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Cardiac Autonomic Neuropathy Predicts All-Cause and Cardiovascular Mortality in Patients With End-Stage Renal Failure: A 5-Year Prospective Study

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Introduction: Chronic renal disease is associated with increased cardiovascular (CV) mortality. Cardiac autonomic neuropathy (CAN) is predictive of mortality for diseases that affect the autonomic nervous system. We prospectively evaluated the prognostic value of indexes of left ventricular (LV) function and CAN in all-cause and CV mortality of patients with end-stage renal failure (ESRF).

Methods: A total of 133 patients with ESRF were recruited. LV function was evaluated by echocardiography, whereas cardiac autonomic function was assessed using the battery of the 4 standardized tests proposed by Ewing.

Results: A total of 123 of 133 (92.5%) patients completed the study and were followed for a mean of 4.9 ± 2.6 years. Mean LV ejection fraction (LVEF) was $50.9 \pm 6.9\%$, whereas 70 (57.9%) patients had CAN. Sixty-nine all-cause and 36 CV deaths were recorded. The survival rates at 3, 5, and 7 years were 77.2%, 57.4%, and 33.7%, respectively. Multivariate analysis after adjustment for waist circumference, current smoking, history of diabetes, and coronary artery disease demonstrated that the only independent predictors of all-cause mortality during follow-up were age, serum triglycerides, LVEF, and presence of CAN. Competing risk regression analysis, after adjusting for waist circumference, coronary heart disease, serum glucose, and triglycerides, indicated that age and presence of CAN were independent risk factors for CV mortality.

Discussion: Age and presence of CAN are independent predictors of all-cause and CV mortality in patients with ESRF. The functionality of the cardiac autonomic nervous system activity can be used for the risk stratification in patients with ESRF.

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Chronic kidney disease (CKD) is a global health problem with major socioeconomic impact. Approximately 16% of the population in Europe and 13% in the United States have CKD.[1] In the last few decades, intense scientific research has investigated increased cardiovascular (CV) and all-cause mortality of patients with CKD, both in predialysis and dialysis stages.[2] Kidney failure that requires treatment with dialysis or transplantation is the most predictable outcome of CKD. However, it is known that most patients with renal insufficiency, irrespective of their stage, die due to cardiovascular disease (CVD), well before reaching hemodialysis or kidney transplantation.[3]

Numerous studies have attempted to identify risk factors for morbidity and mortality in patients with CKD, especially in patients with end-stage renal failure (ESRF) (estimated glomerular filtration rate [eGFR] <15 ml/min/1.73 m^2).[4] Higher systolic blood pressure (SBP) and pulse pressure, lower diastolic blood pressure (DBP), left ventricular (LV) hypertrophy, and anemia have been identified as independent factors of CV morbidity and mortality in ESRF patients.[5,6] CKD is also associated with worse cardiac autonomic function. Heart rate variability (HRV) has been used as marker of cardiac autonomic neuropathy (CAN), and studies of HRV in ESRF patients have shown that a decrement in HRV is predictive of mortality.[7] Coronary

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calcifications, low serum albumin and fetuin-A levels, and increased aortic stiffness have also been associated with increased mortality in dialysis patients.[8–10] However, recent studies have suggested only limited usefulness of either single or multiple biomarkers of inflammation, oxidative stress, anemia, endothelial dysfunction, vascular calcification, and electrolyte imbalance as prognostic tools in patients with CKD.[11]

Additional tools capable of improving risk assessment are clearly warranted in this population. The aim of our study was to prospectively evaluate the prognostic value of indexes of LV function and CAN in all-cause and CV mortality in patients with ESRF, both on dialysis and in the predialysis stage.

MATERIALS AND METHODS

Patients

A total of 133 patients with ESRF were included in the study. Patients were recruited consecutively from the outpatient renal clinic and the hemodialysis center of our hospital. All patients gave written informed consent before participating in the study, which was conducted according to the principles of the Declaration of Helsinki and approved by the hospital's ethics committee.

They all underwent a complete physical examination at the beginning of the study. Established questionnaires were used to evaluate disease history, current disease, and the use of medications. Participants were also classified according to smoking habit as current smokers, ex-smokers, or nonsmokers. Height and weight were measured, and body mass index was calculated. Waist circumference was measured with a soft tape on standing, midway between the lowest rib and the iliac crest. Blood pressure was measured using an appropriate-sized cuff 3 times at 5-minute intervals, with the participant in the sitting position. The mean value of the last 2 measurements was used in the statistical analysis. Arterial hypertension was defined according to current guidelines[12] as SBP $>$140 mm Hg and/or DBP $>$90 mm Hg, or when patients were on antihypertensive treatment. Diabetes status was confirmed from medical records and treatment with antidiabetic medications. Individuals without diabetes had fasting serum glucose levels $<$126 mg/dl and glycosylated hemoglobin levels $<$6.5%. CVD was defined as coronary artery disease, cerebrovascular disease, and/or peripheral arterial disease. Coronary artery disease was defined as history of chronic angina, myocardial infarction, percutaneous transluminal coronary angioplasty, or coronary artery bypass grafting. Peripheral arterial disease was defined as presence of intermittent claudication, history of revascularization at the leg arteries, or an ankle–brachial pressure index $<$0.90. Cerebrovascular disease was defined as history of stroke.

Biochemical and Radioimmunoassay Measurements

Blood was drawn early in the morning after 8 to 10 hours of fasting. Serum lipids (total cholesterol, high-density lipoprotein cholesterol, triglycerides), albumin, and creatinine were measured on a Technicon RA–XT analyzer (Technicon Ltd, Dublin, Ireland). Low-density lipoprotein cholesterol levels were calculated using Friedewald's equation. Serum glucose was measured by the glucose oxidase-peroxidase method (Zafiropoulos, Athens, Greece). Glycosylated hemoglobin levels were determined using a DCA analyzer (DCA 2000+, Bayer HealthCare LLC, Elkhart, Indiana). GFR was calculated according to the Modification of Diet in Renal Disease equation.[13] Parathyroid hormone concentrations were measured by radioimmunoassay (CIS Bio International, Gif-sur-Yvette, France) (coefficient of variance: 4.9 $\pm$ 2.1%).

Assessment of LV Function

Complete 2-dimensional and Doppler echocardiograms were recorded using a Hewlett-Packard Sonos 1000 ultrasound system (Hewlett-Packard, Palo Alto, California). LV chamber dimensions, and septal and posterior wall thicknesses were measured according to the recommendations of the American Society of Echocardiography.[14] LV ejection fraction (LVEF) was also evaluated by Simpson's method. Using pulsed Doppler from the mitral inflow velocity curve, the following parameters were calculated: peak early velocity (E-wave), peak velocity at the time of atrial contraction (A-wave), E/A ratio, and isovolumic relaxation time (IVRT). The average values of 5 beats/min were used for analysis. The Penn convention was used for calculation of LV mass, which was normalized for body surface area (LV mass index [LVMI]).[14,15] The myocardial performance index (Tei index) of the LV, a noninvasive Doppler measurement of both LV systolic and diastolic function, was defined as the sum of the isovolumic contraction time and IVRT divided by the ejection time, and was obtained from Doppler recordings of LV inflow and outflow.[16]

Assessment of Cardiac Autonomic Nervous System Function

Cardiac autonomic function was assessed using the battery of the 4 standardized tests proposed by Ewing *et al.*[17] Heart rate response to deep breathing was assessed by calculating the ratio of the maximum and minimum heart rates during 6 cycles of paced deep breathing (E/I index). Heart rate response to standing

(standing test) was calculated as the ratio of the longest R-R interval (found at approximately beat 30) to the shortest R-R interval (found at approximately beat 15) after standing up (30:15 ratio). Heart rate response to the Valsalva maneuver was assessed by calculating the ratio of the longest R-R interval after the maneuver to the shortest R-R interval during or shortly after the maneuver (Valsalva ratio). All calculations were undertaken by measuring electrocardiographic recordings of R-R intervals automatically, using the computer-aided examination and evaluation system VariaCardio TF5 (Medical Research Limited, Leeds, United Kingdom). The tests were carried out between 7:00 and 9:00 a.m., in an environment with a stable temperature of 22°C to 24°C, and the participants were advised not to eat, smoke, or drink coffee before the examination. The heart rate–based tests were analyzed according to published age-related tables.[18] Orthostatic hypotension was diagnosed when a fall in SBP of >20 mmHg was observed; a fall of 11 to 20 mm Hg was considered borderline, and a fall of <10 mm Hg was considered a normal response.[18] Each normal autonomic function test was graded as 0, each borderline test as 1, and each abnormal test as 2. On the basis of the sum of this score, we calculated the total CAN score, which is the sum of the partial scores (minimum 0, maximum 8). CAN was diagnosed when at least 2 of the 4 tests performed were abnormal.[17,19]

Statistical Analysis

Statistical analysis was performed using the SPSS 17.0 statistical package (SPSS, Inc., Chicago, Illinois) and the Stata (version 13 for Windows; StataCorp, College Station, Texas). Normality of distribution was examined using the Kolmogorov-Smirnov test. Normally distributed continuous variables, presented as means ± SD, were compared using Student's *t*-test. Non-normally distributed continuous variables, presented as median (25th–75th percentile), were compared using the Mann-Whitney test. Categorical variables were compared using the χ^2 test or Fisher exact test, as appropriate. Bivariate correlations for continuous variables were tested using Pearson's or Spearman's correlation coefficients according to the specific indications. The survival rates of the patients were analyzed by the Kaplan-Meier method and compared using the log-rank test. The prognostic significance of each variable with respect to all-cause mortality was examined using Cox's proportional hazards models. The prognostic significance of each variable with respect to CV mortality was calculated with the cumulative incidence competing risk method based on the proportional subhazard model by Fine and Gray, using non-CV mortality as the competing event.[20] The strength of the association between each variable and the outcome was assessed using the subhazard ratio. In both Cox's proportional hazards modeling and the proportional subhazard model by Fine and Gray, prognostic factors were evaluated in univariate and multivariate analyses. Variables associated with the primary endpoint at the 5% level on the basis of univariate models were introduced in the multivariate models. Backward conditional analysis was used for variable selection in the Cox's proportional hazards multivariate model. A 2-sided 95% confidence interval (CI) was calculated around the point estimate of hazard ratio (HR) associated with each study variable. All *P* values were 2-sided, and a *P* value <0.05 was considered statistically significant for all analyses.

RESULTS

Baseline Characteristics

Ten patients attended only the baseline visit and had no follow-up visit afterward. Because only baseline data were available, censoring could not be performed. A total of 123 (92.5%) patients completed the study and were included in the final analysis. Nevertheless, no significant differences were noted in the baseline characteristics of patients who completed the study and those lost to follow-up.

The mean age of the population was 59.5 ± 14.6 years, and 64.2% were men. Diabetes was present in 39% of patients, whereas 58 (47.2%) had a history of CVD at baseline. Mean SBP was 144.3 ± 31.7 mm Hg, and hemoglobin was 10.7 ± 1.6 g/dl for the whole cohort, whereas median eGFR values for patients not on dialysis was 8.8 ml/min/1.73 m^2 (range: 5.9–11.5 ml/min/1.73 m^2). The baseline demographic and clinical characteristics of the study population are shown in Table 1.

The underlying renal diseases for cause-specific ESRF were diabetic nephropathy (38.2%), vascular and/or hypertensive nephropathy (10.6%), primary glomerular diseases (8.9%), obstructive nephropathies (8.1%), polycystic kidney disease (5.7%), glomerular disease secondary to collagen diseases (4.9%), and multiple myeloma (3.3%), whereas the cause of ESRF was unknown in 25 patients (20.3%).

Echocardiography

Mean echocardiographic measurements among study patients are shown in Table 2. Mean LVEF was 50.9 ± 6.9%, the mean value of the LVMI was 159.8 g/m^2 (159.5 ± 39.9 g/m^2 for men and 160.4 ± 39.9 g/m^2 for women). When LV hypertrophy was defined as LVMI >115 g/m^2 in men and >95 g/m^2 in women, 93.5% and 100% of the male and female subjects had LV hypertrophy, respectively. The mean values of left atrial diameter, LVEF, and early diastolic to atrial peak

Table 1. Baseline demographic and clinical characteristics of the study participants

Characteristic	Value
n	123
Age (yr)	59.5 ± 14.6
Male/female	79/44 (64.2/35.8)
Body mass index (kg/m^2)	25.1 ± 4.9
Waist circumference (cm)	90.6 ± 14.1
Systolic blood pressure (mm Hg)	144.3 ± 31.7
Diastolic blood pressure (mm Hg)	80.7 ± 13.2
Current smoking	36 (29.3)
Diabetes	48 (39.0)
Coronary artery disease	28 (22.8)
Cerebrovascular disease	10 (8.1)
Peripheral arterial disease	42 (34.0)
Any cardiovascular disease	58 (47.2)
Hemodialysis	63 (51.2)
Diabetic nephropathy	47 (38.2)
Glomerular filtration rate (ml/min/1.73 m^2)	8.8 (5.9–11.5)[a]
Hemoglobin (g/dl)	10.7 ± 1.6
Total cholesterol (mg/dl)	200.2 ± 48.2
LDL cholesterol (mg/dl)	120.2 ± 40.3
HDL cholesterol (mg/dl)	39.9 ± 14.2
Triglycerides (mg/dl)	179 (122–242)
Albumin (g/dl)	4.26 ± 0.57
Glucose (mg/dl)	109.6 ± 49.6
Parathormone (ng/l)	225.5 (96.0–351.2)
Medications	
β-blockers	18 (14.6)
ACE inhibitor/ARB	29 (23.6)
Calcium channel blocker	66 (53.7)
Diuretic	36 (29.3)
Insulin	34 (70.8)[b]

ACE, angiotensin-converting enzyme; ARB, angiotensin II receptor antagonist; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
Data are shown as mean ± SD, median (25th–75th percentile) or as n (%).
[a]Glomerular filtration rate is calculated for a study participant not on dialysis.
[b]Percentages of insulin use are calculated based on the participants with diabetes.

velocity ratio (E/A peak) were 44.5 ± 5.3 mm, 50.9 ± 6.9%, and 0.84 ± 0.2, respectively.

Cardiac Autonomic Nervous System Function

Seventy (57.9%) patients fulfilled the criteria of CAN diagnosis. Only 40 (33.1%) patients had a normal deep breathing test, whereas 69 (56.1%) had a normal orthostatic hypotension test. Data for Valsalva and the deep breathing index were missing in 2 patients due to poor compliance in performing the test. The indexes of cardiac autonomic function are shown in detail in Table 2.

Table 2. Echocardiographic and cardiac autonomic function measurements of the study participants

Characteristic	Value
Interventricular septum (mm)	12.6 ± 1.2
Left ventricular posterior wall (mm)	12.2 ± 1.2
Left ventricular ejection fraction (%)	50.9 ± 6.9
Left ventricular mass index (g/m^2 of BSA)	159.8 ± 35.6
Early diastolic to atrial peak velocity ratio (E/A ratio)	0.84 ± 0.21
E/I index[a] (normal/borderline/low)	40/17/64 (33.1/14.0/52.9)
30:15 index (normal/borderline/low)	31/24/68 (25.2/19.5/55.3)
Valsalva index[a] (normal/borderline/low)	34/17/70 (28.1/14.0/57.9)
Change in SBP (normal/borderline/low)	69/23/31 (56.1/18.7/25.2)
Total score	5 (3–6)
Presence of CAN	70 (57.9)

BSA, body surface area; CAN, cardiac autonomic neuropathy; SBP, systolic blood pressure.
Data are shown as mean ± SD, median (25th–75th percentile) or as n (%).
[a]Not available in 2 patients due to poor compliance in performing the test.

The total CAN score correlated significantly with LVMI (r = 0.199; P = 0.034), LVEF (r = −0.245; P = 0.009), and serum triglycerides (r = 0.237; P = 0.010). However, no relationship could be demonstrated between CAN score and waist circumference (r = 0.066; P = 0.475). Importantly, the results of the cardiac autonomic function tests did not differ between patients on dialysis and those not on dialysis.

Clinical Outcomes

The patients were followed for a mean of 4.9 ± 2.6 years (median: 5.7, range: 0.1–9 years), adding up to a total of 606.4 patient-years. Fifty-five of 60 (91.6%) pre-dialysis patients began hemodialysis after a mean of 0.9 ± 1.1 years, whereas 16 (13.0%) patients underwent transplantation after a mean of 3.1 ± 2.2 years. A total of 69 deaths were recorded during follow-up; 36 deaths were classified as CV, 21 as non-CV, whereas the cause of death was unidentified in 12 patients. Among the 36 CV deaths, 15 were sudden cardiac deaths, 13 were attributed to coronary artery disease, 4 to heart failure, and 4 to stroke.

The cumulative survival rates at 3, 5, and 7 years were 77.2% (SE: 0.078), 57.4% (SE: 0.149), and 33.7% (SE: 0.249), respectively (Figure 1).

By univariate analysis, age, waist circumference, smoking status, history of diabetes mellitus, history of coronary artery disease, serum glucose and triglycerides, LVEF, LVMI, and presence of CAN were all identified as predictors of all-cause mortality during follow-up (Table 3). However, after adjustment for

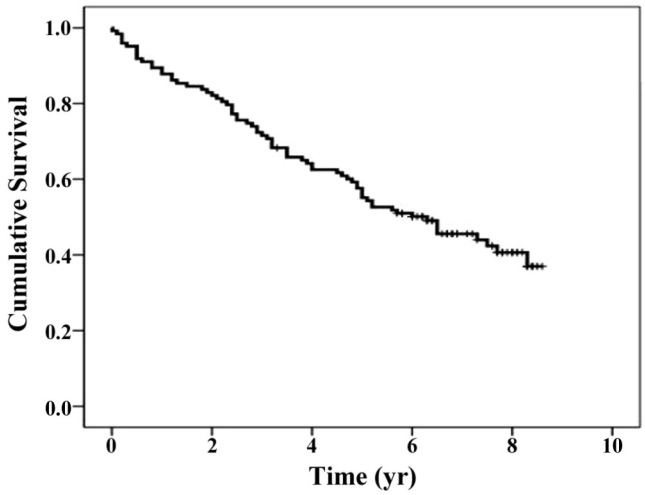

Figure 1. Cumulative survival rates of the study participants.

waist circumference, smoking status, history of diabetes, and history of coronary heart disease, the only independent predictors of mortality were age, serum triglycerides, LVEF, and presence of CAN (Table 3). Based on the mean value of the LVEF, the study population was divided into 2 groups (≤50% and >50%). The cumulative survival rates at follow-up were 30% (SE: 0.422) and 55.4% (SE: 0.365) for patients with a LVEF ≤50% versus >50% (log-rank test: $P = 0.004$) (Figure 2), and 32.9% (SE: 0.369) versus 60.8% (SE: 0.368) for patients with CAN versus without CAN ($P = 0.001$) (Figure 3).

Univariate analysis indicated that age, waist circumference, history of coronary heart disease, serum glucose and triglycerides, and presence of CAN could all predict CV mortality during follow-up (Table 4). After adjusting for waist circumference, history of coronary heart disease, serum glucose, and triglycerides, the independent predictors of CV mortality were age and presence of CAN (Table 4). The cumulative incidence of CV death among patients with and without CAN is depicted in Figure 4.

DISCUSSION

The major novel finding of our study was that the presence of CAN represented an independent risk factor for all-cause and CV mortality in patients with ESRF. The other risk factors identified for all-cause mortality were age, LVEF, and serum triglycerides. Importantly, age and CAN were the only parameters that could prognosticate the incidence of CV death during follow-up, which suggested that abnormalities

Table 3. Univariate and multivariate Cox proportional hazard models for participants' baseline characteristics as determinants of all-cause mortality

	Univariate analysis		Multivariate analysis[a,b]	
Characteristic	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Age (yr)	1.047 (1.025–1.070)	<0.001	1.074 (1.039–1.109)	<0.001
Sex (men vs. women)	1.040 (0.640–1.689)	0.874		
Body mass index (kg/m^2)	1.036 (0.993–1.080)	0.099		
Waist circumference (cm)	1.020 (1.005–1.034)	0.008		
Systolic blood pressure (mm Hg)	1.001 (0.993–1.008)	0.831		
Diastolic blood pressure (mm Hg)	0.988 (0.969–1.006)	0.188		
Current smoking (yes vs. no)	0.554 (0.312–0.984)	0.044		
Diabetes (yes vs. no)	2.357 (1.454–3.822)	0.001		
Coronary artery disease (yes vs. no)	1.759 (1.037–2.977)	0.033		
Cerebrovascular disease (yes vs. no)	2.140 (0.975–4.696)	0.058		
Peripheral artery disease (yes vs. no)	1.617 (0.961–2.720)	0.070		
Any cardiovascular disease (yes vs. no)	1.562 (0.970–2.514)	0.066		
Hemodialysis (yes vs. no)	1.250 (0.777–2.010)	0.358		
Glomerular filtration rate (ml/min/1.73 m^2)	1.039 (0.990–1.090)	0.120		
Hemoglobin (g/dl)	0.925 (0.789–1.085)	0.338		
Total cholesterol (mg/dl)	0.999 (0.994–1.005)	0.849		
LDL cholesterol (mg/dl)	0.996 (0.990–1.003)	0.258		
HDL cholesterol (mg/dl)	0.990 (0.971–1.010)	0.337		
Triglycerides (mg/dl)	1.003 (1.001–1.005)	<0.001	1.004 (1.002–1.007)	0.001
Albumin (g/dl)	0.648 (0.389–1.080)	0.096		
Glucose (mg/dl)	1.005 (1.001–1.009)	0.007		
Parathormone (ng/l)	1.000 (0.998–1.001)	0.574		
β-blocker (yes vs. no)	1.517 (0.813–2.832)	0.190		
ACE inhibitor/ARB (yes vs. no)	1.026 (0.586–1.795)	0.930		
Diuretic (yes vs. no)	1.199 (0.722–1.990)	0.483		
Calcium channel blocker (yes vs. no)	0.981 (0.612–1.574)	0.937		
Insulin (yes vs. no)	0.915 (0.463–1.810)	0.799		
Left ventricular ejection fraction (%)	0.947 (0.918–0.976)	0.001	0.921 (0.866–0.978)	0.008
Left ventricular mass index (g/m^2 of BSA)	1.006 (1.000–1.012)	0.037		
Early diastolic to atrial peak velocity ratio (E/A ratio)	0.853 (0.252–2.892)	0.799		
Cardiac autonomic neuropathy (yes vs. no)	2.347 (1.387–3.969)	0.001	2.977 (1.478–5.997)	0.002

ACE, angiotensin converting enzyme; ARB, angiotensin II receptor antagonist, BSA, body surface area; CI, confidence interval; HDL, high-density lipoprotein; HR, hazard ratio; LDL, low-density lipoprotein.

Sex, current smoking, diabetes mellitus, coronary artery disease, cerebrovascular disease, peripheral arterial disease, any cardiovascular disease, hemodialysis, use of medications, and cardiac autonomic neuropathy (yes vs. no) were analyzed as categorical variables; all the other variables were analyzed as continuous variables in both univariate and multivariate analyses.

[a]After adjustment in addition for waist circumference, smoking status, history of diabetes and history of coronary artery disease.

[b]Left ventricular ejection fraction and not left ventricular mass index was used in the final model as the index of systolic cardiac function; when left ventricular mass index was used in the multivariable analysis, it was rendered insignificant; blood glucose and diabetes were used in turn in the multivariate analysis without affecting the result of the analysis.

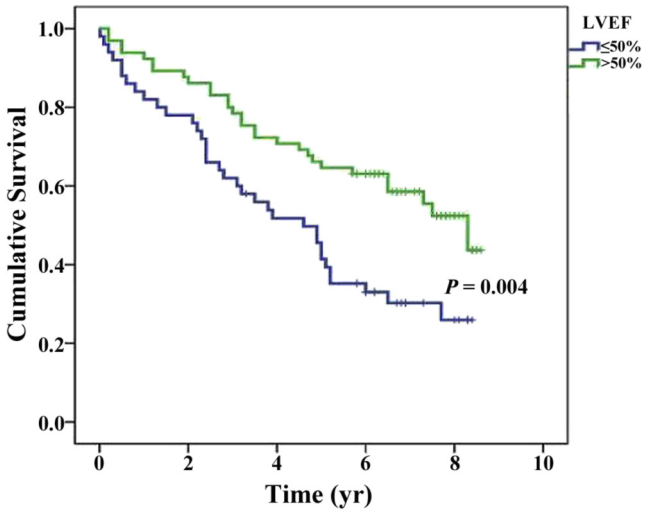

Figure 2. Cumulative survival rates of the study participants according to their left ventricular ejection fraction (LVEF).

of cardiac innervation are one of the most important pathological processes in the cascade of CV-related morbidity and mortality that complicate the end stages of CKD.

CAN was initially recognized and extensively studied in patients with diabetes.[21] The presence of CAN in this group of patients was associated with silent myocardial ischemia,[22] systolic and diastolic myocardial dysfunction even in the absence of coronary artery disease,[23] intra- and perioperative CV instability,[21] stroke,[24] progression of kidney disease,[25] and the appearance of foot ulcers.[26] Importantly, CAN was also recognized as a risk factor for increased mortality in patients with diabetes mellitus.[22]

CAN was also associated with higher triglycerides and higher waist circumference, both components of the metabolic syndrome, in a large study of individuals with impaired glucose tolerance.[27] In accordance with the aforementioned results, the CAN score was

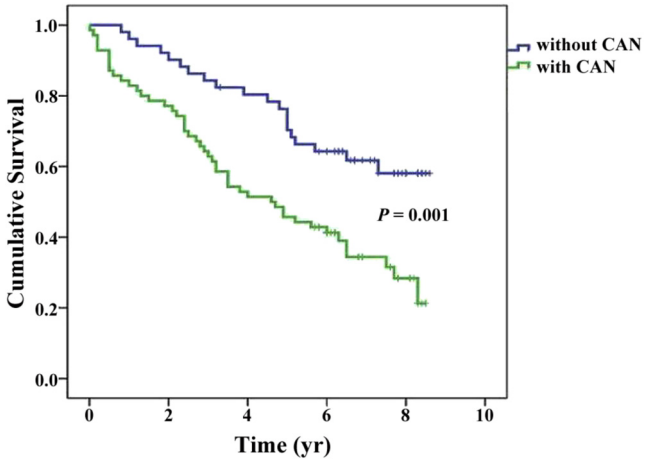

Figure 3. Cumulative survival rates of the study participants according to the presence of cardiac autonomic neuropathy (CAN).

correlated with serum triglycerides in our study ($r = 0.237$; $P = 0.010$). However, no relationship was noted between CAN score and waist circumference ($r = 0.066$; $P = 0.475$). Although hypertriglyceridemia is a common feature of uremic dyslipidemia,[28] neither serum triglyceride levels nor other metabolic abnormalities that commonly appear in patients with ESRF could independently predict CV death. Our finding that only the presence of CAN was a predictor of CV mortality in this group of patients implied that CAN might be a more alarming abnormality than other metabolic derangements that appear in patients with ESRF. Nonetheless, dyslipidemia, and especially, elevated triglycerides, were implicated as potential mechanisms of progression in peripheral neuropathy in subjects with diabetes mellitus.[29] Elevated triglycerides were also shown to independently predict incidence of diabetic kidney disease in patients with diabetes,[30] further reinforcing the notion that a powerful, although poorly investigated, relationship among CAN, serum lipids, and renal function exists.

The association between CAN and morbidity as well as mortality was also examined in other diseases that affect the autonomic nervous system. Apart from congenital diseases, such as familial dysautonomia and Ehlers-Danlos syndrome,[31,32] CAN was associated with chronic obstructive pulmonary disease,[33] liver cirrhosis,[34] hypertension, and hyperuricemia.[35] In patients with chronic heart failure secondary to ischemic or idiopathic-dilated cardiomyopathy, the presence of CAN, as identified by reduced uptake of meta-iodobenzylguanidine labeled with iodine-123 or by pathological HRV measurements, was shown to predict all-cause mortality and sudden death.[36] Importantly, these abnormalities were reversed after long-term circulatory support with a left ventricular assist device in patients with end-stage heart failure.[37] This finding, alongside other studies that reported improvements in cardiac autonomic function with various interventions in persons with diabetes (strict glycemic control,[38] α-lipoic acid, vitamin E, and C-peptide,[24] angiotensin-converting enzyme inhibitors, or cardioselective β-blockers without intrinsic sympathomimetic activity[21]), entails perspectives for therapeutic advances in CAN management.

The association between CAN and renal disease has been known for >40 years.[39] The pathophysiological mechanisms that contribute to the pathogenesis of autonomic dysfunction in CKD are independent of the presence of diabetes and primarily implicate uremia.[40] For this reason, mitigation of levels of uremia, either by means of intensive dialysis or by kidney transplantation, has been shown to improve CAN.[41–43] In accordance with the results of the present study,

Table 4. Univariate and multivariate competing risk regression analyses for participants' characteristics as determinants of cardiovascular mortality

	Univariate analysis		Multivariate Analysis[a]	
Characteristic	**HR (95% CI)**	***P* value**	**HR (95% CI)**	***P* value**
Age (yr)	1.071 (1.039–1.103)	<0.001	1.076 (1.033–1.120)	<0.001
Sex (men vs. women)	0.977 (0.497–1.920)	0.946		
Body mass index (kg/m^2)	1.037 (0.979–1.097)	0.214		
Waist circumference (cm)	1.023 (1.003–1.041)	0.020		
Systolic blood pressure (mm Hg)	1.002 (0.992–1.011)	0.675		
Diastolic blood pressure (mm Hg)	0.989 (0.963–1.014)	0.391		
Current smoking (yes vs. no)	0.656 (0.302–1.425)	0.287		
Diabetes (yes vs. no)	1.874 (0.971–3.615)	0.061		
Coronary artery disease (yes vs. no)	2.211 (1.122–4.358)	0.022		
Cerebrovascular disease (yes vs. no)	1.758 (0.565–5.467)	0.330		
Peripheral artery disease (yes vs. no)	1.302 (0.646–2.625)	0.460		
Any cardiovascular disease (yes vs. no)	1.696 (0.887–3.242)	0.110		
Hemodialysis (yes vs. no)	0.738 (0.382–1.426)	0.367		
Total cholesterol (mg/dl)	1.005 (0.998–1.011)	0.161		
LDL cholesterol (mg/dl)	0.999 (0.992–1.009)	0.962		
HDL cholesterol (mg/dl)	0.988 (0.959–1.018)	0.426		
Triglycerides (mg/dl)	1.004 (1.001–1.006)	0.003		
Albumin (g/dl)	0.853 (0.447–1.628)	0.630		
Glucose (mg/dl)	1.006 (1.001–1.010)	0.008		
Parathormone (ng/l)	1.000 (0.997–1.001)	0.395		
B-blocker (yes vs. no)	1.951 (0.919–4.142)	0.082		
ACE inhibitor/ARB (yes vs. no)	1.154 (0.562–2.372)	0.696		
Diuretic (yes vs. no)	1.709 (0.873–3.344)	0.118		
Calcium channel blocker (yes vs. no)	1.241 (0.641–2.404)	0.521		
Left ventricular ejection fraction (%)	0.962 (0.921–1.005)	0.079		
Left ventricular mass index (g/m^2 of BSA)	1.004 (0.997–1.011)	0.273		
Early diastolic to atrial peak velocity ratio (E/A ratio)	0.169 (0.024–1.171)	0.072		
Cardiac autonomic neuropathy (yes vs. no)	2.392 (1.124–5.088)	0.024	2.360 (1.005–5.541)	0.049

ACE, angiotensin converting enzyme; ARB, angiotensin II receptor antagonist, BSA, body surface area; CI, confidence interval; HDL, high-density lipoprotein; HR, hazard ratio; LDL, low-density lipoprotein.
Sex, current smoking, diabetes mellitus, coronary artery disease, cerebrovascular disease, peripheral arterial disease, any cardiovascular disease, hemodialysis, use of medications, and cardiac autonomic neuropathy (yes vs. no) were analyzed as categorical variables; all the other variables were analyzed as continuous variables in both univariate and multivariate analysis.
[a]After adjustment in addition for waist circumference, history of coronary artery disease, serum glucose, and triglycerides.

abnormalities in cardiac autonomic tests are extremely prevalent among patients with ESRF undergoing hemodialysis.[44] The presence of CAN has been associated

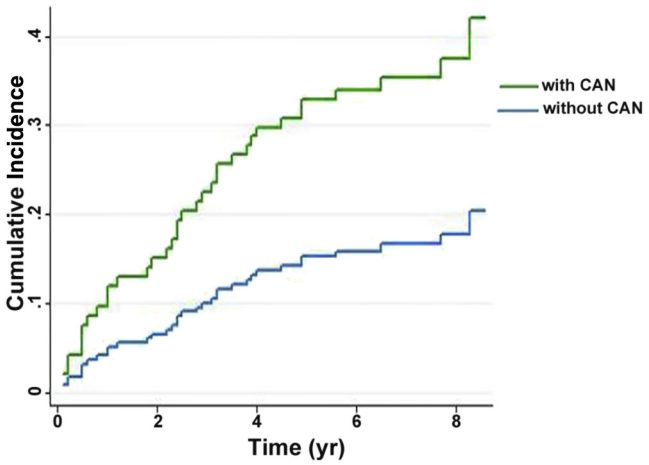

Figure 4. Cumulative incidence of cardiovascular death of the study participants according to the presence of cardiac autonomic neuropathy (CAN).

with increased incidence of arrhythmias and sudden cardiac death in dialysis patients, whereas presence of diabetes, hypertension, uremic dyslipidemia, and other conventional risk factors have been reported to have low power to discriminate patients with advanced CKD at risk for sudden cardiac death.[45,46] In our study as well, 15 of the 36 CV deaths (41.7%) recorded during the follow-up were attributed to sudden cardiac death. CAN has also been related to nocturnal hypoxemia, and to cardiac hypertrophy and remodeling in dialysis patients,[47] findings that are in line with ours. However, a relevant report of the prognostic significance of CAN in predicting long-term all-cause and CV mortality in ESRF patients has been lacking up to now. However, there are some prospective studies that have shown that abnormal HRV parameters are predictive of CV events or all-cause mortality in dialysis patients,[48–50] as well as in patients with advanced CKD not on dialysis.[51] Although HRV has emerged as a simple method to evaluate autonomic nervous system function and has

been used in several clinical trials, it still needs standardization. A definite or confirmed diagnosis of CAN requires at least 2 abnormal tests of the 4 tests proposed by Ewing according to the suggestion of the Diabetic Neuropathy Study Group of the European Association for the Study of Diabetes.[19]

A limitation of our study was the small number of patients. Another possible limitation was the fact that the patients included in the study all had ESRF, and our results could not be extrapolated to patients in earlier stages of CKD. Strengths were the long follow-up period, the robust methods we used to diagnose CAN and indexes of LV structure and function, the high retention rate, and that our results were corroborated by 2 different statistical methods.

Our results might aid to establish readily accessible and easily performed cardiac autonomic function testing as a first-line risk stratification modality in patients with ESRF. Moreover, the fact that adequate hemodialysis has been shown to improve CAN could facilitate tailored intensification of renal replacement therapy in these patients. Finally, data suggesting that CAN is not an innocent bystander of ESRF but a true contributor to CV mortality must instigate research in the field, aimed at elucidating the pathophysiology of the disorder and exploring potential treatment options.

In conclusion, CAN was demonstrated to be an independent risk factor for all-cause and CV mortality in patients with ESRF. These findings strongly suggest that the functionality of the cardiac autonomic nervous system can be assessed and used for risk stratification in patients with ESRF. Further large-scale studies in the field are warranted to investigate if CAN could be improved with certain treatments and whether this improvement would translate to improved CV outcomes.

DISCLOSURE

All the authors declared no competing interests.

REFERENCES

1. Covic A, Kothawala P, Bernal M, et al. Systematic review of the evidence underlying the association between mineral metabolism disturbances and risk of all-cause mortality, cardiovascular mortality and cardiovascular events in chronic kidney disease. *Nephrol Dial Transplant*. 2009;24:1506–1523.
2. Daly C. Is early chronic kidney disease an important risk factor for cardiovascular disease? A background paper prepared for the UK Consensus Conference on early chronic kidney disease. *Nephrol Dial Transplant*. 2007;22 Suppl 9: ix19–ix25.
3. Sarnak MJ, Levey AS, Schoolwerth AC, et al. Kidney disease as a risk factor for development of cardiovascular disease: a statement from the American Heart Association Councils on Kidney in Cardiovascular Disease, High Blood Pressure Research, Clinical Cardiology, and Epidemiology and Prevention. *Circulation*. 2003;108:2154–2169.
4. Block GA, Klassen PS, Lazarus JM, et al. Mineral metabolism, mortality, and morbidity in maintenance hemodialysis. *J Am Soc Nephrol*. 2004;15:2208–2218.
5. Glassock RJ, Pecoits-Filho R, Barberato SH. Left ventricular mass in chronic kidney disease and ESRD. *Clin J Am Soc Nephrol*. 2009;4 Suppl 1:S79–S91.
6. Mehdi U, Toto RD. Anemia, diabetes, and chronic kidney disease. *Diabetes Care*. 2009;32:1320–1326.
7. Drawz PE, Babineau DC, Brecklin C, et al. Heart rate variability is a predictor of mortality in chronic kidney disease: a report from the CRIC Study. *Am J Nephrol*. 2013;38:517–528.
8. Coen G, Pierantozzi A, Spizzichino D, et al. Risk factors of one year increment of coronary calcifications and survival in hemodialysis patients. *BMC Nephrol*. 2010;11:10.
9. Blacher J, Guerin AP, Pannier B, et al. Impact of aortic stiffness on survival in end-stage renal disease. *Circulation*. 1999;99:2434–2439.
10. Hermans MM, Brandenburg V, Ketteler M, et al. Association of serum fetuin-A levels with mortality in dialysis patients. *Kidney Int*. 2007;72:202–207.
11. De Serres SA, Varghese JC, Levin A. Biomarkers in native and transplant kidneys: opportunities to improve prediction of outcomes in chronic kidney disease. *Curr Opin Nephrol Hypertens*. 2012;21:619–627.
12. Mancia G, Fagard R, Narkiewicz K, et al. 2013 ESH/ESC guidelines for the management of arterial hypertension: the Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *Eur Heart J*. 2013;34: 2159–2219.
13. Levey AS, Bosch JP, Lewis JB, et al. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann Intern Med*. 1999;130:461–470.
14. Lang RM, Bierig M, Devereux RB, et al. Recommendations for chamber quantification. *Eur J Echocardiogr*. 2006;7:79–108.
15. Devereux RB, Reichek N. Echocardiographic determination of left ventricular mass in man. Anatomic validation of the method. *Circulation*. 1977;55:613–618.
16. Tei C, Ling LH, Hodge DO, et al. New index of combined systolic and diastolic myocardial performance: a simple and reproducible measure of cardiac function–a study in normals and dilated cardiomyopathy. *J Cardiol*. 1995;26:357–366.
17. Ewing DJ, Martyn CN, Young RJ, et al. The value of cardiovascular autonomic function tests: 10 years experience in diabetes. *Diabetes Care*. 1985;8:491–498.
18. Kahn R. Proceedings of a consensus development conference on standardized measures in diabetic neuropathy. Autonomic nervous system testing. *Diabetes Care*. 1992;15:1095–1103.
19. Tesfaye S, Boulton AJ, Dyck PJ, et al. Diabetic neuropathies: update on definitions, diagnostic criteria, estimation of severity, and treatments. *Diabetes Care*. 2010;33:2285–2293.
20. Verduijn M, Grootendorst DC, Dekker FW, et al. The analysis of competing events like cause-specific mortality–beware of the Kaplan-Meier method. *Nephrol Dial Transplant*. 2011;26: 56–61.

21. Vinik AI, Ziegler D. Diabetic cardiovascular autonomic neuropathy. *Circulation*. 2007;115:387–397.
22. Vinik AI, Maser RE, Mitchell BD, et al. Diabetic autonomic neuropathy. *Diabetes Care*. 2003;26:1553–1579.
23. Sacre JW, Franjic B, Jellis CL, et al. Association of cardiac autonomic neuropathy with subclinical myocardial dysfunction in type 2 diabetes. *JACC Cardiovasc Imaging*. 2010;3:1207–1215.
24. Spallone V, Ziegler D, Freeman R, et al. Cardiovascular autonomic neuropathy in diabetes: clinical impact, assessment, diagnosis, and management. *Diabetes Metab Res Rev*. 2011;27:639–653.
25. Brotman DJ, Bash LD, Qayyum R, et al. Heart rate variability predicts ESRD and CKD-related hospitalization. *J Am Soc Nephrol*. 2010;21:1560–1570.
26. Yun JS, Cha SA, Lim TS, et al. Cardiovascular autonomic dysfunction predicts diabetic foot ulcers in patients with type 2 diabetes without diabetic polyneuropathy. *Medicine (Baltimore)*. 2016;95:e3128.
27. Laitinen T, Lindstrom J, Eriksson J, et al. Cardiovascular autonomic dysfunction is associated with central obesity in persons with impaired glucose tolerance. *Diabet Med*. 2011;28:699–704.
28. Tsimihodimos V, Mitrogianni Z, Elisaf M. Dyslipidemia associated with chronic kidney disease. *Open Cardiovasc Med J*. 2011;5:41–48.
29. Wiggin TD, Sullivan KA, Pop-Busui R, et al. Elevated triglycerides correlate with progression of diabetic neuropathy. *Diabetes*. 2009;58:1634–1640.
30. Russo GT, De Cosmo S, Viazzi F, et al. Plasma Triglycerides and HDL-C levels predict the development of diabetic kidney disease in subjects with type 2 diabetes: The AMD Annals Initiative. *Diabetes Care*. 2016;39:2278–2287.
31. Rubin BY, Anderson SL. The molecular basis of familial dysautonomia: overview, new discoveries and implications for directed therapies. *Neuromolecular Med*. 2008;10:148–156.
32. De Wandele I, Rombaut L, Leybaert L, et al. Dysautonomia and its underlying mechanisms in the hypermobility type of Ehlers-Danlos syndrome. *Semin Arthritis Rheum*. 2014;44:93–100.
33. Rasheedy D, Taha HM. Cardiac autonomic neuropathy: the hidden cardiovascular comorbidity in elderly patients with chronic obstructive pulmonary disease attending primary care settings. *Geriatr Gerontol Int*. 2016;16:329–335.
34. Dumcke CW, Moller S. Autonomic dysfunction in cirrhosis and portal hypertension. *Scand J Clin Lab Invest*. 2008;68:437–447.
35. Liao XP, Zhu HW, Zeng F, et al. The association and interaction analysis of hypertension and uric acid on cardiovascular autonomic neuropathy. *J Endocrinol Invest*. 2015;38:1075–1082.
36. Anastasiou-Nana MI, Terrovitis JV, Athanasoulis T, et al. Prognostic value of iodine-123-metaiodobenzylguanidine myocardial uptake and heart rate variability in chronic congestive heart failure secondary to ischemic or idiopathic dilated cardiomyopathy. *Am J Cardiol*. 2005;96:427–431.
37. Drakos SG, Athanasoulis T, Malliaras KG, et al. Myocardial sympathetic innervation and long-term left ventricular mechanical unloading. *JACC Cardiovasc Imaging*. 2010;3:64–70.
38. Maser RE, Lenhard MJ. An overview of the effect of weight loss on cardiovascular autonomic function. *Curr Diabetes Rev*. 2007;3:204–211.
39. Ewing DJ, Winney R. Autonomic function in patients with chronic renal failure on intermittent haemodialysis. *Nephron*. 1975;15:424–429.
40. Elming MB, Hornum M, Feldt-Rasmussen B, et al. Cardiac autonomic neuropathy in patients with uraemia is not related to pre-diabetes. *Dan Med Bull*. 2011;58:A4244.
41. Laaksonen S, Voipio-Pulkki L, Erkinjuntti M, et al. Does dialysis therapy improve autonomic and peripheral nervous system abnormalities in chronic uraemia? *J Intern Med*. 2000;248:21–26.
42. Yildiz A, Sever MS, Demirel S, et al. Improvement of uremic autonomic dysfunction after renal transplantation: a heart rate variability study. *Nephron*. 1998;80:57–60.
43. Mylonopoulou M, Tentolouris N, Antonopoulos S, et al. Heart rate variability in advanced chronic kidney disease with or without diabetes: midterm effects of the initiation of chronic haemodialysis therapy. *Nephrol Dial Transplant*. 2010;25:3749–3754.
44. Sahin M, Kayatas M, Urun Y, et al. Performing only one cardiovascular reflex test has a high positive predictive value for diagnosing autonomic neuropathy in patients with chronic renal failure on hemodialysis. *Ren Fail*. 2006;28:383–387.
45. Jassal SV, Coulshed SJ, Douglas JF, et al. Autonomic neuropathy predisposing to arrhythmias in hemodialysis patients. *Am J Kidney Dis*. 1997;30:219–223.
46. Ranpuria R, Hall M, Chan CT, et al. Heart rate variability (HRV) in kidney failure: measurement and consequences of reduced HRV. *Nephrol Dial Transplant*. 2008;23:444–449.
47. Zoccali C, Mallamaci F, Tripepi G, et al. Autonomic neuropathy is linked to nocturnal hypoxaemia and to concentric hypertrophy and remodelling in dialysis patients. *Nephrol Dial Transplant*. 2001;16:70–77.
48. Badarau S, Siriopol D, Drugus D, et al. Electrocardiogram abnormalities and heart rate variability in predicting mortality and cardiovascular events among hemodialyzed patients. *Int Urol Nephrol*. 2015;47:1703–1708.
49. Pei J, Tang W, Li LX, et al. Heart rate variability predicts mortality in peritoneal dialysis patients. *Ren Fail*. 2015;37:1132–1137.
50. Fukuta H, Hayano J, Ishihara S, et al. Prognostic value of heart rate variability in patients with end-stage renal disease on chronic haemodialysis. *Nephrol Dial Transplant*. 2003;18:318–325.
51. Chandra P, Sands RL, Gillespie BW, et al. Predictors of heart rate variability and its prognostic significance in chronic kidney disease. *Nephrol Dial Transplant*. 2012;27:700–709.