Diet-sensitive prognostic markers for cardiovascular and renal disease

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Chapter 5

Is the Association of Serum Sodium with Mortality in Patients with Type 2 Diabetes Explained by Copeptin or NT-proBNP? (ZODIAC-46)

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Abstract

Background and Aims. Hyponatremia has been associated with an increased mortality risk in the general population. Diabetes is a condition predisposing for elevated levels of arginine vasopressin (AVP) and heart failure, both common causes of hyponatremia. These factors, however, are also associated with an increased mortality risk. We aimed to investigate whether serum sodium is associated with cardiovascular and all-cause mortality in type 2 diabetes and whether these associations could be explained by copeptin, a surrogate for AVP, or NT-proBNP, a marker of heart failure.

Methods. Patients with type 2 diabetes participating in the ZODIAC study were included. Cox regression analyses were used to investigate the association of serum sodium with mortality.

Results. We included 1,068 patients (age 67 ± 12 years, 45% male, serum sodium 142 ± 3 mmol/L). After 15 years of follow-up, 519 patients (49%) died, with 225 cardiovascular deaths (21%). In univariable analyses, serum sodium, copeptin, and NT-proBNP were all significantly associated with cardiovascular and all-cause mortality. These associations remained significant after combination of these markers in a multivariable model. Serum sodium and NT-proBNP remained significantly associated with mortality after further adjustment for potential confounders, whereas copeptin lost significance after adjustment for SCr and ACR.

Conclusion. Low serum sodium was associated with an increased risk of cardiovascular and all-cause mortality in type 2 diabetes. Moreover, these associations were not explained by copeptin and NT-proBNP. Whether low serum sodium itself leads to poor outcome or is a marker for (unidentified) co-morbidity severity or use of specific medications remains to be elucidated.

Introduction

Hyponatremia, defined as a serum sodium concentration <135 mmol/L, is the most prevalent electrolyte disorder in clinical practice (1). Hyponatremia occurs frequently in hospitalized patients (1,2). The prevalence of hyponatremia in the general population was estimated to be approximately 2-8% (3-5) and was reported to be significantly higher among patients with diabetes mellitus (3,4). Furthermore, hyponatremia has been associated with an increased mortality risk in various study populations including hospitalized patients (2), ambulatory patients with chronic kidney disease (6), heart failure (2,7), and also in the general population (3-5,8).

Hyponatremia is primarily a disorder of water balance, with a relative excess of body water compared to total sodium content, mostly caused by elevated secretion of arginine vasopressin (AVP), also known as anti-diuretic hormone (ADH) (1). For instance, in patients with heart failure, the AVP concentration dramatically increases due to severe effective circulating volume depletion (1,9). The resulting sodium and water retention attenuates the effective circulating volume depletion, but ultimately at the expense of the occurrence of hyponatremia (9).

Importantly, diabetes is a condition predisposing for both elevated levels of AVP and heart failure (10-12). Both factors are common causes of hyponatremia, but are also associated with an increased mortality risk (13-15). Therefore, our aim was to investigate whether serum sodium concentration is associated with cardiovascular and all-cause mortality in patients with type 2 diabetes, and whether a potential association could be explained by copeptin, a surrogate marker for AVP, or NT-proBNP, a marker of heart failure. We additionally aimed to investigate whether copeptin and NT-proBNP are associated with cardiovascular and all-cause mortality independent of serum sodium concentrations.

Materials and Methods

Study group

The Zwolle Outpatient Diabetes Project Integrating Available Care (ZODIAC) study was initiated in the Zwolle region of the Netherlands. The design and details of the ZODIAC study have been published elsewhere (16,17). In brief, the objective was to investigate the effects of a shared-care project for patients with type 2 diabetes that were treated in primary care. Patients who were already treated for diabetes in secondary care, patients with a very short life expectancy (including patients with active cancer) and patients with insufficient cognitive abilities were excluded from participation. In 1998, 1,142 patients with type 2 diabetes were included in this prospective cohort study. In 2001, 546 patients with type 2 diabetes were additionally enrolled, leaving a combined cohort of 1,688 patients (18). Baseline serum sodium, copeptin, and NT-proBNP concentrations were measured in 1,071 patients (63.4%). Three patients with extreme outlying values of serum sodium (severe hypernatremia, i.e., serum sodium ≥155 mmol/L) were excluded from analyses. In the current study, 1,068 patients (63.3%) were included for analyses. The ZODIAC study was approved by the local medical ethics committee, and all patients provided informed consent.

Data collection and measurements

Baseline data were collected in 1998 and 2001, consisting of a full medical history including a history of macrovascular complications, use of medication, and tobacco consumption. Patients were considered to have a history of macrovascular complications if they had a history of angina pectoris, myocardial infarction, percutaneous transluminal coronary angioplasty, coronary artery bypass grafting, stroke or transient ischemic attack. Laboratory and physical assessment data included non-fasting lipid profile, glycated hemoglobin (HbA_{1c}), serum creatinine (SCr), urinary albumin-to-creatinine ratio (ACR), and blood pressure. Albuminuria was defined as ACR >3.5 mg/mmol for women or >2.5 mg/mmol for men. SCr was measured by a kinetic colorimetric Jaffe method (Modular P Analyzer, Roche Almere, the Netherlands). The creatinine-based CKD-EPI equation was used to calculate the estimated glomerular filtration rate (eGFR) (19). Because SCr measurements in this study were not standardized to isotope dilution mass spectrometry, SCr levels were reduced by 5% to calculate the eGFR (20). Serum sodium was measured by indirect ISE (Roche Modular P, Mannheim, Germany). Plasma copeptin was measured using a sandwich immunoassay (BRAHMS GmbH, Hennigsdorf/Berlin, Germany) based on the assay described by Morgenthaler et al. (21). Plasma NT-proBNP was measured using the Roche Modular E170 (Roche

Diagnostics, Mannheim, Germany).

Vital status and cause of death were retrieved from records maintained by the hospital and the general practitioners in 2013. Cause of death was coded according to the International Classification of Diseases, 9th revision (ICD-9).

Statistical analyses

Statistical analyses were performed using SPSS version 22.0 for Windows (IBM Corporation, Chicago, IL), STATA version 11 (StataCorp LP, College Station, TX), and R version 3.0.1 (Vienna, Austria) (http://cran.r-project.org/). Results are presented as mean ± standard deviation (SD) for variables with a normal distribution and as median [interquartile range] for variables with a skewed distribution. Nominal data are presented as the number of patients with percentage (n [%]). A two-sided $P<0.05$ was considered to indicate statistical significance.

Baseline patient characteristics are presented according to five groups of serum sodium concentrations (i.e., ≤139, 140-141, 142-143, 144-145, and ≥146 mmol/L). *P*-values for differences between groups of sodium were assessed using ANOVA for normally distributed data, Kruskal-Wallis test for skewed data, and the χ^2 test for nominal data. Multivariable linear regression analyses were used to investigate whether serum sodium was associated with clinical parameters. Variables with a skewed distribution were logarithmically transformed to fulfill criteria for linear regression analyses.

Several subjects had missing values for one or more baseline variables (i.e., BMI, systolic and diastolic blood pressure, and serum potassium [<0.2%]; smoking and diabetes duration [0.6%]; and ACR [4.6%]). Because excluding subjects with missing values could result in bias, multiple imputation (fully conditional specification [MCMC]) was used to obtain 5 imputed datasets (22,23). Rubin's rules were used to obtain pooled estimates of the regression coefficients and their standard errors across the imputed datasets (24).

We used Cox regression analyses with restricted cubic splines (RCS) with 3 knots positioned at serum sodium concentrations of 139, 142, and 146 mmol/L to test for potential non-linearity of the prospective associations of serum sodium with cardiovascular and all-cause mortality.

Cox regression analyses were used to investigate the associations of baseline serum sodium, copeptin, and NT-proBNP with cardiovascular and all-cause mortality. First, we tested whether the associations of serum sodium, copeptin, and NT-proBNP with mortality differed between the two sub-cohorts (i.e., 1998 and 2001). Since we found no significant differences between the two sub-cohorts, we analyzed the sub-cohorts together using Cox regression in order to take the individual follow-up

times into account. Various Cox regression models were built to adjust for possible confounders. First, the univariable associations of serum sodium, copeptin, and NT-proBNP with cardiovascular and all-cause mortality were investigated. The first multivariable model consisted of serum sodium, copeptin, and NT-proBNP. In model 2, we adjusted for age and sex. In model 3, we additionally adjusted for BMI, smoking, systolic blood pressure, total cholesterol-to-HDL ratio, duration of diabetes, HbA_{1c}, history of macrovascular complications, use of antihypertensive medication including diuretics and RAAS inhibitors, and serum potassium. In our final multivariable model, we additionally adjusted for renal function parameters (i.e., log SCr and log ACR). The assumption of proportional hazards for baseline variables was investigated by inspecting the Schoenfeld residuals and log-log survival plots.

As sensitivity analyses, we repeated the Cox regression analyses in subjects that did not use any kind of antihypertensive drugs at baseline.

Results

Patient characteristics

In this study, 1,068 patients with type 2 diabetes were included. Mean age of included subjects was 67 ± 12 years and 483 subjects were male (45.2%). At baseline, 382 out of 1,068 subjects (36%) had one or more macrovascular complications and 397 subjects (39%) had (micro)albuminuria. Mean serum sodium was 142 ± 3 mmol/L. Fifteen patients (1.4%) had mild hyponatremia (i.e., serum sodium 126-134 mmol/L), 940 patients (88.0%) had serum sodium values within the normal range (i.e., serum sodium 135-145 mmol/L), and 113 patients (10.6%) had mild hypernatremia (i.e., serum sodium 146-154 mmol/L). Baseline patient characteristics according to groups based on serum sodium concentrations are presented in Table 1. Baseline characteristics that were significantly different across groups of serum sodium were BMI, ACR, presence of (micro)albuminuria, copeptin, NT-proBNP, and serum potassium. Furthermore, HbA_{1c} and diabetes duration were significantly higher in subjects with lower serum sodium concentrations. In multivariable linear regression analysis, male sex (β -0.14, $P<0.001$), HbA_{1c} (β -0.28, $P<0.001$), use of antihypertensive drugs (β -0.06, $P=0.04$), and log ACR (β -0.07, $P=0.03$) were inversely associated with serum sodium, whereas $\log_2$ copeptin (β 0.24, $P<0.001$) was positively associated with serum sodium.

Table 1. Baseline patient characteristics of the study population according to five groups of serum sodium concentration.

	Serum sodium (mmol/L)					*P*-value
	≤139 (n=156)	140-141 (n=221)	142-143 (n=349)	144-145 (n=229)	≥146 (n=113)	
Demographics						
Male gender (n, %)	76 (48.7)	107 (48.4)	155 (44.4)	95 (41.5)	50 (44.2)	0.5
Age (years)	67 ± 12	66 ± 12	67 ± 11	68 ± 12	68 ± 11	0.6
Smoking (n, %)	38 (24.4)	44 (19.9)	63 (18.1)	63 (18.1)	23 (14.0)	0.1
Macrovascular complications (n, %)	59 (37.8)	89 (40.3)	117 (33.5)	79 (34.5)	38 (33.6)	0.5
Body composition						
BMI (kg/m^2)	28.2 ± 4.9	29.7 ± 5.1	29.4 ± 4.7	28.8 ± 4.7	29.4 ± 4.6	0.03
Blood pressure						
SBP (mmHg)	151 ± 25	154 ± 23	154 ± 25	152 ± 24	152 ± 23	0.6
DBP (mmHg)	83 ± 10	84 ± 12	84 ± 11	83 ± 10	84 ± 11	0.8
Antihypertensive treatment (n, %)	81 (51.9)	104 (47.1)	184 (52.7)	105 (45.9)	50 (44.2)	0.3
Lipids						
Total cholesterol (mmol/L)	5.5 ± 1.2	5.6 ± 1.2	5.5 ± 1.1	5.5 ± 1.0	5.7 ± 1.1	0.6
HDL cholesterol (mmol/L)	1.2 ± 0.4	1.2 ± 0.3	1.2 ± 0.3	1.2 ± 0.3	1.2 ± 0.6	0.4
Triglycerides (mmol/L)	2.0 [1.4-3.3]	2.3 [1.6-3.2]	2.0 [1.4-3.2]	2.0 [1.4-3.0]	2.1 [1.6-2.8]	0.3
Cholesterol-HDL ratio	5.1 ± 1.6	5.1 ± 1.5	4.9 ± 1.5	4.8 ± 1.4	5.1 ± 1.5	0.1
Use of lipid lowering drugs (n, %)	18 (11.5)	33 (14.9)	54 (15.5)	32 (14.0)	13 (11.5)	0.7
Glucose homeostasis						
Duration diabetes (years)	6 [3-12]	5 [2-10]	5 [2-10]	4 [2-8]	3 [2-7]	0.001
HbA_{1c} (%)	7.8 [6.7-9.0]	7.3 [6.5-8.4]	7.0 [6.3-8.1]	6.6 [6.1-7.3]	6.7 [6.1-7.8]	<0.001
HbA_{1c} (mmol/mol)	62 [50-75]	56 [48-68]	53 [45-65]	49 [43-56]	50 [43-62]	<0.001

Table 1. (Continued).

	Serum sodium (mmol/L)					*P*-value
	≤139 (n=156)	140-141 (n=221)	142-143 (n=349)	144-145 (n=229)	≥146 (n=113)	
Renal function						
ACR (mg/mmol)	2.5 [1.1-8.0]	2.0 [1.0-7.1]	2.0 [0.9-5.7]	1.4 [0.8-4.1]	2.3 [0.8-7.0]	0.006
(Micro)albuminuria (n, %)	64 (41.0)	88 (39.8)	134 (38.4)	67 (29.3)	44 (38.9)	0.04
Serum creatinine (μmol/L)	95 ± 24	94 ± 17	94 ± 19	97 ± 26	96 ± 19	0.5
eGFR (mL/min/1.73m^2)	69 ± 18	70 ± 17	69 ± 17	67 ± 17	66 ± 16	0.1
Other						
Copeptin (pmol/L)	4.0 [2.7-8.8]	5.4 [3.1-8.7]	5.4 [3.1-9.4]	5.6 [3.3-10.1]	8.5 [4.5-14.4]	<0.001
NT-proBNP (ng/L)	92 [36-228]	78 [28-180]	93 [38-218]	99 [48-232]	107 [51-379]	0.01
Serum potassium (mmol/L)	4.5 ± 0.5	4.4 ± 0.3	4.4 ± 0.4	4.4 ± 0.4	4.4 ± 0.4	0.03

Abbreviations: ACR, urinary albumin-to-creatinine ratio; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein.

Serum sodium and mortality

After a follow-up period of 15 years (median 11.1 [5.9-14.1] years), 519 of 1,068 patients (48.6%) died, with 225 deaths (21.1%) attributable to cardiovascular causes. In univariable analyses, we found significant deviances from linear associations of serum sodium with cardiovascular (*P*=0.02) and all-cause mortality (*P*=0.003) (Figure 1). We found no significant deviances from linear associations in multivariable analyses (*P*=0.9 for cardiovascular and *P*=0.7 for all-cause mortality; Figure 1).

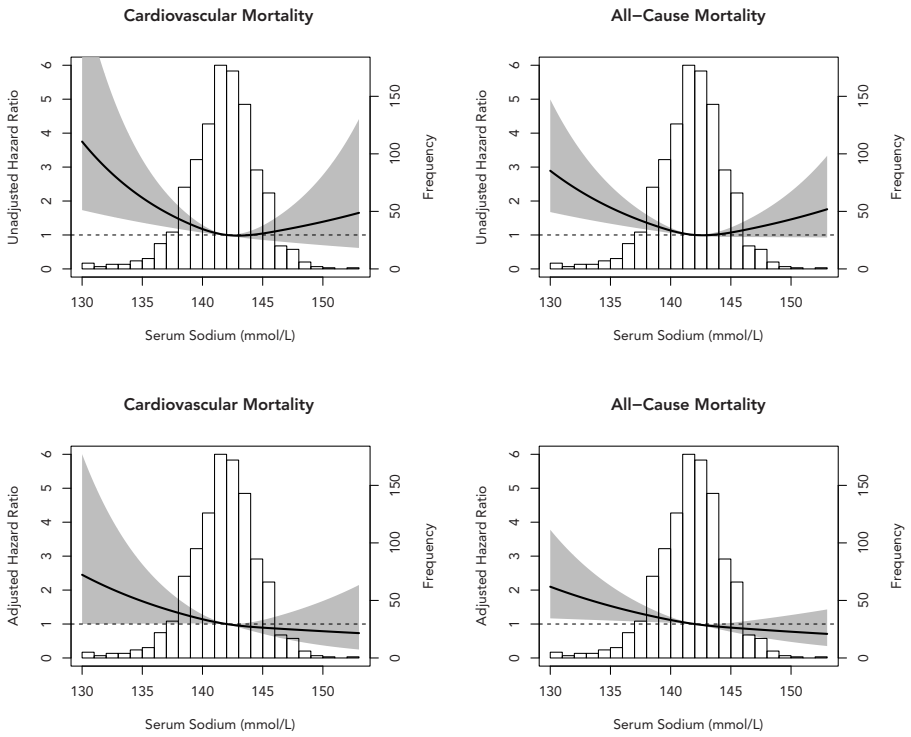

Figure 1. Restricted cubic splines depicting the association of serum sodium concentrations with cardiovascular and all-cause mortality. The line in the graph represents the (un)adjusted risk for cardiovascular and all-cause mortality. The grey area represents the 95% CI of the HR.

Low serum sodium, high copeptin, and high NT-proBNP were significantly associated with cardiovascular and all-cause mortality in univariable Cox regression analyses (Table 2). These associations remained significant after combining these three markers in one multivariable model (Table 2, model 1). The associations of serum sodium and NT-proBNP with mortality remained significant after further adjustment for potential confounders (Table 2, models 2-4). The association of copeptin with cardiovascular mortality, however, lost significance after adjustment for SCr and ACR (Table 2, model 4). Univariable and fully adjusted multivariable associations of copeptin and NT-proBNP with cardiovascular and all-cause mortality are depicted in Supplementary Figures S1 and S2, respectively.

Table 2. Associations of baseline serum sodium, log_2 copeptin, and log_2 NT-proBNP concentrations with cardiovascular and all-cause mortality in univariable and multivariable Cox regression analyses.

	Serum sodium (mmol/L)		Log_2 copeptin (pmol/L)		Log_2 NT-proBNP (ng/L)	
	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
Cardiovascular Mortality[a]						
Univariable	0.89 (0.83-0.96)[b]	0.002	1.51 (1.33-1.71)	<0.001	1.73 (1.61-1.85)	<0.001
Multivariable						
Model 1	0.90 (0.87-0.94)	<0.001	1.37 (1.21-1.55)	<0.001	1.71 (1.60-1.83)	<0.001
Model 2	0.92 (0.88-0.96)	<0.001	1.33 (1.17-1.52)	<0.001	1.51 (1.40-1.66)	<0.001
Model 3	0.94 (0.90-0.99)	0.02	1.23 (1.07-1.42)	0.004	1.51 (1.38-1.64)	<0.001
Model 4	0.95 (0.90-0.99)	0.03	1.13 (0.97-1.31)	0.1	1.44 (1.33-1.57)	<0.001
All-Cause Mortality[c]						
Univariable	0.91 (0.86-0.95)[b]	<0.001	1.40 (1.28-1.52)	<0.001	1.58 (1.51-1.65)	<0.001
Multivariable						
Model 1	0.93 (0.90-0.95)	<0.001	1.30 (1.20-1.42)	<0.001	1.57 (1.50-1.64)	<0.001
Model 2	0.94 (0.91-0.97)	<0.001	1.28 (1.17-1.39)	<0.001	1.34 (1.27-1.41)	<0.001
Model 3	0.95 (0.92-0.98)	0.002	1.21 (1.10-1.33)	<0.001	1.34 (1.27-1.42)	<0.001
Model 4	0.95 (0.92-0.99)	0.004	1.18 (1.08-1.30)	0.001	1.31 (1.24-1.39)	<0.001

Abbreviations: ACEi, angio-converting enzyme inhibitor; ACR, urinary albumin-to-creatinine ratio; ARB, angiotensin receptor blocker; HDL, high-density lipoprotein; SCr, serum creatinine.

[a] Cardiovascular mortality, n_{events}/n_{total} =225/1,068.

[b] HR (95% CI) for serum sodium concentrations ≤142 mmol/L.

[c] All-cause mortality, n_{events}/n_{total} =519/1,068.

Model 1: serum sodium, log_2 copeptin, and log_2 NT-proBNP.

Model 2: as model 1 + age and sex.

Model 3: as model 2 + BMI, smoking, systolic blood pressure, cholesterol-HDL ratio, duration of diabetes, HbA_{1c}, macrovascular complications, use of antihypertensive drugs including diuretics and ACEi/ARBs, and serum potassium.

Model 4: as model 3 + log SCr, and log ACR.

Sensitivity analyses

As sensitivity analyses, we repeated the Cox regression analyses in 544 subjects that did not use any kind of antihypertensive medication at baseline. Hazard ratios of serum sodium for cardiovascular and all-cause mortality were materially unchanged after exclusion of subjects that used antihypertensive drugs at baseline (Table 3).

Table 3. Associations of baseline serum sodium, log_2 copeptin, and log_2 NT-proBNP concentrations with cardiovascular and all-cause mortality in univariable and multivariable Cox regression analyses in subjects without antihypertensive treatment at baseline.

	Serum sodium (mmol/L)		Log_2 copeptin (pmol/L)		Log_2 NT-proBNP (ng/L)	
	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
Cardiovascular Mortality[a]						
Univariable	0.87 (0.78-0.98)[b]	0.03	1.43 (1.16-1.76)	0.001	1.75 (1.58-1.93)	<0.001
Multivariable						
Model 1	0.89 (0.83-0.95)	<0.001	1.57 (1.28-1.93)	<0.001	1.88 (1.68-2.10)	<0.001
Model 2	0.90 (0.84-0.96)	0.002	1.45 (1.17-1.81)	0.001	1.65 (1.45-1.87)	<0.001
Model 3	0.92 (0.89-0.96)	0.03	1.36 (1.08-1.72)	0.009	1.59 (1.40-1.82)	<0.001
Model 4	0.91 (0.84-0.99)	0.02	1.26 (0.99-1.59)	0.06	1.51 (1.32-1.74)	<0.001
All-Cause Mortality[c]						
Univariable	0.91 (0.84-0.98)[b]	0.02	1.20 (1.05-1.37)	0.006	1.61 (1.50-1.72)	<0.001
Multivariable						
Model 1	0.91 (0.87-0.95)	<0.001	1.29 (1.13-1.47)	<0.001	1.68 (1.56-1.80)	<0.001
Model 2	0.92 (0.88-0.96)	<0.001	1.20 (1.04-1.38)	0.01	1.43 (1.32-1.56)	<0.001
Model 3	0.93 (0.89-0.98)	0.003	1.12 (0.96-1.30)	0.1	1.40 (1.29-1.53)	<0.001
Model 4	0.93 (0.89-0.98)	0.004	1.10 (0.94-1.28)	0.2	1.38 (1.26-1.51)	<0.001

Abbreviations: ACR, urinary albumin-to-creatinine ratio; HDL, high-density lipoprotein; SCr, serum creatinine.

[a] Cardiovascular mortality, n_{events}/n_{total} = 96/544.

[b] HR (95% CI) for serum sodium concentrations ≤142 mmol/L.

[c] All-cause mortality, n_{events}/n_{total} = 229/544.

Model 1: serum sodium, log_2 copeptin, and log_2 NT-proBNP.

Model 2: as model 1 + age and sex.

Model 3: as model 2 + BMI, smoking, systolic blood pressure, cholesterol-HDL ratio, duration of diabetes, HbA_{1c}, macrovascular complications, and serum potassium.

Model 4: as model 3 + log SCr, and log ACR.

Discussion

Our study demonstrates that low serum sodium, high copeptin, and high NT-proBNP are associated with an increased cardiovascular and all-cause mortality risk in patients with type 2 diabetes treated in primary care. The association of serum sodium with mortality was not explained by copeptin, a surrogate marker for AVP, and NT-proBNP, a marker for heart failure. Furthermore, the associations of copeptin and NT-proBNP with mortality were independent of serum sodium concentrations. The associations of serum sodium and NT-proBNP with cardiovascular and all-cause mortality remained significant after adjustment for potential confounders, whereas the association of copeptin with cardiovascular mortality lost significance after adjustment for SCr and ACR.

Several studies have reported that the prevalence of hyponatremia was significantly higher among patients with diabetes than in the general population (3,4). In the NHANES cohort, the prevalence of hyponatremia, defined as serum sodium <133 mmol/L for the 1999-2002 survey and <136 for the 2003-2004 survey, was 1.7% in the US general population and 3.3% among subjects with diabetes (4). In line with these previous studies, we found that the prevalence of hyponatremia was 0.7% in a Dutch general population-based cohort (i.e., the PREVEND study [unpublished data]), and that this prevalence was higher (i.e., 1.4%) among patients with type 2 diabetes.

In most hyponatremic disorders, disproportionate AVP secretion is the predominant mechanism causing hyponatremia (25,26). Under normal conditions, AVP secretion depends on effective serum osmolality (osmoregulation) and circulating volume (baroregulation). Several studies have reported that AVP levels are elevated in patients with diabetes mellitus, which could be a result of increased plasma osmolality (10,27). Furthermore, elevated AVP levels have been associated with an increased all-cause and cardiovascular mortality risk in subjects with type 2 diabetes (13,14). We found that the association of serum sodium with mortality was not explained by copeptin. This suggests that, even though hyponatremia might be a secondary reflection of elevated AVP levels, this is not always the case and therefore combination of serum sodium and copeptin may be useful.

Moreover, we found that copeptin was associated with an increased cardiovascular and all-cause mortality risk independent of serum sodium and NT-proBNP, the latter being a marker of the cardiac response to left-ventricular filling pressure and hemodynamic status (28-30). This suggests that the association of copeptin with cardiovascular and all-cause mortality is independent of effects on osmoregulation and volume regulation. The association of copeptin with cardiovascular mortality, however,

lost significance after adjustment for renal function parameters (i.e., SCr and ACR). A number of studies, both experimental and human, have demonstrated adverse effects of vasopressin on renal function and albuminuria (31-33). Because chronic kidney disease (CKD) and albuminuria are also important risk factors for cardiovascular disease (34), elevated vasopressin levels may contribute to an increased cardiovascular risk through its effects on renal function. Adjustment for renal function parameters, which are most likely part of the causal pathway between elevated vasopressin and cardiovascular mortality, may therefore result in material weakening of associations and cause significant associations to disappear (35).

Because diabetes is an important risk factor for development of heart failure (11,12) and hyponatremia is a common condition in patients with advanced heart failure (9), we also investigated whether the association of serum sodium with mortality in patients with type 2 diabetes could be explained by elevated levels of NT-proBNP. In line with a study of Sajadieh et al. (5), we found that low serum sodium was associated with an increased risk for mortality irrespective of plasma NT-proBNP levels. Several additional studies also demonstrated that the association of hyponatremia with mortality was independent of heart failure (4,6,8). However, these studies adjusted for a history of heart failure rather than for continuous NT-proBNP levels.

We acknowledge that this study has several limitations. Given the observational nature of this study it is impossible to draw a definite conclusion about the causality of the association of serum sodium with all-cause and cardiovascular mortality. In addition, hyperglycemia-induced hyponatremia (i.e., hyponatremia caused by dilution due to hyperosmolality) can be present in patients with diabetes. Unfortunately, because no glucose measurements were performed in this outpatient clinical care cohort, we were not able to calculate glucose corrected sodium concentrations. Furthermore, pseudohyponatremia (i.e., a laboratory artefact that can occur when abnormally high concentrations of lipids or proteins in serum interfere with accurate measurement of serum sodium concentrations) may be an issue (1). However, this laboratory artefact is seen less frequently when serum sodium concentration is measured using ion-selective electrodes (1), like it is the case for the present study. Finally, because data on the use of isolated and specific (i.e., thiazide or loop-) diuretics were not available for this study group, we were not able to adjust for use of specific diuretics. However, we repeated Cox regression analyses in subjects that did not use any kind of antihypertensive medication at baseline to eliminate any kind of residual confounding.

A strength of this study is that it is the first to investigate the association of serum sodium with all-cause and cardiovascular mortality in subjects with type 2 diabetes. Furthermore, this study included a relatively large observational cohort of patients with

type 2 diabetes treated in primary care with a long follow-up period and measurement of markers of both osmoregulation and volume regulation (i.e., serum sodium, copeptin, and NT-proBNP).

Conclusion

In this study, we found that low serum sodium was associated with an increased risk of all-cause and cardiovascular mortality in patients with type 2 diabetes. The association of serum sodium with mortality was not explained by copeptin or NT-proBNP. Furthermore, the associations of copeptin and NT-proBNP with mortality were independent of serum sodium concentrations. Whether low serum sodium itself leads to poor outcome or that it is a marker for (unidentified) co-morbidity severity or use of specific medications remains to be elucidated.

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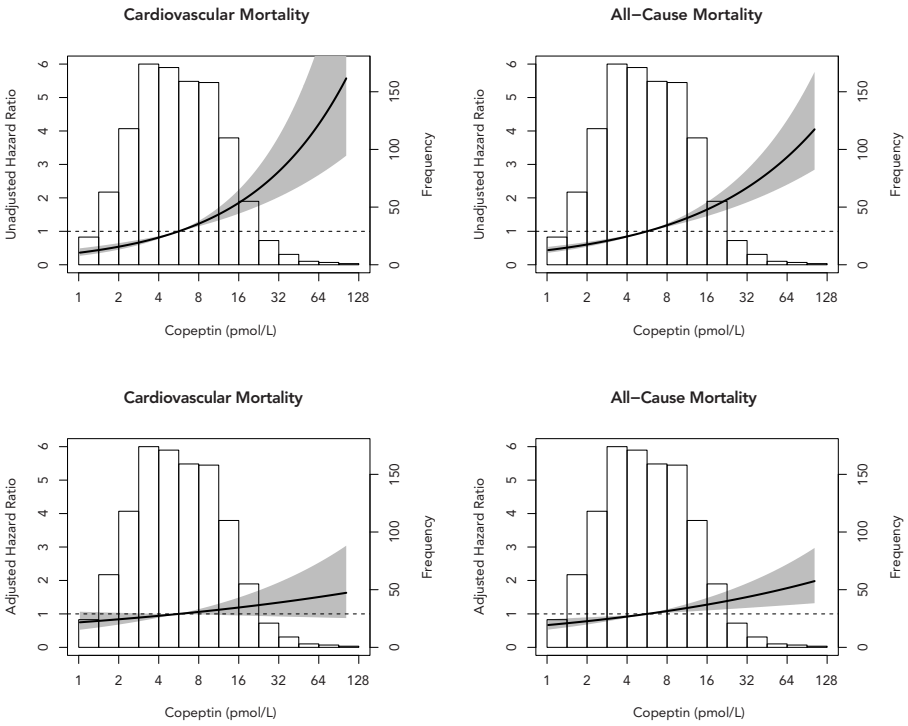
References

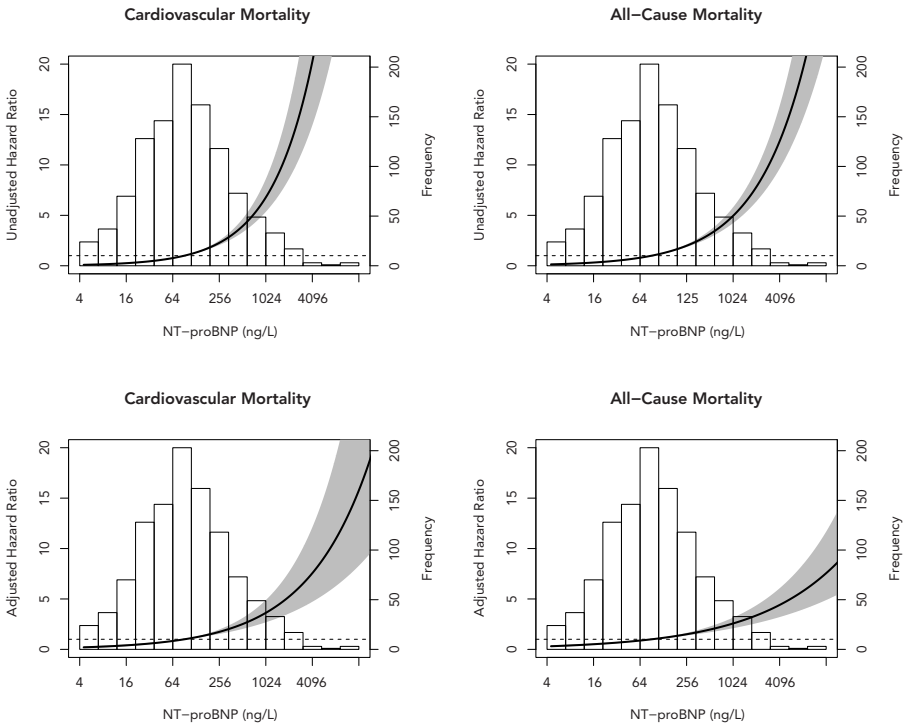
1. Spasovski G, Vanholder R, Allolio B, Annane D, Ball S, Bichet D, et al. Clinical practice guideline on diagnosis and treatment of hyponatraemia. *Nephrol Dial Transplant* 2014; 29 Suppl 2: i1-i39.
2. Corona G, Giuliani C, Parenti G, Norello D, Verbalis JG, Forti G, et al. Moderate hyponatremia is associated with increased risk of mortality: evidence from a meta-analysis. *PLoS One* 2013; 8(12): e80451.
3. Liamis G, Rodenburg EM, Hofman A, Zietse R, Stricker BH, Hoorn EJ. Electrolyte disorders in community subjects: prevalence and risk factors. *Am J Med* 2013; 126(3): 256-63.
4. Mohan S, Gu S, Parikh A, Radhakrishnan J. Prevalence of hyponatremia and association with mortality: results from NHANES. *Am J Med* 2013; 126(12): 1127,37.e1.
5. Sajadieh A, Binici Z, Mouridsen MR, Nielsen OW, Hansen JF, Haugaard SB. Mild hyponatremia carries a poor prognosis in community subjects. *Am J Med* 2009; 122(7): 679-86.
6. Kovesdy CP, Lott EH, Lu JL, Malakauskas SM, Ma JZ, Molnar MZ, et al. Hyponatremia, hypernatremia, and mortality in patients with chronic kidney disease with and without congestive heart failure. *Circulation* 2012; 125(5): 677-84.
7. Milo-Cotter O, Cotter G, Weatherley BD, Adams KF, Kaluski E, Uriel N, et al. Hyponatraemia in acute heart failure is a marker of increased mortality but not when associated with hyperglycaemia. *Eur J Heart Fail* 2008; 10(2): 196-200.
8. Gankam-Kengne F, Ayers C, Khera A, de Lemos J, Maalouf NM. Mild hyponatremia is associated with an increased risk of death in an ambulatory setting. *Kidney Int* 2013; 83(4): 700-6.
9. Schrier RW, Sharma S, Shchekochikhin D. Hyponatraemia: more than just a marker of disease severity? *Nat Rev Nephrol* 2013; 9(1): 37-50.
10. Zerbe RL, Vinicor F, Robertson GL. Plasma vasopressin in uncontrolled diabetes mellitus. *Diabetes* 1979; 28(5): 503-8.
11. He J, Ogden LG, Bazzano LA, Vupputuri S, Loria C, Whelton PK. Risk factors for congestive heart failure in US men and women: NHANES I epidemiologic follow-up study. *Arch Intern Med* 2001; 161(7): 996-1002.
12. Kannel WB, Ho K, Thom T. Changing epidemiological features of cardiac failure. *Br Heart J* 1994; 72(2 Suppl): S3-9.
13. Riphagen IJ, Boertien WE, Alkhalaf A, Kleefstra N, Gansevoort RT, Groenier KH, et al. Copeptin, a surrogate marker for arginine vasopressin, is associated with cardiovascular and all-cause mortality in patients with type 2 diabetes (ZODIAC-31). *Diabetes Care* 2013; 36(10): 3201-7.
14. Fenske W, Wanner C, Allolio B, Drechsler C, Blouin K, Lilienthal J, et al. Copeptin levels associate with cardiovascular events in patients with ESRD and type 2 diabetes mellitus. *J Am Soc Nephrol* 2011; 22(4): 782-90.
15. Miller WL, Grill DE, Struck J, Jaffe AS. Association of hyponatremia and elevated copeptin with death and need for transplantation in ambulatory patients with chronic heart failure. *Am J Cardiol* 2013; 111(6): 880-5.
16. Ubink-Veltmaat LJ, Bilo HJ, Groenier KH, Rischen RO, Meyboom-de Jong B. Shared care with task delegation to nurses for type 2 diabetes: prospective observational study. *Neth J Med* 2005; 63(3): 103-10.
17. Drion I, Kleefstra N, Landman GW, Alkhalaf A, Struck J, Groenier KH, et al. Plasma COOH-Terminal Proendothelin-1: A marker of fatal cardiovascular events, all-cause mortality, and

new-onset albuminuria in type 2 diabetes? (ZODIAC-29). *Diabetes Care* 2012; 35(11): 2354-8.
18. Lutgers HL, Gerrits EG, Sluiter WJ, Ubink-Veltmaat LJ, Landman GW, Links TP, et al. Life expectancy in a large cohort of type 2 diabetes patients treated in primary care (ZODIAC-10). *PLoS One* 2009; 4(8): e6817.
19. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF,3rd, Feldman HI, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med* 2009; 150(9): 604-12.
20. Levey AS, Coresh J, Greene T, Marsh J, Stevens LA, Kusek JW, et al. Expressing the Modification of Diet in Renal Disease Study equation for estimating glomerular filtration rate with standardized serum creatinine values. *Clin Chem* 2007; 53(4): 766-72.
21. Morgenthaler NG, Struck J, Alonso C, Bergmann A. Assay for the measurement of copeptin, a stable peptide derived from the precursor of vasopressin. *Clin Chem* 2006; 52(1): 112-9.
22. de Goeij MC, van Diepen M, Jager KJ, Tripepi G, Zoccali C, Dekker FW. Multiple imputation: dealing with missing data. *Nephrol Dial Transplant* 2013; 28(10): 2415-20.
23. Sterne JA, White IR, Carlin JB, Spratt M, Royston P, Kenward MG, et al. Multiple imputation for missing data in epidemiological and clinical research: potential and pitfalls. *BMJ* 2009; 338: b2393.
24. Harel O, Zhou XH. Multiple imputation: review of theory, implementation and software. *Stat Med* 2007; 26(16): 3057-77.
25. Fenske W, Stork S, Blechschmidt A, Maier SG, Morgenthaler NG, Allolio B. Copeptin in the differential diagnosis of hyponatremia. *J Clin Endocrinol Metab* 2009; 94(1): 123-9.
26. Schrier RW. Body water homeostasis: clinical disorders of urinary dilution and concentration. *J Am Soc Nephrol* 2006; 17(7): 1820-32.
27. Iwasaki Y, Kondo K, Murase T, Hasegawa H, Oiso Y. Osmoregulation of plasma vasopressin in diabetes mellitus with sustained hyperglycemia. *J Neuroendocrinol* 1996; 8(10): 755-60.
28. Richards AM, Crozier IG, Yandle TG, Espiner EA, Ikram H, Nicholls MG. Brain natriuretic factor: regional plasma concentrations and correlations with haemodynamic state in cardiac disease. *Br Heart J* 1993; 69(5): 414-7.
29. Troughton RW, Frampton CM, Yandle TG, Espiner EA, Nicholls MG, Richards AM. Treatment of heart failure guided by plasma aminoterminal brain natriuretic peptide (N-BNP) concentrations. *Lancet* 2000; 355(9210): 1126-30.
30. Slagman MC, Waanders F, Vogt L, Damman K, Hemmelder M, Navis G, et al. Elevated N-terminal pro-brain natriuretic peptide levels predict an enhanced anti-hypertensive and anti-proteinuric benefit of dietary sodium restriction and diuretics, but not angiotensin receptor blockade, in proteinuric renal patients. *Nephrol Dial Transplant* 2012; 27(3): 983-90.
31. Boertien WE, Riphagen IJ, Drion I, Alkhalaf A, Bakker SJ, Groenier KH, et al. Copeptin, a surrogate marker for arginine vasopressin, is associated with declining glomerular filtration in patients with diabetes mellitus (ZODIAC-33). *Diabetologia* 2013; 56(8): 1680-8.
32. Velho G, Bouby N, Hadjadj S, Matallah N, Mohammedi K, Fumeron F, et al. Plasma copeptin and renal outcomes in patients with type 2 diabetes and albuminuria. *Diabetes Care* 2013; 36(11): 3639-45.
33. Bankir L, Bouby N, Ritz E. Vasopressin: a novel target for the prevention and retardation of kidney disease? *Nat Rev Nephrol* 2013; 9(4): 223-39.
34. KDOQI. KDOQI Clinical Practice Guidelines and Clinical Practice Recommendations for Diabetes and Chronic Kidney Disease. *Am J Kidney Dis* 2007; 49(2 Suppl 2): S12-154.
35. Hernan MA, Hernandez-Diaz S, Werler MM, Mitchell AA. Causal knowledge as a prerequisite for confounding evaluation: an application to birth defects epidemiology. *Am J Epidemiol* 2002; 155(2): 176-84.

Chapter
5



Supplementary Figure S1. The risk for cardiovascular and all-cause mortality presented by copeptin concentrations. The line in the graph represents the (un)adjusted risk for cardiovascular and all-cause mortality. The grey area represents the 95% CI of the HR.



Supplementary Figure S2. The risk for cardiovascular and all-cause mortality presented by NT-proBNP concentrations. The line in the graph represents the (un)adjusted risk for cardiovascular and all-cause mortality. The grey area represents the 95% CI of the HR.