Transthyretin Predicts Cardiovascular Outcome in Hemodialysis Patients With Type 2 Diabetes

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OBJECTIVE—BMI and albumin are commonly accepted parameters to recognize wasting in dialysis patients and are powerful predictors of morbidity and mortality. However, both parameters reveal limitations and may not cover the entire range of patients with wasting. The visceral protein transthyretin (TTR) may be helpful in overcoming the diagnostic and prognostic gap. Therefore, the aim of this study was to assess the association of TTR with morbidity and mortality in hemodialysis patients.

RESEARCH DESIGN AND METHODS—The TTR concentration was determined in plasma samples of 1,177 hemodialysis patients with type 2 diabetes. Cox regression analyses were used to determine hazard ratios (HRs) for the risk of cardiovascular end points (CVEs) and mortality according to quartiles of TTR concentration for the total study cohort and the subgroups BMI ≥23 kg/m^2, albumin concentration ≥3.8 g/dL, and a combination of both.

RESULTS—A low TTR concentration was associated with an increased risk for CVE for the total study cohort (HR 1.65 [95% CI 1.27–2.14]), patients with BMI ≥23 kg/m^2 (1.70 [1.22–2.37]), albumin ≥3.8 g/dL (1.68 [1.17–2.42]), and the combination of both (1.69 [1.13–2.53]). Additionally, a low TTR concentration predicted mortality for the total study cohort (1.79 [1.43–2.24]) and patients with BMI ≥23 kg/m^2 (1.46 [1.09–1.95]).

CONCLUSIONS—The current study demonstrated that TTR is a useful predictor for cardiovascular outcome and mortality in diabetic hemodialysis patients. TTR was particularly useful in patients who were not identified to be at risk by BMI or albumin status.

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Transthyretin (TTR), formerly referred to as prealbumin, is known as a sensitive indicator of inflammation and malnutrition (1,2) and has also been described as a marker of body composition (3,4). With regard to hemodialysis patients, TTR is a well-accepted marker of protein-energy wasting (PEW), which is highly prevalent in patients with end-stage renal disease (5,6). PEW represents a syndrome characterized by depletion of body energy and protein stores, inflammation, and development of comorbidities (7) and is highly associated with the risk of death as well as fatal and nonfatal cardiovascular disease (CVD) in hemodialysis patients (8,9). An expert panel suggested a combination of several parameters for the diagnosis of PEW (7); among these, the most frequently and routinely applied are BMI and serum albumin concentration. Both parameters are known to be inversely associated with mortality and morbidity in hemodialysis patients (10–12).

However, BMI and serum albumin concentration have several limitations in diagnosing PEW and subsequently the risk for all-cause mortality and CVD. Among other things, it has been criticized that BMI is not able to discriminate between fat and lean body mass, and a high body mass might be misinterpreted as an appropriate nutritional status. Consequently, patients may be misclassified and wasting may not be recognized. On the other hand, the detrimental effects of being overweight and obesity may also not be recognized or underestimated (13–15). Furthermore, the importance of serum albumin concentration is probably limited since it is more sensitive to inflammation than to nutrition (16), and thus nutrition-related complications are insufficiently considered (13). Additionally, serum albumin is sensitive to hydration status (17,18), and in diabetic patients, the capillary permeability of serum albumin is increased, which might adulterate the serum levels (19).

In this context, the analysis of TTR might be useful in overcoming the resulting diagnostic and prognostic gap since TTR is known to be very sensitive to changes in visceral protein stores, correlated with muscle mass, and not affected by hydration status (3,20). Therefore, we hypothesized that TTR may likewise be a potent predictor of mortality and morbidity in hemodialysis patients beyond BMI and serum albumin concentration. Furthermore, since diabetes is a major risk factor for chronic kidney disease, aggravates PEW, and unequivocally increases the risk for cardiovascular events and mortality (6,21,22), the current study was particularly focused on hemodialysis patients with type 2 diabetes using data from the German Diabetes and Dialysis (4D) study.

RESEARCH DESIGN AND METHODS

Study design and participants

The 4D study was a prospective, randomized, controlled trial evaluating the lipid-lowering effect of atorvastatin in 1,255 diabetic patients undergoing hemodialysis. The study design has been described in detail elsewhere (23). In brief, patients with type 2 diabetes, 18–80 years of age, and on hemodialysis for <2 years were enrolled between March 1998 and October 2002. Patients were randomly assigned to double-blinded treatment with either 20 mg atorvastatin (n = 619) or placebo (n = 636)

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once daily. Study visits took place three times before randomization, at randomization, and at 4 weeks and every 6 months after randomization until the date of death, censoring, or end of the study in March 2004. At each follow-up visit, clinical information was collected, including any adverse events, blood samples were taken, and an electrocardiogram was recorded. The study was approved by the medical ethical committee, and all patients gave their written informed consent before inclusion.

Data collection

Information on age, sex, and smoking status was obtained through patient interviews. Smoking status was classified as never, former, or current. Comorbidities, including the presence of coronary artery disease (CAD) and congestive heart failure (CHF), as well as the duration of diabetes and dialysis treatment, were reported by the patients' nephrologists. CAD was defined by a history of myocardial infarction, coronary artery bypass grafting surgery, and percutaneous coronary intervention and as documented by angiography. Blood pressure was measured in a sitting position. BMI was calculated as weight (kilograms) divided by height (meters) squared.

Laboratory procedures

The laboratory measurements were performed centrally at the Department of Clinical Chemistry, University of Freiburg, Freiburg, Germany. TTR plasma concentration was measured at the University of Potsdam using a noncommercial enzyme-linked immunosorbent assay as described in detail elsewhere (24). Blood samples were taken before start of dialysis and administration of heparin or further drugs, and plasma was kept at −80°C until analysis.

Outcome measures

The primary end point of the 4D study was defined as a composite of death from cardiac causes, stroke, and nonfatal myocardial infarction, whichever occurred first. Death from cardiac causes comprised fatal myocardial infarction (death within 28 days after myocardial infarction), sudden death, death due to CHF, death due to coronary heart disease during or within 28 days after an intervention, and all other deaths ascribed to coronary heart disease. For the present analysis, the primary end points of combined cardiovascular events as well as all-cause mortality were chosen to be separate outcome measures. The 4D study end points were centrally adjudicated by three members of the end-point committee who were blinded to study treatment.

Statistical analysis

Patient characteristics are presented according to quartiles of TTR for the total study cohort as well as the subgroups BMI ≥23 kg/m^2, serum albumin concentration ≥3.8 g/dL, and the combination of both. The subclassification was applied to consider potential underestimation of wasting due to normal to high BMI and albumin levels. The stratification criteria were chosen based on the cutoff values recommended for BMI and serum albumin concentration by the expert panel (7).

Continuous variables were expressed as mean with SD or median with interquartile range as appropriate. Categorical variables were expressed as percentages. We analyzed the effect of TTR concentration on all-cause mortality and combined cardiovascular end points (CVEs) in baseline samples of the 4D study cohort (23) and compared these results to the effects observed for serum albumin concentration. Furthermore, to consider underestimation of wasting based on BMI and albumin levels, we stratified the study population according to the cutoff values recommended by the expert panel (7), resulting in the subgroups BMI ≥23 kg/m^2, albumin ≥3.8 g/dL, and a combination of both. For each of these subgroups, we analyzed the effects of TTR plasma concentration on mortality and CVE separately. Additionally, we analyzed the effect of TTR plasma concentration on the risk of all-cause mortality and reaching a CVE across the spectrum of BMI in the study population, represented by BMI tertiles.

The effects of TTR and albumin concentration on clinical outcomes were assessed by Kaplan-Meier estimates for incidences of all-cause mortality and CVE. Relative risks were derived from Cox regression analyses, i.e., hazard ratios (HRs) and corresponding 95% CIs. Cox regression analyses were additionally performed to analyze the effect of TTR concentration as a continuous variable to estimate the risk for CVE and mortality in BMI tertiles. The Cox regression analyses were adjusted for the confounders' age, sex, atorvastatin treatment, duration of diabetes, time on dialysis, glycated hemoglobin A_{1c} (HbA_{1c}), smoking status, phosphorous, systolic blood pressure, and ultrafiltration volume. Furthermore, we evaluated potential intermediate variables, including total, LDL, and HDL cholesterol, triglycerides, C-reactive protein (CRP), and the history of CAD, coronary heart failure, arrhythmia, and stroke, which may lie in the causal pathway of the effect of TTR and albumin on adverse clinical events. The variables were included one at a time in order to see the magnitude, by which the effect estimate for the risk of TTR and albumin on adverse events changed, respectively. Furthermore, in an analysis aiming for the impact of all intermediate variables together, they were simultaneously added to the main model. All *P* values are reported two sided. Analyses were performed using SPSS version 16.0.

RESULTS—A total of 1,255 hemodialysis patients suffering from type 2 diabetes were recruited between March 1998 and October 2002 and included in the 4D study (23). The mean follow-up period was 3.96 years (median 4.0 years) on atorvastatin and 3.91 years (median 4.08 years) on placebo. In baseline plasma samples of 1,177 patients, TTR concentration was determined. During the follow-up period, 447 of these patients reached one or multiple CVEs, and, overall, 579 patients died.

The clinical as well as biochemical characteristics, including TTR plasma concentration, of the total study cohort (n = 1,177) as well as the subgroups BMI ≥23 kg/m^2 (n = 1,010), serum albumin concentration ≥3.8 g/dL (n = 704), and a combination of BMI ≥23 kg/m^2 and serum albumin concentration ≥3.8 g/dL (n = 611) are shown in Table 1. Patients assigned to atorvastatin treatment and placebo treatment during the follow-up period did not differ in BMI, TTR, and albumin concentration at baseline for the total study cohort as well as the BMI- and/or albumin-specified subgroups. Additionally, changes in BMI and serum albumin concentration, which have been monitored during the follow-up period, did not differ between the treatment groups. Furthermore, the number of events (all-cause mortality and CVE, respectively) during the follow-up period was comparable between the treatment groups for the total study cohort as well as the subgroups.

Risk of all-cause mortality and CVE by quartiles of baseline TTR and serum albumin concentration for the total study cohort

For the total study cohort, Kaplan-Meier curves (Fig. 1) revealed a significant

Table 1—*Characteristics of the study population according to wasting classification*

	All patients (n = 1,177)	BMI ≥23 kg/ m^2 (n = 1,010)	Albumin ≥3.8 g/dL (n = 704)	BMI ≥23 kg/ m^2 and albumin ≥3.8 g/dL (n = 611)
Age, years	66 (8)	65 (8)	65 (8)	65 (8)
Sex, % male	54	53	60	60
BMI, kg/m^2	27.6 (4.8)	28.7 (4.3)	27.5 (4.5)	28.5 (4.0)
Atorvastatin treatment, %	51	50	52	50
SBP, mmHg	146 (22)	145 (22)	146 (21)	145 (21)
DBP, mmHg	76 (11)	76 (11)	76 (11)	76 (11)
Smoker/ex-smoker, %	40	38	45	44
HbA_{1c}, %	6.7 (1.3)	6.8 (1.3)	6.7 (1.3)	6.7 (1.2)
Diabetes duration, years	18.2 (8.8)	18.3 (8.9)	18.1 (9.0)	18.2 (9.1)
Time on dialysis, months	8 (7)	8 (7)	9 (7)	9 (7)
History of CAD, %	29	28	29	28
History of CHF, %	36	35	33	33
History of arrhythmia, %	19	19	20	20
Total cholesterol, mg/dL	219 (43)	221 (43)	222 (42)	223 (42)
LDL cholesterol, mg/dL	126 (30)	126 (30)	128 (29)	128 (29)
HDL cholesterol, mg/dL	36 (13)	36 (13)	37 (14)	36 (13)
Triacylglycerides, mg/dL	223 (149–327)	230 (154–337)	228 (154–325)	236 (156–337)
Phosphate, mmol/L	6.0 (1.6)	6.0 (1.6)	6.1 (1.5)	6.1 (1.5)
Sensitive CRP, mg/L	5.1 (2.3–12.4)	5.1 (2.4–12.4)	4.2 (2.0–9.5)	4.2 (2.1–9.9)
Ultrafiltration volume, kg*	2.3 (1.2)	2.3 (1.2)	2.3 (1.3)	2.3 (1.3)
Albumin, mg/dL	3.8 (0.30)	3.8 (0.30)	4.0 (0.19)	4.0 (0.19)
TTR, μmol/L	3.4 (2.5–4.5)	3.4 (2.6–4.5)	3.7 (2.8–4.9)	3.7 (2.8–4.9)

Data are mean (SD) or median (interquartile range) unless otherwise indicated. DBP, diastolic blood pressure; SBP, systolic blood pressure. *The ultrafiltration volume was calculated based on the body weight before and after dialysis at the randomization visit.

inverse association of baseline TTR and albumin concentration with the risk of death as well as reaching a CVE during the follow-up period. These results were confirmed by Cox regression analyses, presented in Table 2.

The results of the unadjusted Cox regression analyses indicated that patients with a low TTR plasma concentration (first quartile, ≤2.5 μmol/L) revealed an increased risk for reaching a CVE (HR 1.65 [95% CI 1.27–2.14], $P < 0.001$) in comparison with patients with a high TTR plasma concentration (fourth quartile, >4.5 μmol/L). Thereby, the increased risk for CVE was mainly attributed to an increased risk for sudden death and stroke (data not shown). With regard to albumin, comparable results for the association with the risk of CVE were obtained. Low serum albumin concentrations (first quartile, ≤3.6 g/dL; 1.81 [1.36–2.39], $P < 0.001$) were associated with an increased risk in comparison with those with high serum albumin concentration (fourth quartile, >4.0 g/dL). Also, after adjustment, the risk of reaching a CVE remained inversely associated with TTR (1.51 [1.12–2.04], P = 0.007) as well as albumin concentration (1.47 [1.07–2.01], P = 0.018).

With regard to the risk of all-cause mortality, the results of unadjusted Cox regression analyses indicated that patients with a low TTR as well as those with a low albumin concentration revealed an approximately twofold higher mortality (TTR: HR 1.79 [95% CI 1.43–2.24], $P < 0.001$; albumin: 1.80 [1.41–2.29], $P < 0.001$) in comparison with patients with high TTR and high albumin concentrations, respectively. After adjustment, the risk of death remained significantly increased in patients with a low TTR concentration (1.37 [1.05–1.77], P = 0.019) in comparison with those with a high TTR concentration. With regard to albumin, a low serum concentration indicated an increased risk of death also after adjustment (1.34 [1.02–1.76], P = 0.039) in comparison with those with a high albumin concentration; however, the association was less pronounced than for TTR.

Risk for reaching a CVE and all-cause mortality by quartiles of TTR plasma concentration in patients with BMI ≥23 kg/m^2

The results of adjusted Cox regression analyses for the risk of death and of reaching a CVE according to TTR quartiles for patients with BMI ≥23 kg/m^2 (n = 1010) are presented in Table 3. For these patients, a low TTR plasma concentration (≤2.5 μmol/L) was associated with an increased risk of death (HR 1.41 [95% CI 1.05–1.88], P = 0.021) and also of reaching a CVE during the follow-up period (1.58 [1.13–2.21], P = 0.007) in comparison with patients with a TTR plasma concentration >4.5 μmol/L. Interestingly, patients with a moderately reduced TTR plasma concentration (>2.5 to ≤3.4 μmol/L) also revealed an increased risk for reaching a CVE (crude HR 1.41 [1.05–1.88], P = 0.021; data not shown); however, this association disappeared during the adjustment process (1.32 [0.96–1.81], P = 0.087).

Risk of all-cause mortality and reaching a CVE by quartiles of TTR plasma concentration in patients with serum albumin concentration ≥3.8 g/dL

The results of adjusted Cox regression analysis for the risk of reaching a CVE and of all-cause mortality according to TTR quartiles for patients with a

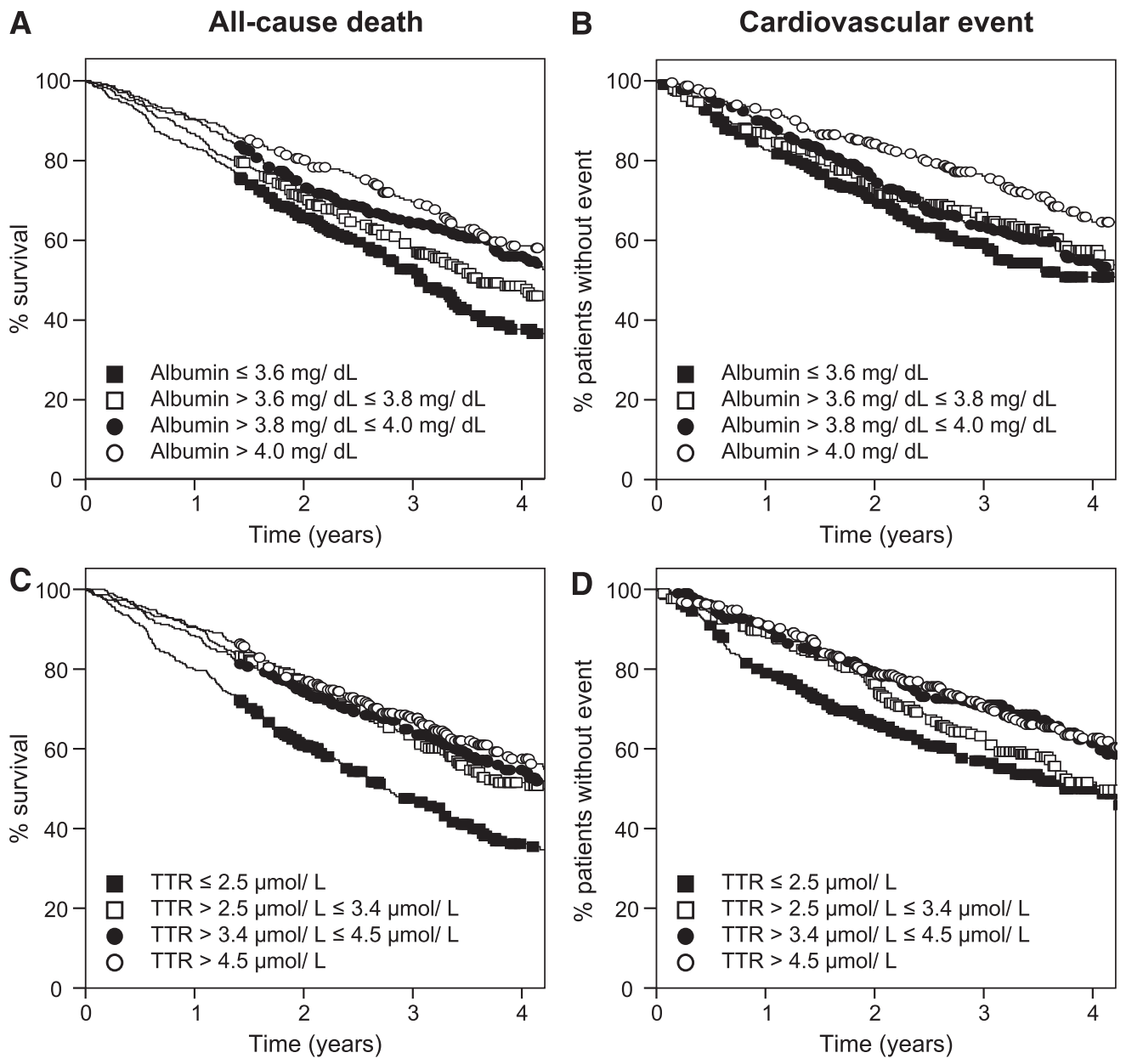

Figure 1—*Kaplan-Meier curves for the estimated cumulative incidence of all-cause mortality* (A *and* C) *and cardiovascular events* (B *and* D) *in subgroups of patients according to quartiles of baseline serum albumin concentration* (A *and* B) *and TTR plasma concentration* (C *and* D).

serum albumin concentration ≥3.8 g/dL (n = 704) are presented in Table 3. Among these patients, a total of 269 reached a CVE during the follow-up period, whereas the highest risk was present in patients with a TTR plasma concentration ≤2.5 µmol/L (HR 1.80 [95% CI 1.23–2.65], P = 0.003). With regard to the risk of death, a low TTR plasma concentration was also associated with an increased all-cause mortality (crude HR 1.41 [1.03–1.92], P = 0.030; data not shown); however, this effect was not consistent during adjustment (HR 1.15 [0.82–1.63], P = 0.418).

Risk of all-cause mortality and reaching a CVE by quartiles of TTR plasma concentration in patients with BMI ≥23 kg/m^2 and serum albumin concentration ≥3.8 g/dL

For patients who revealed a BMI ≥23 kg/ m^2 in combination with a serum albumin concentration ≥3.8 g/dL (n = 611), the results of the adjusted Cox regression analyses according to the TTR quartiles are presented in Table 3. In these patients, a low TTR plasma concentration (≤2.5 µmol/L) was associated with a 1.8-fold increased risk of reaching a CVE during the follow-up period (95% CI 1.16–2.79, P = 0.009) in comparison with patients with a TTR plasma concentration >4.5 µmol/L. Additionally, patients with a moderately reduced TTR plasma concentration (>2.5 to ≤3.4 µmol/L) revealed an increased risk for reaching a CVE (HR 1.49 [95% CI 1.00–2.21], P = 0.046). In contrast, no association of TTR plasma concentration and the risk of death could be observed for this subcohort of patients either for crude or for adjusted analyses.

Table 2—*Risk of all-cause mortality and CVE by quartiles of baseline TTR and albumin*

TTR quartiles	Quartile 1 (≤2.5 μmol/L)	Quartile 2 (>2.5 to ≤3.4 μmol/L)	Quartile 3 (>3.4 to ≤4.5 μmol/L)	Quartile 4 (>4.5 μmol/L)
n	295	294	294	294
CVE				
Number of events	128 (43%)	116 (40%)	97 (33%)	106 (36%)
Crude	1.65 (1.27–2.14)	1.28 (0.98–1.67)	1.02 (0.77–1.34)	1[b]
	$P < 0.001$	$P = 0.068$	$P = 0.913$	
Adjusted[a]	1.51 (1.12–2.04)	1.20 (0.90–1.60)	0.92 (0.68–1.24)	1[b]
	$P = 0.007$	$P = 0.217$	$P = 0.584$	
All-cause death				
Number of events	178 (60%)	133 (45%)	134 (46%)	134 (46%)
Crude	1.79 (1.43–2.24)	1.12 (0.88–1.43)	1.08 (0.85–1.38)	1[b]
	$P < 0.001$	$P = 0.353$	$P = 0.520$	
Adjusted[a]	1.37 (1.05–1.77)	0.96 (0.74–1.25)	0.92 (0.71–1.19)	1[b]
	$P = 0.019$	$P = 0.753$	$P = 0.528$	
Albumin	Quartile 1 (≤3.6 g/dL)	Quartile 2 (>3.6 to ≤ 3.8 g/dL)	Quartile 3 (>3.8 to ≤ 4.0 g/dL)	Quartile 4 (>4.0 g/dL)
n	330	303	307	237
CVE				
Number of events	127 (39%)	113 (37%)	123 (40%)	84 (35%)
Crude	1.81 (1.36–2.39)	1.51 (1.13–2.01)	1.44 (1.09–1.90)	1[b]
	$P < 0.001$	$P = 0.005$	$P = 0.011$	
Adjusted	1.47 (1.07–2.01)	1.35 (1.00–1.84)	1.39 (1.04–1.87)	1[b]
	$P = 0.018$	$P = 0.052$	$P = 0.026$	
All-cause death				
Number of events	173 (52%)	152 (50%)	138 (45%)	116 (49%)
Crude	1.80 (1.41–2.29)	1.49 (1.16–1.90)	1.13 (0.88–1.45)	1[b]
	$P < 0.001$	$P = 0.002$	$P = 0.334$	
Adjusted[a]	1.34 (1.02–1.76)	1.23 (0.95–1.61)	1.03 (0.79–1.34)	1[b]
	$P = 0.039$	$P = 0.122$	$P = 0.848$	

Cox proportional hazards regression is presented with 95% CIs. [a]Cox proportional hazards regression adjusted for age, sex, atorvastatin treatment, duration of diabetes, time on dialysis, HbA_{1c}, smoking status, phosphorous, systolic blood pressure, ultrafiltration volume, total, LDL, and HDL cholesterol, triacylglycerides, CRP, and history of CAD, CHF, arrhythmia, and stroke. [b]The group of patients with baseline TTR and albumin levels in the fourth quartile served as reference for each of the other three quartiles, respectively.

Risk of all-cause mortality and reaching a CVE by TTR status (continuous variable) and in strata of BMI tertiles

For these analyses, patients were stratified according to BMI tertiles at baseline (BMI ≤25.1 kg/m^2, BMI >25 to ≤29.1 kg/m^2, and BMI >29.1 kg/m^2), and the effect of differences in TTR plasma concentration on the risk of death and reaching a CVE was analyzed. A low TTR concentration had the strongest predictive value of CVE in the highest BMI tertile. Adjusted Cox regression analysis revealed 56% risk decrease for CVE per unit increase of TTR (HR 0.44 [95% CI 0.26–0.74], $P = 0.002$). TTR was furthermore significantly associated with mortality across tertiles of BMI in crude analyses. In the highest BMI tertile, this association of TTR with mortality was slightly attenuated after adjustment for confounders.

CONCLUSIONS—BMI and serum albumin concentration are routinely used for the assessment of PEW and represent powerful predictors of mortality and morbidity in hemodialysis patients (7,10,12). However, both parameters reveal limitations, which lessen their reliability in risk discrimination, and especially the beneficial effect of a high BMI is under debate (15,16). Therefore, the current study was designed to analyze the potential of TTR plasma concentration in overcoming the prognostic gap in a cohort of diabetic hemodialysis patients. In this context, our most important finding is that in diabetic hemodialysis patients with a BMI ≥23 kg/m^2, a serum albumin concentration ≥3.8 g/dL, or a combination of both, a low TTR plasma concentration at baseline was a reliable predictor for reaching a CVE during the follow-up period. Besides, we were able to show that with the entire study cohort included, a low TTR plasma concentration was principally associated with mortality and the incidence of CVE.

TTR, formally known as prealbumin, is mainly synthesized in the liver, and its main function is the transport of thyroid hormones and vitamin A (1). The plasma concentration of TTR is decreased during inflammation, and it is a well-characterized, negative, acute-phase protein (1). Additionally, TTR is a sensitive marker of nutritional status, and its hepatic synthesis is promptly reduced even in early stages of protein and energy deficiency (25). Especially for hemodialysis patients, several studies demonstrated that TTR plasma concentration is an appropriate indicator of nutritional status and predictor of mortality and morbidity (4,26–29). In the current study, we confirmed these results and demonstrated that, particularly in hemodialysis patients with type 2 diabetes, TTR plasma

Table 3—*Risk of CVEs and all-cause mortality for the subgroups BMI ≥23 kg/m² (n = 1,010), serum albumin concentration ≥3.8 g/dL (n = 704), and BMI ≥23 kg/m² + serum albumin concentration ≥3.8 g/dL (n = 611) according to TTR quartiles*

	≤2.5 μmol/L	>2.5 to ≤3.4 μmol/L	>3.4 to ≤4.5 μmol/L	>4.5 μmol/L
TTR quartiles (BMI ≥23 kg/m²)				
n	243	259	248	260
CVE				
Number of events	100 (41%)	102 (39%)	80 (32%)	97 (33%)
Adjusted[a]	1.58 (1.13–2.21)	1.32 (0.96–1.81)	0.97 (0.70–1.35)	1[b]
	P = 0.007	*P* = 0.087	*P* = 0.853	
All-cause death				
Number of events	140 (58%)	116 (45%)	100 (40%)	113 (43%)
Adjusted[a]	1.41 (1.05–1.88)	1.00 (0.75–1.33)	0.85 (0.63–1.14)	1[b]
	P = 0.021	*P* = 0.973	*P* = 0.267	
Albumin ≥3.8 g/dL				
n	128	155	203	218
CVE				
Number of events	57 (45%)	63 (41%)	76 (37%)	73 (33%)
Adjusted[a]	1.80 (1.23–2.65)	1.33 (0.92–1.90)	1.13 (0.80–1.60)	1[b]
	P = 0.003	*P* = 0.133	*P* = 0.486	
All-cause death				
Number of events	67 (52%)	71 (46%)	94 (46%)	99 (45%)
Adjusted[a]	1.15 (0.82–1.63)	0.94 (0.67–1.30)	0.87 (0.64–1.19)	1[b]
	P = 0.418	*P* = 0.695	*P* = 0.379	
BMI ≥ 23 kg/m² and albumin ≥3.8 g/dL				
n	106	138	172	195
CVE				
Number of events	42 (40%)	58 (42%)	65 (38%)	61 (31%)
Adjusted[a]	1.80 (1.16–2.79)	1.49 (1.00–2.21)	1.20 (0.82–1.75)	1[b]
	P = 0.009	*P* = 0.046	*P* = 0.347	
All-cause death				
Number of events	51 (48%)	63 (46%)	70 (41%)	86 (44%)
Adjusted[a]	1.12 (0.76–1.66)	0.93 (0.66 (1.33)	0.79 (0.56–1.11)	1[b]
	P = 0.575	*P* = 0.705	*P* = 0.171	

[a]Cox proportional hazards regression models were adjusted for age, sex, atorvastatin treatment, diabetes duration, duration of dialysis, HbA_{1c}, phosphate, smoking status, history of CAD, CHF, arrhythmia, and stroke, systolic blood pressure, total, LDL, and HDL cholesterol, triacylglycerides, CRP, and ultrafiltration volume. [b]The group of patients with baseline TTR and albumin levels in the fourth quartile served as reference for each of the other three quartiles, respectively.

concentration is a potent long-term predictor for all-cause mortality and reaching a CVE (Fig. 1 and Table 2). Additionally, we illustrated that in this context, the predictive power of TTR and albumin concentration seems to be comparable.

Beyond this, we were able to show that TTR plasma concentration is a potent predictor for CVE when traditional risk factors, namely BMI and albumin, failed. In detail, ~38% of the total study population reached a CVE during the follow-up period (Table 2). Actually, the same incidence rate could be observed for patients with a BMI ≥23 kg/m², serum albumin concentration ≥3.8 g/dL, or a combination of both, who are, per definition, regarded to reveal a lower risk (Table 3). For these patients, a low TTR plasma concentration (<2.5 μmol/L) was associated with a risk increase of 60–80%. Additionally, the association of low TTR and an increased risk for reaching a CVE was analyzed for the BMI spectrum represented by BMI tertiles. This analysis indicated that a low TTR concentration was particularly detrimental for patients within the highest BMI tertile (>29 kg/m²) supporting the above findings.

Several studies demonstrated that an elevated BMI is linked to an improved outcome in hemodialysis patients (12,30). Inversely, it has also been shown that this beneficial effect is linked to a normal or high muscle mass (15). However, BMI is not able to discriminate between fat and lean mass (14). In this context, TTR has been shown to represent not only an indicator of nutritional status but also of total body nitrogen metabolism (25), emphasized by the correlation of TTR plasma concentration with total fat-free and muscle mass in the general population (3) as well as hemodialysis patients (31,32). Therefore, it might be assumed that a low TTR plasma concentration in patients with a high BMI is an indicator of a reduced lean mass and is consequentially associated with an increased fat mass. Since a high fat mass in hemodialysis patients is as detrimental as for the general population and promotes the development of CVD (13,15), this might explain the increased incidence of CVE in patients of the current study cohort with a low TTR and a high BMI. Consequently, future therapeutic strategies should be developed that take into account the preservation of lean mass and a reduction of fat mass. This function of TTR plasma concentration might also account for the prediction of CVE in patients of the current study with a serum albumin concentration ≥3.8 g/dL. In fact, 87% (*n* = 611) of these patients have a BMI ≥23 kg/m², and actually only 2.3% (*n* = 16) reveal a BMI <20 kg/m². Hence, a low

TTR plasma concentration might also indicate in these patients a reduction of lean and an elevation of fat body mass, with all the detrimental effects enhancing the risk of reaching a CVE.

Taken together, the present results indicate that particularly in diabetic hemodialysis patients with a high BMI, TTR plasma concentration might be an appropriate indicator of body composition and thereby a marker of cardiovascular outcome. This association is most likely also responsible for the power of TTR plasma concentration as long-term predictor of CVEs in the current study (mean follow-up time 4 years). Body composition is known to be comparatively stable over time, but nonetheless it is subjected to subtle changes during aging, which manifest in a reduction of lean and increase of fat mass (33,34). In hemodialysis patients, this rearrangement of body composition is aggravated by metabolic acidosis and the hemodialysis process per se and has been associated with an adverse outcome (17,18). Additionally, with regard to the current study cohort, it has to be considered that only patients with type 2 diabetes are included, which presumably aggravates the wasting situation, since diabetes is known to independently promote muscle protein breakdown (35,36). In this context, the long-term predictive effect of TTR plasma concentration is probably based on the fact that the unfavorable changes in body composition are hardly correctable and instead persist (17). Furthermore, it should also be mentioned that the long-term predictive effect of baseline TTR plasma concentration is potentially biased by the sensitivity of TTR to inflammation and nutritional supply, which are associated with short-term decreases of TTR plasma concentration (25,37). Therefore, a temporary period of inflammation or malnutrition before baseline sampling may distort the results of the current study. However, high-sensitivity CRP levels were included in Cox regression analyses as covariate to consider the confounding effects of inflammation.

Additionally, in contrast to CVE, baseline TTR concentration revealed shortcomings in predicting the risk of mortality in patients with albumin concentration ≥3.8 g/dL and patients with a combination of albumin concentration ≥3.8 g/dL and BMI ≥23 kg/m^2 (Table 3). However, a low TTR plasma concentration was associated with an increased mortality in patients with a BMI ≥23 kg/m^2. This might be attributed to the substantial amount of patients in the latter subgroup with an albumin concentration <3.8 g/dL (n = 399), which, in combination with a low TTR plasma concentration, supposedly indicates a deteriorated general health status, contributing to an increased mortality (16). In this context, the high BMI might also be interpreted as overhydration and indicates inadequacies in dialysis status (38). However, these assumptions are speculative and remain to be elucidated.

Potential limitations of the study need to be acknowledged. It was a post hoc analysis within a selected cohort of German patients with type 2 diabetes on hemodialysis. Therefore, the relationship between TTR and adverse outcome may not be generalizable to other patient populations. We did not have other detailed methods of body composition measurements available, like dual-energy X-ray absorptiometry or computed tomography scan analyses, and cannot exactly distinguish protein wasting and inflammation. Despite careful adjustments for possible confounders, we cannot rule out residual confounding. However, since the known important confounders were considered, the effect of potential residual confounding is likely to be small. The main strengths of this study were the high incidence of prespecified and centrally adjudicated end points, adequate sample size, and long-term follow-up of dialysis patients.

In conclusion, we demonstrated that in a cohort of hemodialysis patients with type 2 diabetes, a low TTR plasma concentration (<2.5 μmol/L) is an effective long-term predictor of mortality, and reaching CVE and in this context is as potent as serum albumin concentration. Moreover, we could show that for patients with a BMI ≥23 kg/m^2 and/or serum albumin concentration ≥3.8 g/dL, usually associated with a low risk, a low TTR plasma concentration was a reliable predictor for the incidence of CVE. This effect is thereby probably most likely explained by the association of TTR plasma concentration with lean body mass.

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A.H. interpreted data and drafted the manuscript. K.M.E. and C.D. performed statistical analysis and contributed to interpretation of data and preparation of the manuscript. C.W. contributed to study design and critically revised the manuscript for important intellectual content. V.K. contributed to study design and data collection. J.R., B.H., and F.J.S. critically revised the manuscript for important intellectual content. All authors read and approved the final manuscript. A.H. and C.D. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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