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Received for publication: 2.2.11; Accepted in revised form: 22.4.11

Nephrol Dial Transplant (2012) 27: 700–709
doi: 10.1093/ndt/gfr340
Advance Access publication 17 July 2011

Predictors of heart rate variability and its prognostic significance in chronic kidney disease

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Abstract

Background. Heart rate variability (HRV), a noninvasive measure of autonomic dysfunction and a risk factor for cardiovascular disease (CVD), has not been systematically studied in nondialysis chronic kidney disease (CKD).

Methods. HRV was assessed using 24-h Holter monitoring in 305 subjects from the Renal Research Institute-CKD Study, a four-center prospective cohort of CKD (Stages 3–5). Multiple linear regression was used to assess predictors of HRV (both time and frequency domain) and Cox regression used to predict outcomes of CVD, composite of CVD/death and end-stage renal disease (ESRD).

Results. A total of 47 CVD, 67 ESRD and 24 death events occurred over a median follow-up of 2.7 years. Lower HRV was significantly associated with older age, female gender, diabetes, higher heart rate, C-reactive protein and phosphorus, lower serum albumin and Stage 5 CKD. Lower HRV (mostly frequency domain) was significantly associated with higher risk of CVD and the composite end point of CVD or death. Significantly, lower HRV (frequency domain) was associated with higher risk of progression to ESRD, although this effect was relatively weaker.

Conclusions. This study draws attention to the importance of HRV as a relatively under recognized predictor of adverse cardiovascular and renal outcomes in patients with nondialysis CKD. Whether interventions that improve HRV will improve these outcomes in this high-risk population deserves further study.

Keywords: autonomic nervous system; cardiovascular disease risk factors; cardiovascular outcomes; cohort study; end-stage renal disease

Introduction

Increased cardiovascular (CV) morbidity and mortality is well documented in chronic kidney disease (CKD) [1]. Sudden cardiac death accounts for about a third of total mortality among dialysis patients, that could in part, be due to autonomic nervous system (ANS) dysfunction [2] and increased sympathetic activity, in particular [3]. Increased sympathetic activity is also noted in patients with CKD [4] and likely contributes to the higher risk of cardiovascular disease (CVD) and also renal damage.

Heart rate variability (HRV) is a noninvasive measure of autonomic function that reflects beat-to-beat variability in heart rate. It is best assessed by continuous electrocardiography over a 24-h period, although shorter recordings have also been utilized. It is quantified by 'time domain' or

'frequency domain' measures. Time domain measures assess the variability in the individual's RR interval. For frequency domain analysis, cyclical changes in heart rate are transformed by fast Fourier transformation into a spectral representation, which plots magnitude against frequency of these cyclical changes [5–7]. HRV is influenced by age, race, gender, blood pressure and certain lifestyle factors [8, 9]. It declines after myocardial infarction (MI) and is lower among those with coronary artery disease (CAD) and diabetes mellitus (DM) [10, 11]. Lower HRV has been associated with adverse CVD outcomes in settings such as post-MI, CAD, congestive heart failure (CHF), DM [5–7] and end-stage renal disease (ESRD) [12, 13]. However, it has not been systematically studied in the nondialysis CKD population.

Our study aims were to explore the distribution of several HRV parameters across CKD Stages 3–5, investigate important predictors of HRV and examine independent associations of HRV with the outcomes of CVD, progression to ESRD and mortality, in a multicenter cohort of CKD patients.

Materials and methods

The Renal Research Institute (RRI)-CKD Study is a four-center prospective cohort study of adults with moderate to severe CKD (Stages 3–5), enrolled between June 2000 and February 2006 ($n = 834$). The study methodology has been published previously [14]. Eligibility criteria included age $\geq$18 years and estimated glomerular filtration rate (eGFR) $\leq$50 mL/min by the Cockroft–Gault formula. Subsequently, the abbreviated (four-variable) Modification of Diet in Renal Disease (MDRD) equation was used and eGFR was between 50 and 60 mL/min/1.73m^2 in 13 subjects. To exclude patients with transient renal impairment prior to enrollment, glomerular filtration rate (GFR) was estimated on two occasions at least 1 month apart. At enrollment and follow-up visits, data on demographic characteristics, anthropometric measures, cause of CKD, symptoms, laboratory values and medications were collected.

From 1 January 2003 onwards, individuals from the original RRI-CKD cohort ($n = 627$) were invited to undergo noninvasive CV studies, including 24-h Holter monitoring as part of a CV substudy. Figure 1 displays patient recruitment flow into this CV substudy. Of the 408 patients still alive and active at this time from the RRI-CKD study, 149 (37%) were consented into the new CV substudy. Those who consented were significantly healthier than those who did not: they were younger (mean age 58 versus 64), had higher mean eGFR (27 versus 25) and fewer had diabetes (30 versus 42%) or a history of CVD (37 versus 58%). An additional 199 patients were newly recruited into this CV substudy from the renal clinics at the four participating centers. Compared to the 149 consenters from the original cohort, the 199 newly recruited patients were similar with respect to age, diabetes, hypertension, history of CVD, race, gender and medication use but had significantly higher mean eGFR (32 versus 24). Finally, of the total 348 patients consented into the CV substudy, 43 patients declined Holter monitoring. Except for significantly older mean age (67 versus 60), these patients were otherwise similar compared to the 305 who underwent the procedure. A subset of the 305 patients who underwent Holter monitoring also had echocardiographic data ($n = 204$). Study coordinators at each site were trained in Holter monitoring and other study procedures at the University of Michigan data coordinating center to ensure uniformity of technique and compliance with the study's manual of operations. During the 24-h Holter, patients went about their usual activities but were asked to refrain from heavy physical activity. The Holter data were transferred electronically via a file transfer protocol to the data coordinating center where HRV was analyzed using SyneTec Holter analysis software, version 1.20 (Ela Medical, Paris, France) under the supervision of the study cardiologist (S.R.). The 24-h tracing was examined and masking was used to exclude areas of artifact. Abnormal atrial and ventricular rhythms were manually verified. Time and frequency domain measures were calculated for the 24-h period, day (8 a.m.–9 p.m.) and nighttime periods. The terminology/abbreviations pertaining to the HRV parameters collected in this study are shown in Table 1. Patients on nitroglycerin patch, with defibrillator, active pacing or with allergy to electrode adhesive material were excluded.

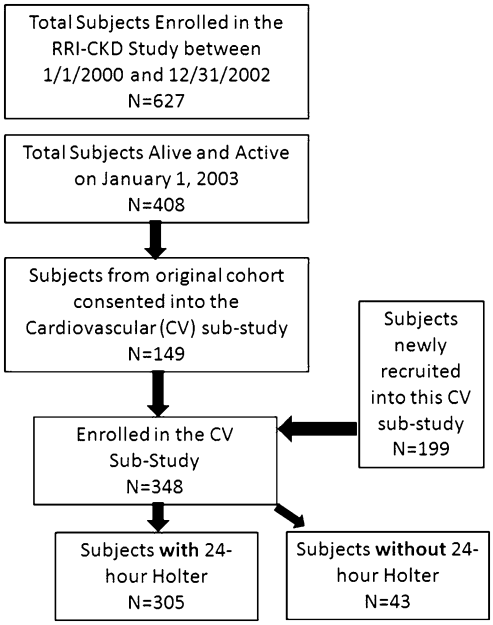

Fig. 1. Patient recruitment flow into the CV substudy of the RRI-CKD study (n = 305).

The outcomes studied were CVD, composite of CVD/death and ESRD (dialysis or preemptive kidney transplant). CVD events included those related to CAD, cerebrovascular disease, peripheral arterial disease, CHF and cardiac arrest. ESRD was defined by documented initiation of dialysis or preemptive renal transplantation. All outcomes were ascertained on an ongoing basis by study coordinators from regular review of electronic health records, direct patient contact in clinic and periodic telephone communication. CVD events to be collected were prespecified and identified using a CVD event data collection form. Follow-up of patients ended on 31 December 2006. To compare HRV in CKD patients with HRV in the general population, we used published data on healthy controls [10]. The study protocol was approved by the Institutional Review Boards of participating centers and written informed consent was obtained from all study subjects.

Statistical analysis

Multiple linear regression models were used to investigate determinants of the HRV parameters that were most predictive of CVD events, CVD/death and/or ESRD. All variables from Table 2 including demographics, medical history, laboratory values and medications were included in the multivariable model selection. Cox regression was used to analyze time to event outcomes. The method of best subsets with the R-squared selection criterion was used to guide the model selection process [15]. Since the inclusion of left ventricular mass index (LVMI) in the model selection restricted the sample size, model selection was performed with and without LVMI. Comparisons of HRV parameters between this CKD cohort and the general North American population were made using summary statistics from published literature, using *t*-tests for unequal variance. All analyses were conducted using SAS, version 9.2 (SAS Institute Inc., Cary, NC).

Results

Baseline patient characteristics

Baseline (at the time of the Holter) patient characteristics by CKD stage are displayed in Table 2. The average age was 59.5 $\pm$ 14.7 years, 51% were male, 78% white and 18% black. A majority were hypertensive (89%), 31% were diabetic and 37% had a history of CVD.

Table 1. Overview of HRV parameters[a]

Frequency domain HRV measures		Unit	Description
VLF	Power in very low frequency range	ms^2	Physiological correlate unclear; may reflect vasomotor function, RAS and/or parasympathetic influence.
LF	Power in low frequency range	ms^2	Reflects sympathetic or sympathetic parasympathetic influence.
HF	Power in high frequency range	ms^2	Reflects short-term heart rate variations, under parasympathetic influence.
LF/HF	Ratio of low to high frequency power	n/a	Reflects sympathovagal balance.
TP	Total power	ms^2	Estimate of overall HRV.
Time domain HRV measures		**Unit**	**Description**
SDNN	SD of all normal R–R (NN) intervals	ms	Estimate of overall HRV. When measured over 24 h, it reflects both long- and short-term fluctuations in HRV over 24-h period.
SDANN	SD of the averages of 5-min NN intervals over 24 h	ms	Estimate of long-term components of HRV (i.e. changes in heart rate due to cycles >5 min).
ASDNN	Average of all 5-min SDNN over 24 h (aka SDNN index)	ms	Estimate of short-term components of HRV (i.e. changes in heart rate due to cycles <5 min).
rMSSD	The square root of the mean of the square of successive NN intervals	ms	Estimate of short-term variations in heart rate. Reflects high frequency variations and parasympathetic influence.

[a]References: [5, 6].

HRV measurements

HRV measures by CKD stage are presented in Table 3. The majority of the HRV measures were significantly lower in diabetics compared to nondiabetics (data not shown). Although some HRV measures significantly differed across CKD stage, this difference was most notable in CKD Stage 5 (i.e. eGFR <15) compared with Stages 3 and 4 (i.e. eGFR ≥15). In univariate analysis, several HRV measures (SDNN, SDANN, VLF and LF/HF) were significantly lower in patients with eGFR <15 mL/min/1.73m^2 compared to those with eGFR ≥15 mL/min/1.73m^2 (Table 3).

Crude comparisons of HRV frequency (VLF, LF, HF, LF/HF) and time (SDNN) domain parameters between the RRI-CKD cohort and healthy population from the literature [10] are shown in Figure 2. All frequency domain and most time domain measures in the RRI-CKD cohort were significantly lower than healthy controls (Supplementary Table A).

Associations of HRV with baseline clinical characteristics

Table 4 shows associations of HRV with certain patient characteristics. HRV measures were lower in older patients, women, nonwhites, diabetics, those with a higher mean 24-h heart rate, higher LVMI, elevated serum phosphorus, higher C-reactive protein (CRP), higher high-density lipoprotein (HDL), lower albumin, higher urine albumin/creatinine ratio (ACR), among those with an eGFR <15 mL/min/1.73m^2 and in those prescribed beta-blockers.

Association of HRV with clinical outcomes

Patients that had undergone HRV measurements had a median follow-up of 2.7 years. During this time, 47 (15%) had at least one CVD event, 67 (22%) reached ESRD and 24 (8%) patients died. Supplementary Table B presents the types of clinical events and major outcomes during the follow-up period. Frequency domain measures of HRV (LF, LF/HF, VLF and TP) were the most predictive of outcomes. Among the time domain measures, higher ASDNN was associated with lower risk of CVD and CVD/death outcomes but not progression to ESRD. Supplementary Table C displays the 24-h, day and night HRV measures as predictors of CVD/death and ESRD, while Figure 3 displays 24-h, day and night HRV measures for CVD events. For the purposes of this study, we focused only on the 24-h HRV measures.

The relative risk of time to first CVD event was significantly higher with older age, history of CVD, higher phosphorus and lower serum albumin [Table 5(IA)]. Measures of HRV were added one at a time to this 'base' model. Higher HRV was significantly associated with a lower risk of CVD [Figure 3 and Table 5(IIA)]. The probability of CVD events by LF/HF ratio (dichotomized at the median) is shown in Figure 4.

Table 5(IB) shows the base model predicting time to the composite endpoint of CVD/death. The risk was significantly higher with older age, history of CVD, diabetes and lower albumin. After inclusion of HRV measures to this model, several HRV measures were strongly predictive of the composite event with higher values of HRV associated with lower risk of the composite outcome [Table 5(IIB)].

Table 5IC shows the time to ESRD base model, which includes age, gender, baseline eGFR, urine ACR, LVMI and hemoglobin. Because ACR was strongly correlated with serum albumin ($r = –0.38$; $P < 0.0001$), models with serum albumin instead of ACR provided similar fit. After including HRV measures to this model, higher 24-h LF/HF ratio was the only 24-h HRV measure significantly associated with lower risk of ESRD [Table 5(IIC)]. When LVMI

Table 2. Baseline patient characteristics[a,b]

	Overall ($n = 305$)	CKD Stage 3 $30 \leq$ eGFR < 60 ($n = 126$)	CKD Stage 4 $15 \leq$ eGFR < 30 ($n = 140$)	CKD Stage 5 eGFR <15 ($n = 39$)
Demographics/anthropometrics				
Age	59.5 ± 14.7	**61.7 ± 14.4**	**58.9 ± 14.7**	**54.8 ± 14.7**
Gender				
Male	50.5% (154)	46.8% (59)	54.3% (76)	48.7% (19)
Race				
White	78.4% (239)	73.8% (93)	83.6% (117)	74.4% (29)
Black	17.7% (54)	23.0% (29)	12.1% (17)	20.5% (8)
Other	3.9% (12)	3.2% (4)	4.3% (6)	5.1% (2)
Body mass index (kg/m^2)	29.3 ± 6.7	28.7 ± 6.1	29.3 ± 6.3	31.1 ± 9.1
Smoker	11.5% (35)	11.1% (14)	12.1% (17)	10.3% (4)
CV indices				
Systolic blood pressure (mmHg)	138.9 ± 23.4	140.8 ± 21.2	137.2 ± 24.5	138.6 ± 26.4
Diastolic blood pressure (mmHg)	78.6 ± 12.9	79.1 ± 11.9	78.2 ± 13.7	77.9 ± 12.9
LVMI (g/m^2)	109.7 ± 36.8	106.8 ± 32.9	108.2 ± 36.7	119.4 ± 43.1
Comorbidities				
Diabetes	30.8% (94)	27.8% (35)	30.7% (43)	41.0% (16)
Hypertension	88.9% (271)	84.9% (107)	90.0% (126)	97.4% (38)
CVD	36.7% (112)	40.5% (51)	33.6% (47)	35.9% (14)
Cause of CKD[c]				
Diabetes	25.9% (79)	23.8% (30)	24.3% (34)	38.5% (15)
Hypertension	47.9% (146)	50.0% (63)	47.1% (66)	43.6% (17)
Other	57.0% (174)	54.8% (69)	60.7% (85)	51.3% (20)
Medications				
Diuretics	50.2% (153)	44.4% (56)	51.4% (72)	64.1% (25)
ACE inhibitors	46.9% (143)	45.2% (57)	47.9% (67)	48.7% (19)
A-II receptor blockers	30.8% (94)	33.3% (42)	31.4% (44)	20.5% (8)
Beta-blocker	51.1% (156)	47.6% (60)	52.9% (74)	56.4% (22)
Calcium channel blocker	42.3% (129)	40.5% (51)	42.9% (60)	46.2% (18)
Erythropoiesis-stimulating agents	24.3% (74)	**17.5% (22)**	**22.9% (32)**	**51.3% (20)**
Statin	48.2% (147)	48.4% (61)	50.0% (70)	41.0% (16)
Laboratory values				
CRP (mg/L)	2.5 (0, 94)	2.5 (0, 49.9)	2.6 (0.1, 94.4)	1.5 (0, 35.3)
Intact parathyroid hormone (pg/mL)	114.0 (5, 1150)	**93.0 (6, 991)**	**122.0 (5, 534)**	**324 (5, 1,150)**
eGFR (mL/min/1.73m^2)	28.2 ± 11.5	**39.4 ± 7.2**	**22.7 ± 4.4**	**11.7 ± 2.3**
Serum creatinine (mg/dL)	2.4 (1, 11)	**1.7 (1, 2.9)**	**2.8 (1.8, 5)**	**4.9 (3.4, 10.5)**
Serum albumin (g/dL)	4.0 ± 0.5	4.0 ± 0.4	4.0 ± 0.5	4.0 ± 0.4
Blood urea nitrogen (mg/dL)	37.5 (14, 131)	**28.0 (14, 90)**	**41.0 (15, 116)**	**70.5 (29, 131)**
Serum corrected calcium (mg/dL)	9.2 ± 0.6	9.2 ± 0.6	9.2 ± 0.6	9.2 ± 0.8
Serum glucose (mg/dL)	97.0 (46, 393)	98.0 (55, 312)	96.0 (57, 393)	103.0 (46, 361)
Serum phosphorus (mg/dL)	3.7 ± 0.9	**3.5 ± 0.6**	**3.7 ± 0.8**	**4.9 ± 1.1**
Serum potassium (mEq/L)	4.5 ± 0.6	**4.4 ± 0.6**	**4.6 ± 0.6**	**4.6 ± 0.6**
Hemoglobin (g/dL)	12.1 ± 1.6	12.2 ± 1.7	12.2 ± 1.5	11.6 ± 1.5
Total cholesterol (mg/dL)	191.9 ± 51.2	195.5 ± 53.0	189.3 ± 49.2	189.4 ± 52.8
Low-density lipoprotein (mg/dL)	109.0 ± 39.7	111.8 ± 41.9	106.7 ± 37.6	108.0 ± 40.5
High-density lipoprotein (mg/dL)	42.6 ± 14.5	44.5 ± 15.5	41.7 ± 13.5	39.7 ± 13.7
Triglycerides (mg/dL)	150.3 ± 90.6	151.6 ± 92.1	153.3 ± 96.2	134.7 ± 58.8
Urine albumin/creatinine ratio	192.0 (2, 9259)	**81.2 (2, 5012.9)**	**243.5 (3, 9258.7)**	**465 (2, 5738.5)**

[a]Continuous variables are reported as mean ± SD for normally distributed variables and as median (min, max) for skewed variables. Significant differences among CKD stages are shown in **bold** ($P < 0.05$).
[b]Based on ANOVA.
[c]Multiple causes of CKD are possible.

was excluded from the model, HRV was no longer significantly associated with ESRD (data not shown).

Discussion

To the best of our knowledge, this study represents the first detailed analysis of HRV obtained by 24-h Holter monitoring in a fairly large prospective cohort of referred nondialysis CKD (Stages 3–5) patients. Lower HRV was significantly associated with several traditional and nontraditional risk factors for CVD including diabetes, lower albumin, higher phosphorus, higher CRP, higher ACR and eGFR <15 mL/min/1.73m^2. Lower HRV was independently associated with higher risk of CVD events, composite of CVD/death and ESRD. In general, frequency domain measures of HRV demonstrated greater prognostic significance compared with time domain measures.

Determinants of HRV in CKD

Older age, female gender and diabetes were strongly associated with lower HRV. We found an inverse relationship

Table 3. Baseline HRV measurements ($n = 305$)[a]

	Overall ($n = 305$)	CKD Stage 3 $30 \leq$ eGFR < 60 ($n = 126$)	CKD Stage 4 $15 \leq$ eGFR < 30 ($n = 140$)	CKD Stage 5 eGFR <15 ($n = 39$)
Heart rate (obtained during Holter monitoring)				
Mean 24-h (b.p.m.)	73.5 ± 10.4	73.6 ± 10.6	73.5 ± 10.0	73.1 ± 11.6
Mean day/night difference	7.9 ± 9.7	8.0 ± 8.0	8.3 ± 7.4	6.1 ± 5.9
Time domain (ms)				
SDNN	107.4 ± 37.5	**107.6 ± 36.7**	**110.4 ± 37.4**	**95.7 ± 39.3**
SDANN	87.4 (25, 258)	**88.0 (25, 258)**	**91.7 (27, 202)**	**69.3 (34, 147)**
ASDNN	47.1 ± 26.1	41.0 (12, 188)	42.5 (12, 195)	37.0 (18, 187)
RMSSD	24.6 (6, 267)	26.0 (6, 267)	22.8 (9, 259.5)	22.9 (8, 262)
Frequency domain (ms^2)				
VLF	1005.5 (36, 7532)	**1019.0 (520, 5908)**	**1084.0 (36, 6252)**	**589.0 (162, 7532)**
LF	310.0 (13, 11 977)	292.5 (13, 6688)	327.0 (16, 11 977)	241.0 (27, 7151)
HF	121.5 (5, 15 123)	141.0 (5, 11 247)	112.0 (16, 15 123)	102.0 (11, 11 445)
LF/HF ratio	2.5 (0.2, 14)	**2.3 (0.2, 12)**	**2.9 (0.2, 14)**	**2.1 (0.3, 9)**
Total power	1555.0 (118, 30 828)	1560.5 (132, 25 058)	1645.0 (118, 30 828)	1131.0 (306, 25 436)

[a]Continuous variables are reported as mean ± SD for normally distributed variables and as median (min, max) for skewed variables. Significant differences for CKD Stage 5 versus 3/4 are shown in bold ($P < 0.05$).

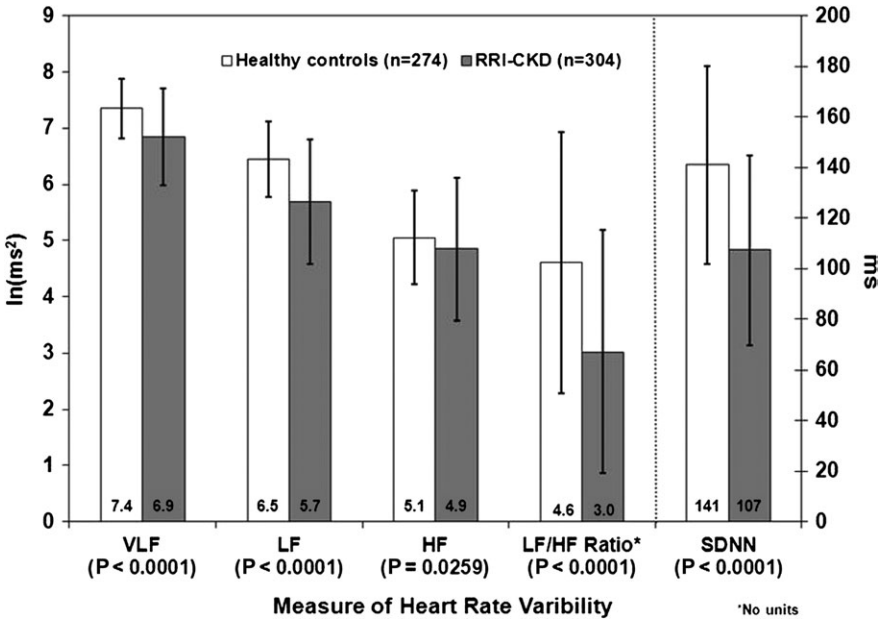

Fig. 2. Comparisons of HRV parameters among this CKD cohort (dark gray) and healthy controls [10] (white). Bars and whiskers represent the mean ± SD.

between CRP and HRV; CRP is a known risk factor for CVD and autonomic dysregulation may be worse among those with inflammation [16, 17]. Similarly, serum phosphorus was also inversely associated with HRV. Higher phosphorus is associated with increased CVD mortality in patients on dialysis [18] and CKD [19]. We also found that HRV was inversely associated with LVMI, as has also been noted in the literature [20]. Medications had variable associations with HRV in this cohort. Those on beta-blockers had lower HRV, despite adjustment for history of CAD and CHF. Beta-blockers have previously been shown to be associated with improved HRV in settings such as CHF [21] and acute MI [22]. We believe that the observed association of lower HRV with medications in our study very likely represents confounding by indication, as these medications are preferentially used in sicker patients who tend to have lower HRV. No association was observed between angiotensin converting enzyme inhibitors or angiotensin II receptor blocker use and HRV. Drugs that decrease sympathetic activity including alpha- and beta-blocking agents, central sympatholytic agents, ACE and ARBs have been shown to improve HRV in non-CKD populations. The effect of these drugs on sympathetic activity has also been studied in patients with kidney disease as reviewed by Kooman *et al.* [4]. Further studies are clearly needed to explore the potentially beneficial effect of these and possibly other agents (e.g. aldosterone antagonists) in improving HRV in patients with CKD. The association of higher HDL with lower HRV in this cohort is contrary to what has been observed in the general

Table 4. Multiple linear regression models predicting 24-h HRV parameters (log transformed)

	Frequency domain				Time domain
Predictors	LF (ln ms^2) ($n = 201$)	VLF (ln ms^2) ($n = 303$)	LF/HF ratio ($n = 301$)	Total power (ln ms^2) ($n = 285$)	SDANN (ln ms) ($n = 289$)
	$R^2 = 0.23$	$R^2 = 0.33$	$R^2 = 0.21$	$R^2 = 0.24$	$R^2 = 0.27$
DM	$\beta = -0.71^{***}$	$\beta = -0.51^{***}$	$\beta = -0.21^{***}$	$\beta = -0.46^{***}$	$\beta = -0.12^{**}$
Mean 24-h heart rate (b.p.m.)	$\beta = -0.03^{***}$	$\beta = -0.03^{***}$	n/a	$\beta = -0.03^{***}$	$\beta = -0.01^{***}$
Age	n/a	$\beta = -0.01^{***}$	$\beta = -0.01^{***}$	n/a	$\beta = -0.003$*
Gender: male	n/a	$\beta = 0.22^{*}$	$\beta = 0.17^{**}$	[d]	n/a
Serum phosphorus (mg/dL)	n/a	n/a	$\beta = -0.08^{*}$	n/a	n/a
CKD Stage 5 versus Stage 3/4	n/a	$\beta = -0.42^{**}$	[c]	n/a	$\beta = -0.15$*
CRP[a]	n/a	n/a	n/a	$\beta = -0.13^{*}$	n/a
High-density lipoprotein (mg/dL)	$\beta = -0.02^{***}$	n/a	n/a	$\beta = -0.01^{***}$	n/a
Beta-blocker (Y/N)	[b]	$\beta = -0.26^{**}$	n/a	$\beta = -0.24^{*}$	$\beta = -0.19$***
LVMI[a] (g/m^2)	$\beta = -0.70^{**}$	n/a	n/a	n/a	n/a
Serum albumin (g/dL)	n/a	$\beta = 0.23^{*}$	n/a	n/a	n/a
Urine albumin/creatinine ratio	n/a	n/a	n/a	n/a	$\beta = -0.03$**
Race: white	n/a	n/a	n/a	n/a	$\beta = 0.11$*

[a] Log-scale
[b]Due to the limitation on the samples size when LVMI was included, model selection without LVMI was performed. When LVMI was excluded, use of beta-blocker was significantly associated with LF (β for beta-blocker =−0.41, P = 001; $n = 299$).
[c]Competing model: substituting CKD Stage 5 versus Stage 3/4 for phosphorus yields similar results (β for Stage 5 CKD = −0.20, P = 0.0099).
[d]Competing model: substituting Gender for HDL yields similar results (β for gender = 0.30, P = 0.001; $n = 285$).
$^{*}P < 0.05$, $^{**}P < 0.005$, $^{***}P < 0.0005$.

population [23]. Crude comparisons with data from published literature from the USA revealed that the RRI-CKD patients had lower HRV than subjects from the general population, which is not surprising.

Recently, Mylonopoulou *et al.* [24] evaluated HRV in patients with diabetic and nondiabetic CKD Stage 4 ($n = 50$) and found that several time and frequency measures were lower in diabetic versus nondiabetic patients. Patients were excluded if they were on medications affecting ANS. These patients were followed until they started dialysis and HRV was repeated after 3 months of dialysis; some time domain measures improved after dialysis. In our CKD cohort, we found that the majority of time and frequency HRV measures were lower in diabetics than nondiabetics. In addition, even among nondiabetics, HRV was significantly lower than healthy controls ($P < 0.05$, data not shown). We studied a larger number of patients with CKD, reported on multiple clinically relevant variables associated with low HRV including medications affecting ANS and found associations of HRV with clinical outcomes.

HRV as a predictor of clinical outcomes in CKD

In general, higher values of HRV were independently associated with lower risk of all outcomes in this cohort. Our findings indicate that similar to non-CKD populations, low HRV appears to be a significant and independent marker of adverse clinical outcomes, both with respect to CV events (including the composite of CVD and mortality) [5, 6] and possibly even progression to ESRD. Findings derived from multivariable analysis suggest that the effect of HRV is independent of other clinical variables associated with increased CVD risk, and given the strength of the association (30–40% lower risk of, CVD event for each unit higher HRV; Table 5) and its biological plausibility, likely to be clinically important. Furthermore, in this patient population, certain clinical markers were associated with low HRV (e.g. higher CRP, P, lower albumin, etc.). This would suggest a potential link between these clinical variables and autonomic dysregulation. These findings are relevant because the CKD population is at particularly high risk for CVD and the study of novel CV risk factors is a research priority that can assist in the discovery of newer targets for therapy.

LF and LF/HF ratio were associated with improved outcomes and may indicate better sympathovagal balance. LF has been variably thought to represent sympathetic activity by some and sympathovagal balance by others [5–7, 25, 26]. Though the physiological correlate of VLF is unclear [thought to represent parasympathetic versus vasomotor versus renin–angiotensin–aldosterone system (RAS)], it has been shown to predict cardiac- and all-cause mortality in the post-MI setting [6].

It is useful to compare our findings with studies on HRV performed in patients on dialysis. Vita *et al.* [27] found that LF was reduced in dialysis patients compared with controls. Similar to our results, in patients on dialysis, reduced HRV predicted cardiac [12] and all-cause and CV death [13].

In this study, higher HRV was associated with lower risk of progression to ESRD; however, LF/HF ratio was the only 24-h measure associated with risk to ESRD and this association was weaker when LVMI was not included in the multivariable ESRD model. There was significant correlation observed between LVMI and HRV (LF/HF ratio), which likely explains this relationship. Hence, the independent effect of HRV in predicting ESRD cannot be definitively concluded from our study. In patients with diabetes, autonomic dysfunction has been associated with increased risk of progression of nephropathy [28, 29]. The association of low HRV with increased risk of progression to ESRD is biologically plausible. Renal failure is associated with increased sympathetic activity which has been

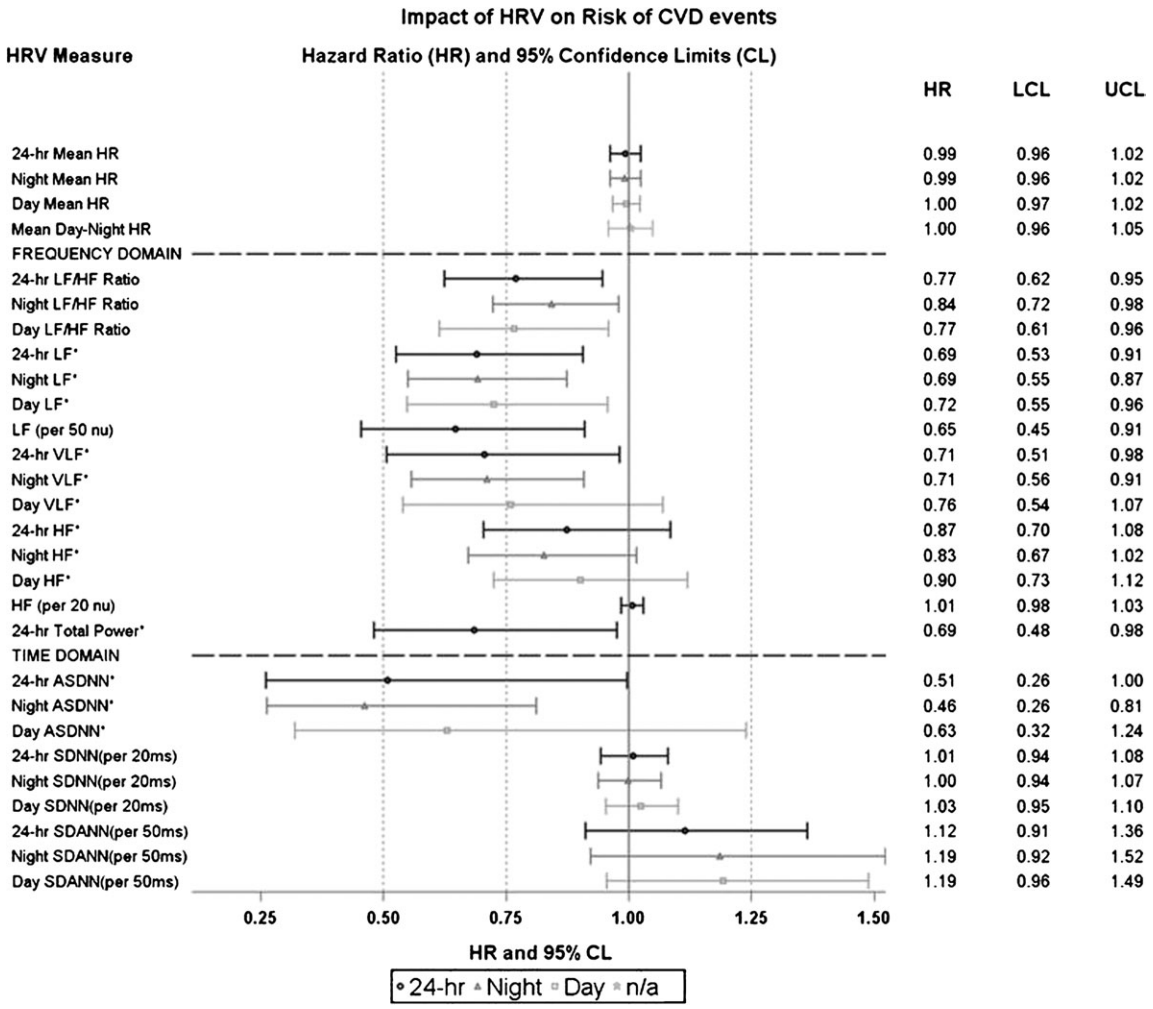

Fig. 3. Impact of HRV parameters on risk of CVD events. Hazard ratios and 95% confidence intervals associated with increases in HRV parameters, after adjustment for age, history of CVD events, gender, serum phosphorus and albumin, are presented. Measures marked with an asterisk are plotted on the log scale.

implicated in causing both vascular and glomerular injury to the kidney [4]. Brotman *et al.* [30] have recently shown that low HRV was significantly associated with increased incidence of ESRD and CKD-related hospitalization in a population-based cohort with mean eGFR of 93 mL/min/1.73m^2; in comparison, we have reported results on subjects who already have moderately advanced CKD (mean eGFR = 28 mL/min/1.73m^2).

Both autonomic withdrawal and high level of sympathetic input can lead to decreased HRV [5]. In patients with kidney disease, the pathogenesis of altered autonomic function is thought to be multifactorial. Increased sympathetic activity can be attributed to (i) increased activity of RAS, (ii) afferent signals released in response to ischemia from diseased kidneys which in turn cause increased central sympathetic outflow, (iii) peripheral denervation hypersensitivity, (iv) decreased nitric oxide availability due to decreased nitric oxide (NO) synthase activity from increased levels of NO inhibitors such as asymmetrical dimethylarginine which are known to accumulate in renal failure, (v) other contributing factors including diabetes, hypertension, obesity, inflammation, smoking etc., which coexist in patients with CKD and can contribute to increased sympathetic activity [2–4] and (vi) in addition, there is evidence of decreased parasympathetic activity in patients with kidney disease [27].

Despite its strengths, limitations of our study should be acknowledged. This is an observational study and any associations found do not prove causality. Our study was based on HRV data collected at baseline in this CKD cohort. We therefore cannot definitively comment on the modification of risk of CVD or ESRD if HRV could be improved, as that would require an interventional study. GFR was estimated using the four-variable MDRD equation and not measured by more accurate isotope methods. Serum creatinine was assayed by each institution's own laboratory, not centrally. We did not study healthy controls directly, although we compare our findings with HRV measures obtained in healthy controls in a US population. The potential for 'survival bias' affecting our results deserves mention, as patients enrolled in the cardiac substudy were, at least in part, survivors from among those initially enrolled in the RRI-CKD study. However, this survival bias should have led to the attenuation of the association between HRV and adverse outcomes, suggesting that the documented association of adverse outcomes with

Table 5. (I) Cox regression models predicting time to (A) first CVD event (B) composite of CVD/death and (C) ESRD endpoints and (II) each HRV measure was added to these 'base' models to further assess the relationship between HRV and outcomes[a]

(I) Base model		(II) Base model + HRV parameter			
Covariate	HR (95% CI) for covariate	HRV parameter	HR (95% CI) for HRV		
(A) First CVD event	$n = 302$, $R^2 = 0.14$	(A) First CVD event	$n = 290$	P-value	R^2
Age	1.04 (1.01–1.06)**	LF/HF ratio	0.70 (0.48–1.02)	0.0608	0.15
Males	1.60 (0.87–2.94)	LF (ln ms^2)	0.69 (0.52–0.91)*	0.0077	0.16
Phosphorus (mg/dL)	1.53 (1.17–1.99)**	VLF (ln ms^2)	0.70 (0.51–0.98)*	0.0381	0.15
Albumin (g/dL)	0.35 (0.19–0.65)**	ASDNN (ln ms)	0.51 (0.26–3.13)	0.0491	0.15
History of CVD	2.74 (1.47–5.13)**	Total power (ln ms^2)	0.69 (0.48–0.98)*	0.0358	0.15
(B) CVD or death	$n = 304$, $R^2 = 0.14$	(B) CVD or Death	$n = 292$	P-value	R^2
Age	1.04 (1.02–1.06)***	LF/HF ratio	0.62 (0.45–0.87)	0.0050	0.16
Males	1.55 (0.91–2.63)	LF (ln ms^2)	0.70 (0.55–0.90)	0.0053	0.16
Diabetes	1.86 (1.10–3.15)*	VLF (ln ms^2)	0.72 (0.54–0.96)	0.0263	0.15
Albumin (g/dL)	0.43 (0.24–0.75)**	ASDNN (ln ms)	0.57 (0.31–1.03)	0.0641	0.15
History of CVD	1.79 (1.03–3.09)*	Total power (ln ms^2)	0.72 (0.52–0.98)	0.0387	0.15
(C) ESRD end point	$n = 182$, $R^2 = 0.48$	(C) ESRD end point	$n = 174$	P-value	R^2
Age	0.98 (0.96–1.00)	LF/HF ratio	0.66 (0.44–0.98)	0.0372	0.50
Males	1.41 (0.78–2.56)	LF (ln ms^2)	0.93 (0.74–1.17)	0.5387	0.49
Ln (ACR)	1.21 (1.01–1.45)*	VLF (ln ms^2)	0.74 (0.54–1.03)	0.0768	0.50
Hemoglobin (g/dL)	0.77 (0.60–0.97)*	ASDNN (ln ms)	0.83 (0.47–1.46)	0.5186	0.49
Ln (LVMI) (g/m^2)	7.95 (3.14–20.13)***	Total power (ln ms^2)	0.90 (0.67–1.21)	0.4978	0.49
eGFR (mL/min/1.73m^2)	0.84 (0.80–0.88)***				

[a]Hazard ratios (HR) and 95% confidence intervals (CI) for selected HRV parameters (log transformed) are displayed above. Ratios for all HRV measures, including day and night, are displayed in Supplementary Table C.
*P-value < 0.05, **P-value < 0.005, ***P-value < 0.0005.

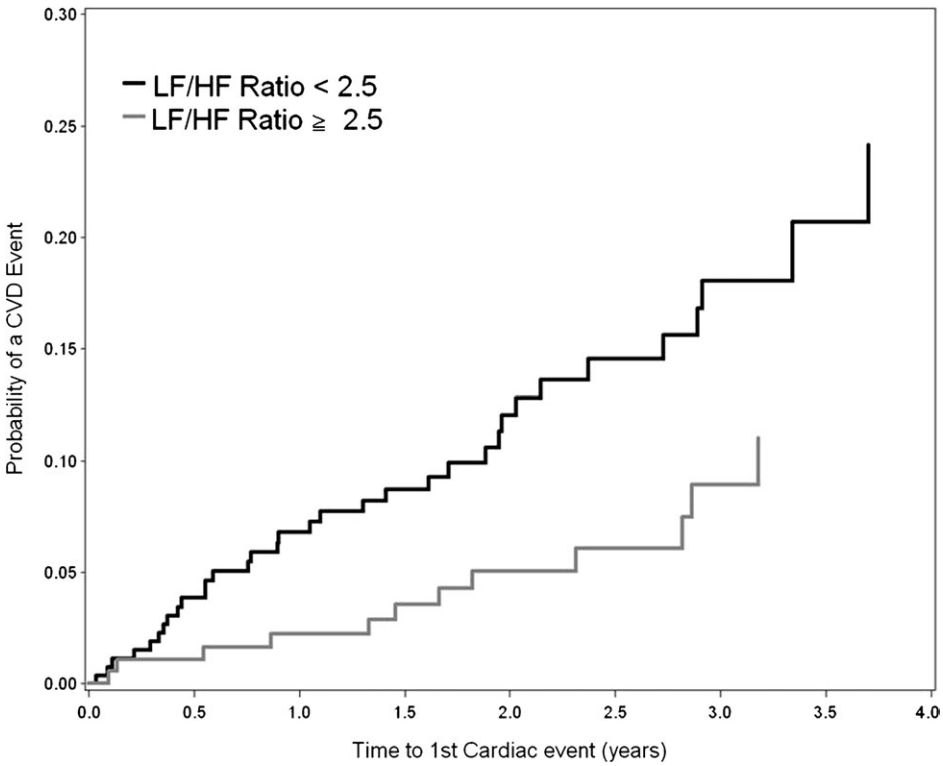

Fig. 4. The cumulative probability of CVD events over time since Holter monitoring by LF/HF ratio (most predictive measure) above and below the median. An LF/HF ratio below the median (<2.5) was associated with a significantly higher risk compared to an LF/HF ratio ≥2.5 (HR = 2.52, P = 0.002). Plotted values were calculated based on Cox regression stratified by LF/HF ratio and adjusted for mean values of: age (60 years), phosphorus (3.7), albumin (4.0), proportion with history of CVD (0.37) and proportion male (0.50).

low HRV in this study is likely to have been even stronger had the study measured HRV in all patients originally enrolled in RRI-CKD. We did not have simultaneous measurement of sympathetic activity (e.g. muscle sympathetic activity) to determine if low HRV was associated with high sympathetic activity. However, in comparison to healthy controls from the literature, almost all HRV measures were lower in our CKD cohort compared with healthy population. In frequency domain analysis, LF (which is variably thought to represent sympathetic activity versus sympathovagal balance) as well as HF (measure of parasympathetic activity) were reduced in CKD compared with healthy population.

Summary and conclusions

Our study describes the spectrum of HRV obtained by 24-h Holter monitoring in a prospective cohort of CKD (Stages 3–5) patients. We find several clinically important variables including comorbidities and laboratory values which are associated with lower HRV in this CKD population. Importantly, lower HRV was independently associated with higher risk of CVD, the composite of CVD/death and a weaker association with ESRD. Our results suggest that HRV has the potential to be a useful predictive tool for risk stratification as well as an intermediate outcome for clinical trials designed to test therapies that could eventually also improve hard outcomes in patients with CKD. This study is hypothesis generating at best due to its observational nature, and we propose that more studies should be done to further explore the practical utility of HRV as a clinical tool to help identify those at higher CVD/ESRD risk in this patient population. Further studies are also needed to investigate if HRV can be improved with certain classes of medications and whether this improvement in HRV will translate to improved CVD and other clinical outcomes.

Supplementary data

Supplementary Tables A–C are available online at http://ndt.oxfordjournals.org.

Acknowledgements. The study was funded by the RRI, New York, NY. We are grateful to all study coordinators and Kerri Briesmiester, Project Manager, for training all the study coordinators in the Holter monitoring technique.

P.C., MD, is supported by a research fellowship from the National Kidney Foundation. These results have previously been presented in part at the American Society of Nephrology and National Kidney Foundation meetings in 2009 as poster presentations.

Drs R.S., MD, and B.W.G., PhD, are members of the project 'Establishing a Surveillance System for CKD' supported under a cooperative agreement from the Centers for Disease Control and Prevention through grant number 5U36CD319276, AAMC ID number MM-1149-10/10.

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Received for publication: 24.2.11; Accepted in revised form: 16.5.11

Nephrol Dial Transplant (2012) 27: 709–715
doi: 10.1093/ndt/gfr288
Advance Access publication 26 May 2011

Testosterone deficiency is a cause of anaemia and reduced responsiveness to erythropoiesis-stimulating agents in men with chronic kidney disease

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Abstract

Background. Hypogonadism or testosterone deficiency is a prevalent condition in men with chronic kidney disease (CKD). Testosterone stimulates erythropoiesis via production of haematopoietic growth factors and possible improvement of iron bioavailability. We hypothesized that testosterone deficiency predisposes to anaemia and reduced responsiveness to erythropoiesis-stimulating agents (ESAs) in CKD men.

Materials and methods. We studied associations between endogenous testosterone and haemoglobin in 239 ESA-naïve nondialysed CKD Stages 1–5 male patients. Additionally, we studied associations between endogenous testosterone levels and ESA dose (U/kg/week) in 126 ESA-treated men undergoing haemodialysis (HD).

Results. Among ESA-naïve males, patients with anaemia presented lower testosterone values. Endogenous testosterone was negatively associated with haemoglobin levels in uni- and multivariate models. Testosterone-deficient patients (total testosterone <10 nmol/L) were 5.3 (95% confidence interval 2.2–12.5) times more likely to be anaemic (Hb < 13.0 g/dL) than testosterone-sufficient patients. In ESA-treated men undergoing HD, higher ESA doses (above the median value of 121 IU/kg body weight/week) are associated with lower testosterone levels and higher percentage of hypochromic red blood cells (RBC). The inverse association between testosterone levels and ESA doses persisted after multivariate adjustment for age, sex hormone-binding globulin, comorbidities, C-reactive protein and s-albumin but was lost after further adjustment for iron medication and hypochromic RBC.

Conclusions. Hypogonadism may be an additional cause of anaemia and reduced ESA responsiveness in men with CKD. Our results raise the possibility that restoration of testosterone levels in hypogonadal CKD males may translate into lower prevalence of anaemia and better ESA responsiveness.

Keywords: androgens; chronic kidney disease; erythropoietin; sex hormones

Introduction

Anaemia and hyporesponsiveness to erythropoiesis-stimulating agents (ESA) are important phenomena