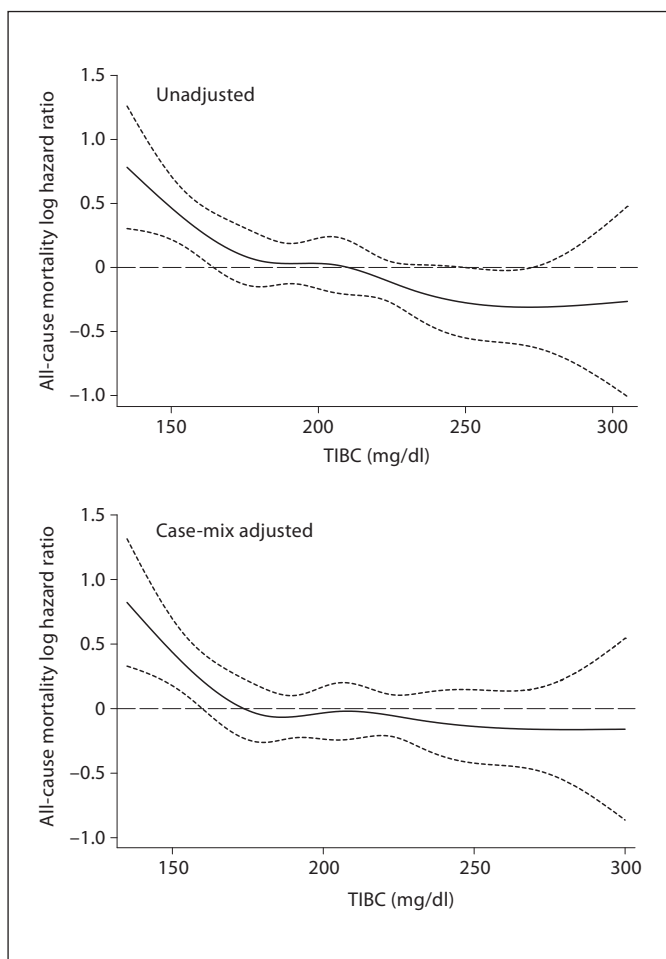
Discussion

We found that serum TIBC, an indirect measurement of serum transferrin concentration, is correlated with several markers of nutrition and inflammation in 807 MHD patients who participated in a prospective 5-year study in Southern California. Patients with lower TIBC levels, i.e. <150 mg/dl, had lower BMI, triceps and biceps skin folds and mid-arm muscle circumference compared to those with higher serum TIBC. A trend of low self-reported HR-QoL and poor survival was observed with the lowest and/or highest serum TIBC concentrations even after exhaustive multivariate adjustment for other surrogates of malnutrition and inflammation. A fall in serum TIBC beyond 20 mg/dl over 6 months was associated with 57% increased risk of death in the subsequent years.

These findings may indicate that serum TIBC is a useful and robust marker of nutritional status with clinically relevant associations with inflammatory markers, iron stores and survival and that its longitudinal changes over time can modify the risk of death in MHD patients.

Individuals with CKD have an exceptionally high mortality rate and a high burden of cardiovascular disease [26]. About one of every 5 of the 400,000 MHD patients in the United States die every year [28]. Even though half of all these deaths are attributed to cardiovascular disease [28], measures of PEW, and not traditional mortality risk factors, are the strongest predictors of mortality in MHD patients [2]. Therefore, reliable markers of PEW with stronger and more robust associations with morbidity and mortality in MHD patients are needed, so that patients at risk can better be identi-

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Fig. 3. Unadjusted (upper panel) and case-mix adjusted (lower panel) spline models with 95% CI reflecting mortality predictability of serum TIBC in 807 maintenance hemodialysis patients (Oct. 2001 to Jan. 2007).

Fig. 4. Mortality predictability of serum TIBC in 807 maintenance hemodialysis patients (Oct. 2001 to Jan. 2007). Case-mix variables: age, gender, race/ethnicity, diabetes mellitus, dialysis vintage, primary insurance, marital status, Charlson co-morbidity score, dialysis dose (Kt/V), kidney residual urine (KRU), and serum iron. MICS variables: albumin, erythropoietin dose, creatinine, hemoglobin, phosphorus, normalized protein catabolic rate (nPCR), bicarbonate, calcium, ferritin, WBC, lymphocyte percentage, body mass index, and vitamin D dose. Inflammatory variables: CRP, IL-6, TNF- α .

fied for focused nutritional and anti-inflammatory interventions.

We found that serum TIBC had the strongest positive correlation with serum iron and negative correlation with serum ferritin concentrations (table 3; fig. 2). The positive association of serum TIBC with iron in our current study, as well as in previous studies [7], appears to be in sharp contradistinction to its known reverse association with iron in the general population. Indeed, in the non-CKD populations, iron deficiency is associated with concurrent decreased serum iron and increased TIBC levels, so that iron divided by TIBC, also known as iron saturation ratio, is usually quite low [12]. In dialysis patients, on the contrary, TIBC could be low in the setting of malnutrition, inflammation or iron deficiency. Hence, use of traditionally calculated iron saturation ratio, i.e., iron divided by TIBC, to rule out iron deficiency may be misleading, since this simple algebraic fraction may erroneously be normal to high in the setting of MICS or

even true iron deficiency. Furthermore, the negative association of TIBC with ferritin, a positive acute-phase reactant, underscores the hypothesis that TIBC is a negative acute-phase reactant.

In our study, the associations between baseline serum TIBC and death were attenuated after multivariate adjustment, especially after exhaustive adjustment for a number of markers of iron stores, nutrition and inflammation. This may be due to the collinearity between serum transferrin, as a nutritional marker and possibly a negative acute-phase reactant per se, and other markers of MICS. However, the fall in transferrin over time remained a robust predictor of worsening risk of death even after exhaustive multivariate adjustment for serum albumin, CRP, IL-6 and other covariates. Our study also examined the mortality predictability of longitudinal changes in serum TIBC over time. Patients whose serum TIBC decreased after 6 months subsequently had a 57% higher risk of death compared to those with stable serum

Table 4. Hazard ratios (HRs) of 5-year mortality according to the four a priori selected groups of TIBC in 807 maintenance hemodialysis patients (Oct. 2001 to Jan. 2007)

TIBC group	Proportional hazard model									
	unadjusted		case-mix ¹ adjusted		case-mix + albumin adjusted		case-mix + MICS ² adjusted		case-mix + MICS + inflammation adjusted (full model) ³	
	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p
<150 mg/dl	2.4 (1.5–3.9)	<0.001	2.6 (1.6–4.4)	<0.001	1.8 (1.1–3.1)	0.03	1.8 (1.0–3.1)	0.05	1.4 (0.8–2.5)	0.24
150 to <200 mg/dl	1.2 (0.9–1.6)	0.24	1.1 (0.8–1.5)	0.50	1.0 (0.7–1.4)	0.99	1.0 (0.7–1.4)	0.97	1.0 (0.7–1.3)	0.78
200 to <250 mg/dl	1.0 (reference)		1.0 (reference)		1.0 (reference)		1.0 (reference)		1.0 (reference)	
≥250 mg/dl	0.8 (0.5–1.2)	0.29	0.9 (0.6–1.5)	0.85	0.9 (0.6–1.5)	0.81	0.9 (0.5–1.5)	0.62	0.9 (0.5–1.6)	0.76

¹ Case-mix variables include age, gender, race/ethnicity, diabetes, dialysis vintage, insurance (medicare), marital status, Charlson comorbidity score, dialysis dose (log Kt/V), kidney residual urine (KRU), and serum iron.

² MICS variables include albumin, log erythropoietin dose, creatinine, hemoglobin, phosphorus, normalized protein catabolic rate (nPCR), bicarbonate, calcium, log ferritin, WBC, lymphocyte percent, body mass index, log vitamin D dose.

³ Full model consists of case-mix variables, MICS, and three inflammatory markers: CRP, IL-6, and TNF-α.

Table 5. Hazard ratios (HRs) of 5-year mortality according to the three a priori selected groups of change in TIBC in 625 maintenance hemodialysis patients (Oct. 2001 to Jan. 2007)

Change in TIBC	Adjusted for baseline serum TIBC		Baseline serum TIBC + case-mix ¹ adjusted		Baseline serum TIBC + case-mix + albumin adjusted		Baseline serum TIBC + case-mix + MICS ² adjusted		Baseline serum TIBC + case-mix + MICS + inflammation adjusted (full model) ³	
	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p
<-20 mg/dl (n = 151)	1.6 (1.1–2.3)	0.01	1.4 (0.95–2.0)	0.09	1.4 (1.0–2.0)	0.07	1.5 (1.0–2.3)	0.03	1.6 (1.1–2.4)	0.02
-20 to <+20 mg/dl (n = 368)	1.0 (reference)		1.0 (reference)		1.0 (reference)		1.0 (reference)		1.0 (reference)	
≥+20 mg/dl (n = 106)	0.9 (0.6–1.4)	0.84	1.0 (0.7–1.5)	0.99	0.9 (0.6–1.4)	0.73	1.0 (0.6–1.6)	0.98	0.9 (0.6–1.4)	0.64

¹ Case-mix variables include age, gender, race/ethnicity, diabetes, dialysis vintage, insurance (medicare), marital status, Charlson comorbidity score, dialysis dose (log Kt/V), kidney residual urine (KRU), and serum iron.

² MICS variables include albumin, log erythropoietin dose, creatinine, hemoglobin, phosphorus, normalized protein catabolic rate (nPCR), bicarbonate, calcium, log ferritin, white blood count (WBC), lymphocyte percent, body mass index, log vitamin D dose.

³ Full model consists of case-mix variables, MICS, and three inflammatory markers: CRP, IL-6, and TNF-α.

TIBC (table 5; fig. 5). Although the opposite association, i.e. between a rise in TIBC and improved survival, was not evident except for a lower mortality rate observed in the Kaplan-Meier graphs (fig. 5). These general findings may imply that monitoring changes in serum TIBC can help identify patients at risk, who may benefit from nutritional interventions, especially if the TIBC fall is greater than 20 mg/dl over 6 months. To our knowledge, this is the first study that has examined outcome predictability of both baseline TIBC and its changes over time and the confounding effect of other measures of inflammation and wasting on these associations.

A potential limitation of the present study is a selection bias during enrollment [13]. However, since the mortality in our cohort was less than the base population, it might be argued that a selection bias with such a direction generally would lead to a bias toward the null hypothesis, so without this bias, our positive results may have been even stronger. The strengths of our study include the sample size, which was moderately large, the comprehensive clinical and laboratory evaluations with repeated measures of serum TIBC, concomitant assessment of HR-QoL and body composition measures, and detailed evaluation of comorbid states by study physicians at baseline. Unlike previous cohorts that have been

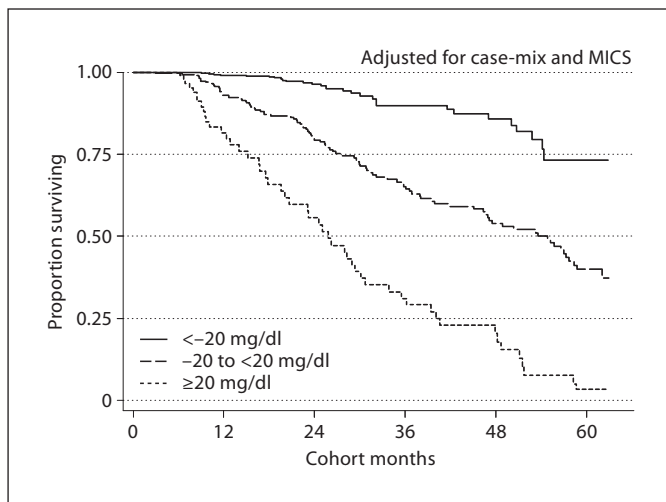


Fig. 5. Kaplan-Meier proportion of surviving MHD patients after 5 years of observation according to the three a priori selected groups of changes in TIBC during the first 6 months of observation in 625 MHD patients (adjusted for case-mix and MICS variables). Case-mix variables: age, gender, race/ethnicity, diabetes mellitus, dialysis vintage, primary insurance, marital status, Charlson co-morbidity score, dialysis dose (Kt/V), and kidney residual urine (KRU), and serum iron. MICS variables: albumin, erythropoietin dose, creatinine, hemoglobin, phosphorus, normalized protein catabolic rate (nPCR), bicarbonate, calcium, ferritin, WBC, lymphocyte percentage, body mass index, and vitamin D dose.

studied, ours has been extensively characterized for markers of inflammation and nutritional status, including direct total body fat measurements. The availability of these measures allowed us to demonstrate that the serum transferrin concentration was strongly associated with mortality risk independent of influences from other known inflammatory markers or comorbid states in this group of MHD patients. Another strength of this cohort is that the subjects were selected randomly without having any prior knowledge of their inflammation status. Finally, the very same blood specimens that were utilized to measure markers of PEW and cytokines were also used for the TIBC measurements.

In conclusion, we found that serum transferrin, as measured by TIBC, correlated with several surrogates of body composition and several components of HR-QoL in MHD patients and that a low baseline serum transferrin (i.e. <150 mg/dl) and especially its decline over time was independently associated with a trend towards increased risk of death. These data may also justify the inclusion of serum TIBC or transferrin as one of the 10 components

of the ‘Malnutrition-Inflammation Score’ [4]. Understanding the role of TIBC or transferrin in the MHD population may lead to more useful strategies to identify patients at risk of PEW and to the development of focused nutritional interventions to improve nutritional status and, hence, survival in almost half a million dialysis patients and over the 20 million individuals with CKD in the United States and many more throughout the world.

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Potential Conflict of Interests

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