Serum β-Trace Protein and Risk of Mortality in Incident Hemodialysis Patients

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Summary

Background and objectives Residual kidney function in dialysis patients is associated with better survival, but there are no simple methods for its assessment. β-Trace protein is a novel endogenous filtration marker of kidney function that is not removed during hemodialysis and may serve as a marker for residual kidney function similar to serum creatinine in patients not on dialysis. The objective of this study was to determine the association of serum β-trace protein with mortality in incident hemodialysis patients.

Design, setting, participants, & measurements Serum β-trace protein was measured in baseline samples from 503 participants of a national prospective cohort study of incident dialysis patients with enrollment during 1995–1998 and follow-up until 2004. Outcomes were all-cause and cardiovascular disease mortality analyzed using Cox regression adjusted for demographic, clinical, and treatment factors.

Results Serum β-trace protein levels were higher in individuals with no urine output compared with individuals with urine output (9.0±3.5 versus 7.6±3.1 mg/L; $P<0.001$). There were 321 deaths (159 deaths from cardiovascular disease) during follow-up (median=3.3 years). Higher β-trace protein levels were associated with higher risk of mortality. The adjusted hazard ratio and 95% confidence interval for all-cause mortality per doubling of serum β-trace protein was 1.36 (1.09–1.69). The adjusted hazard ratios (95% confidence intervals) for all-cause mortality in the middle and highest tertiles compared with the lowest tertile were 0.95 (0.69–1.32) and 1.72 (1.25–2.37). Similar results were noted for cardiovascular disease mortality.

Conclusions The serum level of β-trace protein is an independent predictor of death and cardiovascular disease mortality in incident hemodialysis patients.

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Due to the number of contributing authors, the affiliations are provided in the Supplemental Material.

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Introduction

Optimizing care for dialysis patients remains a major challenge. Although the US costs for treatment of ESRD rose to $26.8 billion in 2008, accounting for 5.9% of the Medicare budget, the 5-year death rate for dialysis patients remains dismally high at 70% (1). Impressive improvements in morbidity and mortality have been documented in many chronic diseases over the last decade, but most of the recent randomized controlled trials of therapies in dialysis patients have yielded mostly negative results (2–4).

Native kidney function in dialysis patients, also referred to as residual kidney function (RKF), is strongly associated with better survival, with an 11%–48% lower risk of death per 1-ml/min per 1.73 m^2 higher GFR. (5–11). RKF, even at the low GFR levels seen in dialysis patients, plays a crucial role in the clearance of uremic toxins that are not cleared by dialysis and also prevents volume overload and its sequelae, such as left ventricular hypertrophy and congestive heart failure. Although it is generally agreed that the contribution of RKF to clearance is important to quantify, the only method currently available for assessing RKF and residual Kt/V_{urea} is a 24- to 48-hour urine collection between dialysis days. This method is cumbersome; as a result, it is performed in fewer than 5% of all US incident hemodialysis patients (12) and is even difficult to perform in clinical trials (6). Thus, simpler methods for assessing kidney function similar to serum creatinine measurement in nondialysis patients, are needed.

β-Trace protein (BTP), a 23- to 29-kD serum protein, is a novel endogenous filtration marker. Unlike urea, creatinine, and cystatin C, it is not removed by hemodialysis because of its higher molecular weight. Serum levels of BTP are highly correlated with measured GFR (13–15) and have been shown to correlate with RKF (16), but their association with long-term outcomes in dialysis patients has not been studied. The aim of this study was to examine the association of serum BTP with mortality in incident hemodialysis participants of the Choices for Healthy Outcomes in Caring for ESRD (CHOICE) study.

Table 1. Baseline characteristics of 503 incident hemodialysis participants of the Choices for Healthy Outcomes in Caring for ESRD Study by tertiles of serum β-trace protein

	N	Serum β-Trace Protein Tertiles (range, mg/L)			Linear Trend *P* Value
		Lowest Tertile (1.8–6.1)	Middle Tertile (6.1–8.8)	Highest Tertile (8.8–21.1)	
Number of participants	503	168	168	167	
Serum β-trace protein (mg/L)[a]	503				
mean (SD)		4.6 (1.0)	7.3 (0.8)	11.4 (2.5)	
median (25th to 75th percentiles)		4.8 (3.8–5.5)	7.3 (6.7–7.9)	10.8 (9.8–12.2)	
median time from dialysis initiation to specimen collection (months; 25th to 75th percentiles)		4.5 (3.5–5.9)	4.8 (3.8–6.0)	5.1 (4.1–6.2)	0.003
Demographic					
age	503	59 (14)	57 (15)	58 (15)	0.54
sex (percent female)	503	52	42	43	0.15
race (percent white)	503	64	68	59	0.26
education (percent high school graduate)	489	37	33	33	0.60
employment (percent working)	503	11	12	6	0.15
marital status (percent married)	503	52	49	47	0.65
Clinical					
smoking status (percent ever smoker)	489	56	59	61	0.64
index of coexistent disease score (percent)	502				0.33
≤1		33	25	31	
2		38	42	44	
3		29	33	25	
baseline comorbid conditions (%)					
diabetes	502	59	60	52	0.27
CVD	502	56	55	55	0.97
CHF	502	50	49	48	0.91
LVH	502	29	23	29	0.35
BMI median (kg/m^2; 25th to 75th percentiles)	475	27.2 (22.7–32.1)	25.9 (22.8–31.0)	25.0 (21.9–28.3)	0.001
systolic BP (mmHg)	484	149 (19)	153 (17)	152 (18)	0.10
diastolic BP (mmHg)	484	78 (10)	81 (10)	81 (10)	0.03
pulse pressure (mmHg)	484	71 (15)	73 (13)	72 (14)	0.59
urine output≥1 cup/d					
status at baseline (percent yes)	487	90	87	75	0.001
status at year 1	425				<0.001
not at baseline or year 1 (%)		11	15	28	
at baseline but not year 1 (loss of RKF; %)		52	49	52	
at baseline and year 1 (preserved RKF; %)		37	36	20	
urine volume (ml)	231				<0.001
mean (SD)		906 (584)	815 (548)	605 (381)	
median (25th to 75th percentiles)		738 (500–1150)	600 (400–1100)	525 (300–800)	
ESRD-related					
eGFR at dialysis initiation (ml/min per $1.73\ m^2$)[b]	476	11 (4)	10 (3)	9 (4)	<0.001
assigned primary cause of renal failure (%)	503				<0.001
diabetes		50	55	43	
hypertension		18	11	23	
glomerulonephritis		10	13	22	
late referral (%; <4 months)		31	29	31	0.62
average dialysis Kt/V[c]	400	1.37 (0.28)	1.31 (0.26)	1.39 (0.27)	0.42
average dialysis duration in minutes	434	216 (23)	218 (23)	219 (22)	0.18
Laboratory					
predialysis BUN (mg/dl)	404	57 (14)	59 (15)	61 (15)	0.02
predialysis creatinine (mg/dl)	501	6.6 (2.0)	8.2 (2.4)	9.8 (3.1)	<0.001

Table 1. (Continued)

	N	Serum β-Trace Protein Tertiles (range, mg/L)			Linear Trend P Value
		Lowest Tertile (1.8–6.1)	Middle Tertile (6.1–8.8)	Highest Tertile (8.8–21.1)	
potassium (mEq/L)	500	4.6 (0.6)	4.7 (0.6)	4.9 (0.6)	<0.001
albumin (g/dl)	503	3.4 (0.6)	3.5 (0.5)	3.7 (0.6)	<0.001
calcium (mg/dl)	500	9.8 (0.7)	9.8 (0.9)	9.7 (0.8)	0.56
phosphorus (mg/dl)	500	5.1 (1.3)	5.8 (1.5)	5.9 (1.7)	<0.001
hemoglobin (g/dl)	500	10.9 (1.1)	11.2 (1.1)	10.9 (1.1)	0.48
C-reactive protein (mg/dl; median [25th to 75th percentiles])	502	0.63 (0.30–1.39)	0.58 (0.25–1.42)	0.64 (0.22–1.70)	0.77
Baseline medications					
diuretics (%)	503	30	29	24	0.38
ACE inhibitors (%)	503	28	30	29	0.89
calcium channel blockers (%)	503	60	57	68	0.07
β-blockers (%)	503	27	23	24	0.66
phosphate binders (%)	503	88	90	93	0.24
EPO dose (units/wk)[d]	338	42,184 (25,024)	47,388 (24,157)	48,956 (26,621)	0.06

Numbers presented are mean (SD) or percent unless otherwise specified. Conversion factors for units: albumin in g/dl to g/L, ×10; calcium in mg/dl to mmol/L, ×0.2495; phosphorus in mg/dl to mmol/L, ×0.3229; hemoglobin in g/dl to g/L, ×10; BUN in mg/dl to urea in mmol/L, ×0.357; creatinine in mg/dl to umol/L, ×88.4. No conversion is necessary for potassium in mEq/L to mmol/L. CVD, cardiovascular disease; CHF, congestive heart failure; LVH, left ventricular hypertrophy; BMI, body mass index; RKF, residual kidney function; eGFR, estimated GFR; ACE, angiotensin converting enzyme; EPO, erythropoietin.

[a]Normal reference range of serum BTP has not been determined. In the Atherosclerosis Risk in Communities Study, among those individuals in the highest eGFR group (>96.7 ml/min per 1.73 m^2), serum β-trace protein (BTP) was 0.6±0.1 mg/L, and among those individuals in the lowest eGFR group (<60 ml/min per 1.73 m^2), serum BTP was 1.1±0.9 mg/L (52).

[b]eGFR at dialysis initiation is eGFR at the time of initiation of renal replacement therapy. It is calculated using the four-variable Modification of Diet in Renal Disease Study formula from serum creatinine reported on Form 2728 (53).

[c]Dialysis dose (Kt/V) was calculated using the Daugirdas formula and was available for 327 (65%) participants at baseline (54).

[d]EPO dose is average weekly erythropoietin dose requirements during the first 6 months after enrollment.

Materials and Methods

Study Design

The CHOICE Study is a national prospective cohort study of incident dialysis patients (17). From October of 1995 to June of 1998, 1041 participants (767 on hemodialysis) from 19 US states were enrolled a median of 45 days after initiation of dialysis (95% within 3.5 months) and followed through December 31, 2004. Eligibility criteria were new onset of long-term dialysis therapy in the preceding 3 months, ability to provide informed consent, age>18 years, and ability to speak English or Spanish. A specimen bank was established among the Dialysis Clinics, Inc. participants of the CHOICE study. Nonfasting, predialysis serum specimens were centrifuged within 30–45 minutes of blood draw and sent overnight on ice to the Dialysis Clinics, Inc. Central Laboratory. Each blood draw was aliquoted into multiple vials and stored at −80°C. This study population includes 503 hemodialysis participants with banked sera. Included participants were younger (58 versus 62 years; $P<0.001$), less likely to be white (64% versus 76%; $P=0.001$) or have cardiovascular disease (56% versus 68%; $P=0.001$), and had lower estimated GFR at dialysis initiation (10.1 versus 11.3 ml/min per 1.73 m^2; $P=0.01$) than those participants not included. Median time from dialysis initiation to blood draw was 4.8 months (25th to 75th percentiles=3.8–6.1 months). The Johns Hopkins Medicine Institutional Review Board (Baltimore, Maryland) and the clinical centers' review boards approved the study, and participants provided written informed consent.

Data Collection

Exposure. Serum BTP was measured by particle-enhanced immunonephelometric assays with a Siemens Dade Behring ProSpec analyzer (Siemens Healthcare Diagnostics, Newark, Delaware) at the University of Minnesota Medical Center, Fairview's Collaborative Studies Clinical Laboratory. BTP is stable for 14 days on room temperature and multiple freeze–thaw cycles (18). The coefficient of variation (CV) for BTP was 4.0% at 1.8 mg/L and 5.7% at 0.6 mg/L. The reliability coefficient for BTP in 5% masked replicates assayed on different days was 0.885, and after the removal of two outliers, it was 0.968.

Outcomes. The outcomes were all-cause and cardiovascular disease (CVD) mortality. Mortality information was independently adjudicated using information from clinic report, medical records, National Death Index, Centers for Medicare & Medicaid Services death notification forms, and Social Security records as previously described (19).

Other Variables. Participants self-reported demographics, work history, medical history, and predialysis care. Body mass index (BMI; weight in kg/height in m^2) was calculated based on the height and weight reported on the 2728 form. Late referral was defined as <4 months between first nephrologist evaluation and start of dialysis. The presence and severity of comorbid conditions were

assessed using the Index of Coexistent Disease, a validated medical record-derived index that captures both presence and severity of comorbid conditions (20,21). Index of Coexistent Disease scores range from zero to three, with three as the highest severity level. Data on use of medications at baseline were abstracted from patient charts. Self-reported ability to make at least 1 full cup (250 ml) of urine daily was ascertained from study questionnaires at baseline and 1-year follow-up. Urine volume, measured as part of routine patient care, was available for a subsample (46%) of the participants at baseline. Data from routine patient care were available for BP, interdialytic weigh gain, serum calcium, phosphorus, potassium, blood urea nitrogen, and hemoglobin. High-sensitivity C-reactive protein (CRP) was measured using a colorimetric competitive enzyme-linked immunosorbent assay (CV=8.9%) as previously described (22). Serum albumin was measured in the same specimen as BTP at the University of Minnesota using bromocresol purple (CV=1.9%).

Statistical Analyses

Baseline characteristics of participants were compared by tertiles of serum BTP, and *P* values for linear trend were obtained using Pearson's chi-squared test or linear regression as appropriate. Survival analysis techniques were used to analyze the risk of mortality, with serum BTP modeled categorically as tertiles and continuously as natural log of BTP. Individuals were censored at transplantation or the end of the study period (December 31, 2004). Missing data for variables were as follows: educational status (2.8%), smoking history (2.8%), BMI (5.6%), systolic and diastolic BP (3.8%). Missing data values were imputed with 10 data replicates using multiple imputation by the chained equations method implemented by the *ice* program and analyzed using the *mim* program in Stata 10.1 (Stata Corp.; www.stata.com). Cox proportional hazards regression was used to model the risk of death. Proportional hazards assumptions were checked graphically and by hypothesis-based tests (P=0.69). Hazard ratios (HRs) were calculated to assess the risk of death in unadjusted models and after adjustment for *a priori*-defined confounders, including demographic characteristics and clinical and treatment factors. Adjusted relative hazard of death was displayed with serum BTP modeled as a restricted cubic spline with knots at 10th, 50th, and 90th percentiles, with the 25th percentile of the lowest tertile as the reference point (23). In exploratory analyses, to assess the construct validity of serum BTP as a marker of RKF, we analyzed the association between BTP and self-described urine output, urine volume, and interdialytic weight gain among subgroups with available data. Statistical analyses were performed using Stata software, version 10.1 (Stata Corp.; www.stata.com). Statistical significance was defined as $P<0.05$ using two-tailed tests.

Results

Characteristics Associated with BTP

Baseline characteristics of the participants stratified by tertiles of serum BTP are listed in Table 1. The median time to specimen collection from start of dialysis was slightly longer among those individuals with higher BTP. Individuals with higher levels of BTP were less likely to self-report≥1 cup urine/d and had lower urine volumes. Higher levels of BTP were also associated with lower BMI, higher diastolic BP, lower estimated GFR at dialysis initiation, higher serum BUN, creatinine, potassium, phosphorus, and albumin. Importantly, there was no association between BTP and CRP, baseline Kt/V, or average duration of dialysis sessions.

All-Cause Mortality

Of the 503 participants at baseline, 321 participants died during 1814 person-years of follow-up (median=3.3 years). Analyzed as a continuous variable, doubling of serum BTP was associated with 36% higher risk of death in the fully adjusted models (Table 2) (HR=1.36; 95% confidence interval [CI]=1.09–1.69; P=0.007). Compared with the lowest tertile of BTP, the highest tertile was associated with a 45% higher risk of death in unadjusted models (Table 3) (HR=1.45; 95% CI=1.09–1.92). After adjustment for demographic and clinical factors, compared with the lowest tertile, the highest tertile was associated with a 72% higher risk of death (HR=1.72; 95% CI=1.25–2.37). Figure 1A displays the relative hazard of death with serum BTP adjusted for mean values of confounders. With serum BTP of 3.8 mg/L as the reference point (25th percentile for tertile 1), the initial rise in hazard is steep followed by flattening in hazard at the highest levels.

CVD Mortality

There were 159 CVD deaths over the follow-up period. The specific causes of death were coronary artery disease=120 (75.5%) stroke=23 (14.5%) peripheral arterial disease=10 (6.3%) and ischemic bowel=6 (3.8%) Analyzed as a continuous variable, doubling of serum BTP was associated with a trend to higher mortality, but it did not reach statistical significance (Table 2). Analyzed as tertiles, the highest BTP tertile was associated with a 63% higher risk of CVD mortality compared with the lowest BTP tertile after adjustment (Table 3) (HR=1.63; 95% CI= 1.03–2.58). Figure 1B displays the relative hazard of CVD mortality with serum BTP adjusted for mean values of confounders.

Other Exploratory Analyses

Urine volume (n=231; 46%) was the highest among those participants with the lowest tertile of BTP, intermediate among those participants in the middle tertile, and lowest among those participants in the highest tertile (Table 1). A similar trend was seen with self-reported urine output with the highest BTP levels in those participants without urine output at baseline or year 1. Figure 2 displays the distribution of serum BTP by self-reported urine output. Those participants with urine output at baseline (Figure 2A, solid black line) and those participants with urine output at baseline and year 1, indicating preserved RKF, (Figure 2B, solid black line) have lower BTP levels (to the left of the graph) and more people with lower levels (taller peak). Higher BTP levels were associated with greater interdialytic weight gain (Table 4). There was no significant effect modification by BMI on the association between serum BTP and

Table 2. Association between serum β-trace protein and mortality in 503 incident hemodialysis participants of the Choices for Healthy Outcomes in Caring for ESRD Study

	All-Cause Mortality		CVD Mortality	
	Estimate (95% CI)	*P* Value	Estimate (95% CI)	*P* Value
Events				
number of deaths/participants	321/503		159/503	
unadjusted incidence rate per 1000 person-years (95% CI)	177 (159–198)		88 (75–102)	
Risk of death[a] [HR (95% CI)]				
unadjusted	1.21 (0.99–1.45)	0.06	1.11 (0.84–1.47)	0.45
adjusted				
demographic[b]	1.19 (0.97–1.47)	0.09	1.08 (0.80–1.45)	0.63
plus clinical factors[c]	1.36 (1.09–1.69)	0.007	1.20 (0.86–1.66)	0.29

CVD, cardiovascular disease; HR, hazard ratio; CI, confidence interval.
[a]Cox proportional hazards regression. Serum β-trace protein (BTP) is modeled as a natural log of BTP. HR presented represents hazard for each doubling of serum BTP.
[b]Demographic characteristics: age, race (white or other), sex, educational status (completed high school or not), marital status (married or not), and employment status (employed or not employed).
[c]Clinical and treatment factors in addition to demographic characteristics: smoking history (ever smoked), pulse pressure, body mass index, primary cause of kidney failure (diabetes, hypertension, glomerulonephritis, or other), Index of Coexistent Disease score (zero to three), CVD, congestive heart failure, left ventricular hypertrophy, diabetes, and serum albumin.

mortality (*P* interaction=0.19 for all-cause mortality; *P*=0.17 for CVD mortality).

Discussion

Mortality among hemodialysis patients remains dismally high, and there is a great need to identify new ways of improving hemodialysis care. In this report from a large, national prospective cohort study of incident hemodialysis patients in the United States, we show that serum BTP, a novel endogenous marker of kidney function, is associated with all-cause and CVD mortality, independent of a rigorously assessed list of covariates. Higher BTP was related to self-reported low urine output and measured volume, suggesting that it is acting (as hypothesized) as a measure of RKF. Thus, BTP provides a promising blood measure of RKF that could facilitate existing recommendations to integrate regular assessment of RKF, hitherto thwarted by the inconvenience and inaccuracy of 48-hour urine collection, into the care of hemodialysis patients.

RKF is increasingly being recognized as an important factor associated with survival among hemodialysis patients. Preserved RKF in hemodialysis patients was highly associated with better survival in a number of large representative populations (6,9,24). RKF in hemodialysis patients may prevent volume overload and its complications such as left ventricular hypertrophy and hypertension. Loss of RKF contributes to hyperkalemia and hyperphosphatemia (25), and it is also associated with accumulation of uremic toxins such as β-2-microglobulin and p-cresol (6,26). Higher BTP levels in our study, indicating lower RKF, were associated with higher diastolic BP, greater interdialytic weight gain, and higher serum potassium and phosphate, in addition to mortality.

Measurement and estimation of RKF in dialysis patients remains a challenge in clinical practice. Serum creatinine cannot be used for estimation of GFR in dialysis patients, and gold-standard measures of GFR, such as inulin, iothalamate, or iohexol clearance, can be cumbersome in routine clinical care. Estimation of RKF and calculation of renal Kt/V_{urea} are integral components of peritoneal dialysis (PD) prescription (27). RKF in PD patients is generally estimated from urea clearance in a 24-hour urine collection and then incorporated into the dialysis dose. Given the cumbersome nature of the urine collections, there has been increasing interest in filtration markers obtainable through a single blood draw that can obviate the need for urine collection. In PD patients, serum cystatin C is correlated with RKF (r^2~0.7), and a number of recent studies have explored its use for estimation of RKF (28–31). Cystatin C, a 13.2-kD protein, is about the same size as β-2 microglobulin (11.8 kD) and is removed effectively by high-flux hemodialysis, limiting its use as a serum marker of RKF in hemodialysis patients (17,32,33).

BTP is a 23- to 29-kD, 168-amino acid glycoprotein. Serum levels of BTP are highly correlated with measured GFR, and BTP seems comparable with serum creatinine in accuracy for estimation of GFR in nondialysis patients (13–15). Two major forms are recognized; brain-type BTP is a member of the lipocalin superfamily, and hematopoietic BTP is a member of the glutathione synthase class. Serum BTP assays measure only brain-type BTP (Mary Lou Gantzer, personal communication, 2010), which is produced by the epithelial cells of the choroid plexus in the central nervous system. From the cerebrospinal fluid, it diffuses into the systemic circulation. Serum BTP has a narrow range of distribution in healthy individuals, suggesting a constant rate of production (34,35). However, it is certainly possible that there may be non-GFR determinants of serum BTP, and large studies in populations

Table 3. Association between serum β-trace protein tertiles and mortality in 503 incident hemodialysis participants of the Choices for Healthy Outcomes in Caring for ESRD Study

	Overall	Serum β-Trace Protein Tertiles (mg/L)			*P* for Linear Trend
		Lowest Tertile (1.8–6.1)	Middle Tertile (6.1–8.8)	Highest Tertile (8.8–21.1)	
All-cause mortality					
events					
number of deaths/participants	321/503	103/168	100/168	118/167	
unadjusted incidence rate per 1000 person-years (95% CI)	177 (159–198)	155 (128–189)	166 (136–202)	216 (180–258)	0.03
risk of death HR (95% CI)[a]					
unadjusted		Referent	1.04 (0.77–1.40)	1.45 (1.09–1.92)	0.009
adjusted					
demographic[b]		Referent	0.89 (0.65–1.21)	1.39 (1.04–1.86)	0.01
plus clinical factors[c]		Referent	0.95 (0.69–1.32)	1.72 (1.25–2.37)	<0.001
CVD mortality					
events					
number of deaths/participants	159/503	52/168	49/168	58/167	
unadjusted incidence rate per 1000 person-years (95% CI)	88 (75–102)	78 (60–103)	81 (61–108)	106 (82–136)	0.03
risk of death HR (95% CI)[a]					
unadjusted		Referent	0.95 (0.62–1.45)	1.41 (0.94–2.10)	0.08
adjusted					
demographic[b]		Referent	0.73 (0.47–1.14)	1.31 (0.87–1.98)	0.13
plus clinical factors[c]		Referent	0.77 (0.47–1.24)	1.63 (1.03–2.58)	0.02

CI, confidence interval; HR, hazard ratio; CVD, cardiovascular disease.
[a]Cox proportional hazards regression.
[b]Demographic characteristics: age, race (white or other), sex, educational status (completed high school or not), marital status (married or not), and employment status (employed or not employed).
[c]Clinical and treatment factors in addition to demographic characteristics: smoking history (ever smoked), pulse pressure, body mass index, primary cause of kidney failure (diabetes, hypertension, glomerulonephritis, or other), Index of Coexistent Disease score (zero to three), CVD, congestive heart failure, left ventricular hypertrophy, diabetes, and serum albumin.

with measured GFR may be able to address this important question. BTP glycoforms lacking *N*-acetylneuraminic acid residues are rapidly removed by liver, and the remaining are excreted in the urine (36). Similar to cystatin C and other low molecular weight proteins, BTP is freely filtered by the glomerulus, and it is completely reabsorbed and metabolized by the proximal tubule (37). Serum BTP levels increase as urine output declines in hemodialysis patients (16). BTP is not removed by low- or high-flux hemodialysis membranes during conventional hemodialysis (16). Hemodiafiltration, a specialized type of dialysis not performed for maintenance dialysis in the United States, can remove BTP (16,33). These characteristics suggest that serum BTP may serve as a useful biomarker for GFR estimation in hemodialysis patients. Estimation of renal Kt/V_{urea} by a simple blood test may allow for adjustment of hemodialysis frequency and delivered dose, especially in those individuals with significant RKF at start of hemodialysis and patients performing frequent hemodialysis, such as home hemodialysis patients. Although there is a growing body of literature on the use of BTP as an endogenous filtration marker, to our knowledge, no studies have analyzed the association between serum BTP levels and outcomes in dialysis patients.

In a recent small study (n=66), higher cystatin C levels, measured immediately before dialysis initiation, were associated with a higher risk of cardiovascular events in the follow-up period (38). In our study, higher serum BTP levels were independently associated with all-cause and CVD mortality. The effect was most pronounced comparing the lowest with the highest tertiles of serum BTP, and those individuals in the highest tertile (median level=10.8 mg/L) had 72% greater hazard of death (95% CI=25%–237% higher risk) compared with the lowest tertile of BTP (median level=4.8 mg/L) after adjustment for confounders. The middle tertile (median level=7.3 mg/L) did not have higher risk compared with the lowest tertile, likely reflecting the relatively narrow range of BTP in this group (6.1–8.8 mg/L) and similar incidence rates of mortality in the lowest and middle tertile. These findings are consistent with the role of BTP as a marker of RKF, with the early rise in BTP levels reflecting declining RKF and later values representing those individuals with no RKF. In our study, we did not measure cystatin C, and as a result, we

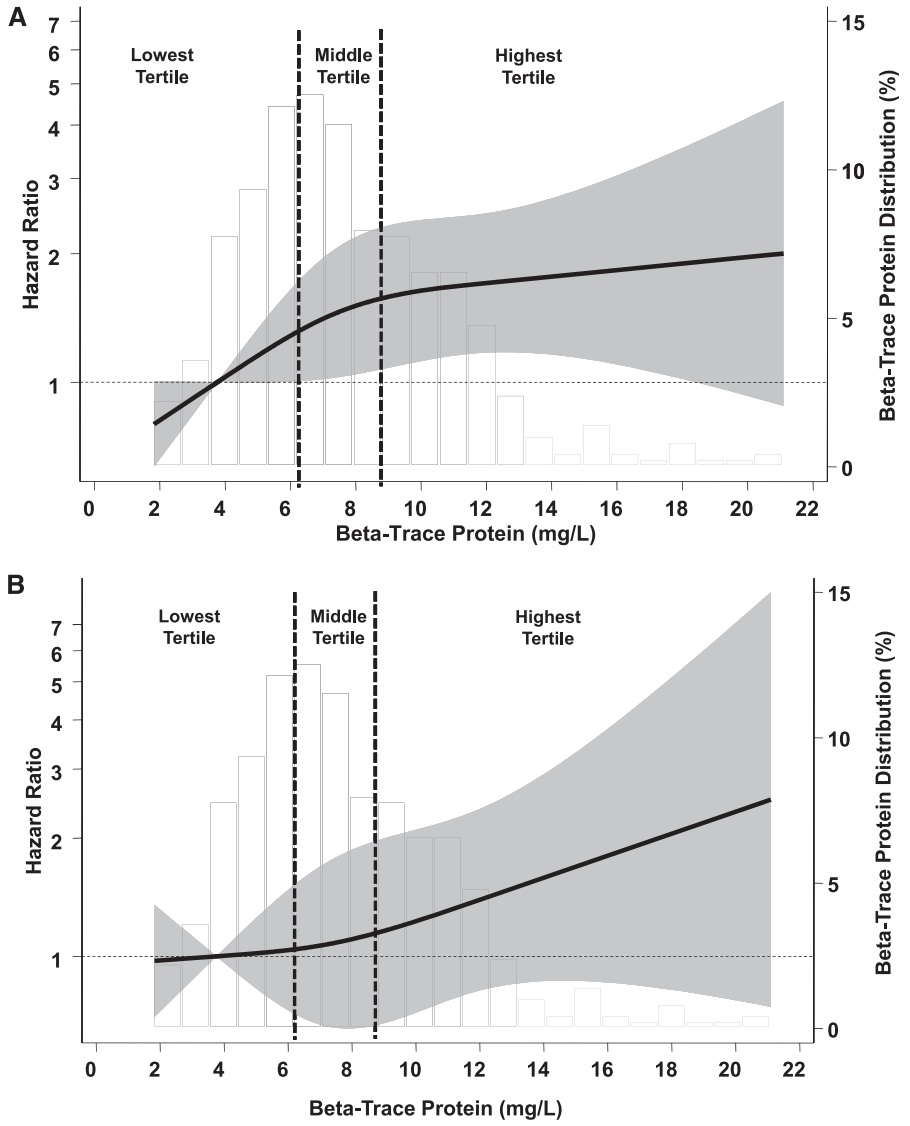

Figure 1. | **Serum β-trace protein and adjusted risk of death.** Adjusted relative hazard of all-cause mortality (A) and cardiovascular disease mortality (B) with serum β-trace protein (BTP) in 503 incident hemodialysis participants of the Choices for Healthy Outcomes in Caring for ESRD (CHOICE) Study. Relative hazard predicted using Cox proportional hazards regression adjusted for demographic characteristics (age, race [white or other], sex, educational status [completed high school or not], marital status [married or not], and employment status [employed or not]) and clinical and treatment factors (smoking history [ever smoked], pulse pressure, body mass index, primary cause of kidney failure [diabetes, hypertension, glomerulonephritis, or other], Index of Coexistent Disease score [zero to three], cardiovascular disease, congestive heart failure, left ventricular hypertrophy, diabetes, and serum albumin). Bars present the distribution of serum BTP. Solid line represents the adjusted hazard ratio of serum BTP with mortality; BTP of 3.8 mg/L (25th percentile of the lowest tertile) is used as the reference point (hazard ratio=1). Gray bands surrounding the solid line represent the 95% confidence interval of the hazard ratios. Vertical dotted lines represent BTP tertiles: lowest tertile (<6.1 mg/L), middle tertile (6.1–8.8 mg/L), and highest tertile (>8.8 mg/L).

cannot directly compare the risk of mortality in BTP versus cystatin C in hemodialysis patients.

We noted a trend toward higher albumin levels in patients in the highest BTP tertile. Higher BTP levels were also associated with lower BMI, despite higher albumin levels. No previous associations between BMI and BTP have been reported, and it is difficult to determine if this cross-sectional association is causal or incidental. We did not find any effect modification by BMI on the association between BTP and mortality. Importantly, there was no association between inflammation, measured using CRP, and serum BTP tertiles. Future studies in healthy populations and different disease states will be required to determine the non-GFR determinants of serum BTP.

BTP binds lipophilic substances in blood, such as retinoids, thyroid hormone, and bilirubin, and it may have a role in extracellular transport of these substances (39,40). Biologically, BTP increases conversion of prostaglandin H2 to prostaglandin D2. Prostaglandin D2 regulates body temperature, sleep–awake cycle, tactile pain response, and inflammation (41,42). In animal models, prostaglandin D2 can induce coronary vasoconstriction and reduce left

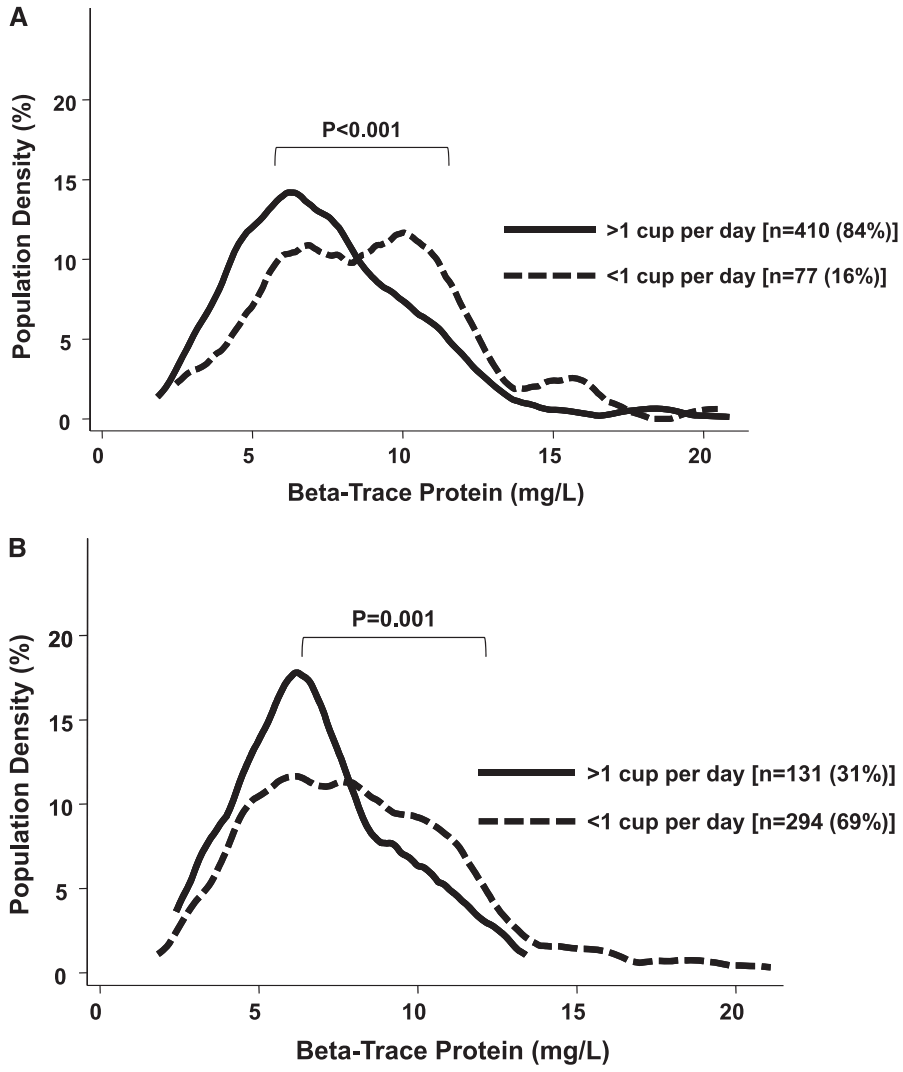

Figure 2. | **Serum β-trace protein (BTP) and self-reported urine output.** Serum BTP distribution by self-reported urine output at baseline (A) and year 1 (B) in incident hemodialysis participants of the CHOICE Study. Vertical axis (population density) represents the percent population at any given level of BTP. Solid line represents individuals with >1 cup/d self-reported urine output, and broken line represents individuals with <1 cup/d self-reported urine output.

Table 4. Association between serum β-trace protein and interdialytic weight gain

BTP	Interdialytic Weight Gain, kg			
	0–3 Months (n=225)	4–6 Months (n=450)	7–9 Months (n=428)	10–12 Months (n=403)
Tertiles				
lowest tertile	2.5 (1.0)	2.6 (1.1)	2.6 (1.1)	2.8 (1.2)
middle tertile	2.6 (1.0)	2.7 (1.1)	2.8 (1.1)	2.8 (1.2)
highest tertile	2.9 (1.0)	2.9 (1.0)	3.0 (1.0)	3.0 (1.0)
P value for linear trend	0.02	0.02	0.004	0.07
Continuous				
per doubling of BTP	0.33 (0.11, 0.55)	0.18 (0.19, 0.35)	0.24 (0.08, 0.40)	0.18 (−0.002, 0.36)
P value	0.004	0.03	0.004	0.05

Months represent time period after BTP measurement. BTP, β-trace protein.

ventricular contractility (43,44). BTP has been shown in human coronary artery atherosclerotic plaques, especially those plaques causing >75% luminal stenosis, and on immunohistochemistry, BTP seems to be localized to intima more than media (45). BTP has also been implicated in the progression of subclinical atherosclerosis (46–49). Thus, it is possible that, beyond marking lower RKF, accumulation of BTP in dialysis patients may have direct toxicity and contribute to the high prevalence of CVD, sudden cardiac death, and sleep apnea.

Our study has some limitations. First, assessment of RKF was based on self-report, and we did not have measurement of residual urea and creatinine clearance. However, this self-reported urine output itself is independently associated with all-cause mortality in hemodialysis patients (6,9,24). Serum BTP levels tended to be the highest in participants with lower urine volumes and lower self-reported urine output. Second, dialysis dose (Kt/V) was available for a limited number of participants. It is, however, unlikely that BTP levels would have differed by levels of Kt/V, because it is not cleared with conventional hemodialysis (16). Third, in the context of biomarker research, our study represents a second-phase evaluation (prospective validation) of a new biomarker (50,51). Although we had an adequate sample size and number of events in our cohort to support our findings, demonstration of the clinical use of BTP will need validation in future studies. Fourth, as with any cohort study, there is always the possibility of residual confounding as a result of unmeasured factors, and causal associations cannot be established. These limitations are balanced by the several strengths of our study, including prospective design with inclusion of only incident hemodialysis patients; detailed and precise information for demographic, clinical, and treatment factors; and systematic adjudication of baseline comorbid conditions as well as outcomes. These comprehensive data allowed us to extensively adjust for potential confounders in our analysis.

In summary, we found that serum BTP is independently associated with all-cause and CVD mortality in hemodialysis patients. Validation of these results in other studies may allow the use of BTP as a practical serum marker of RKF in hemodialysis patients to improve care and enhance prognostic ability.

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Disclosures

None.

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