The Associations of Heart Rate Variability with Stroke Outcomes: Mediating Effects of Inflammatory Markers

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Abstract

Background

Heart rate variability (HRV), as a sensitive index of autonomic nervous system (ANS) function, was supposed to be associated with risk of recurrent stroke and functional disability, and negatively related with inflammatory marker levels. Some studies showed that significant changes in HRV metrics might precede the development of asymptomatic or inflammatory flare. This study aimed to validate the association of HRV with stroke outcomes and inflammation, and further to investigate whether inflammatory markers were mediators of the association between HRV and stroke outcome.

Methods

Patients were derived from the Third China National Stroke Registry from August 2015 to March 2018. HRV and inflammatory markers was measured at baseline. The primary outcome (ischemic stroke) and secondary outcomes (new stroke, composite vascular events, all-cause death and disability) were assessed at 1-year follow-up after stroke symptom onset. Mediation analysis was performed to estimate the mediation effect of inflammatory markers between HRV and stroke outcomes.

Results

A total of 4,592 patients were included in this analysis. The standard deviation of N-N intervals (SDNN) of HRV was negatively associated with inflammatory markers, including high-sensitive C-reactive protein (hs-CRP) and interleukin-6 (IL-6). The adjusted hazard ratio/odds ratio for the fourth vs. the first quartile of SDNN for Primary and all secondary outcomes were significant ($P<0.05$). The associations between SDNN and ischemic stroke, composite vascular events, all-cause death and disability at one year were mediated by hs-CRP were 3%, 4%, 7%, and 5%, respectively. The mediation proportions of association SDNN with ischemic stroke, composite vascular events, all-cause death and disability by IL-6 were 8%, 7%, 14%, and 13%, respectively.

Conclusions

SDNN was an independent predictor for stroke outcomes and had a negative relationship with inflammatory markers. Inflammatory markers may partially mediate the relationship between heart rate variability and stroke outcomes at one year. However, the specific role of inflammation in HRV and stroke outcomes needs to be verified by more studies, and our results might provide clues for future studies.

Introduction

Autonomic nervous system (ANS) dysfunction was closely associated with increased recurrent stroke, functional disability and mortality in patients with stroke[1–4]. Heart rate variability (HRV) was the quantitative assessment of variation in heartbeat intervals and is a sensitive index of ANS function. Previous studies pointed that HRV was strongly associated with stroke, atherosclerosis and neurological dysfunction[5–7].

Converging shreds of evidence suggested that the ANS could regulate the inflammatory reflex, and HRV indicators were usually used to reflect how the ANS handled the activity of neurophysiological pathways to the inflammatory process.[8–10] Some studies showed that significant changes in HRV metrics might precede the development of asymptomatic or inflammatory flare and systematic inflammation was associated with increased risk of stroke recurrence[11–13]. However, whether the association of HRV with stroke outcome was mediated through inflammatory markers was still unclear. Therefore, we aimed to validate the association of HRV with stroke outcomes and inflammation in a national prospective stroke registry in China, and then further to investigate whether inflammatory markers were potential mediators of the association between HRV and stroke outcome.

Methods

Study inclusion and participants

Data were derived from the Third China National Stroke Registry (CNSR-III), a prospective national registry of patients with acute ischemic stroke or transient ischemic attack (TIA) from August 2015 to March 2018 in China. The rationale, design and method of CNSR-III have been previously published in detail[14]. In brief, the study was designed to find the imaging and biological markers for the prognosis of the ischemic cerebrovascular events and identify the patients at high risk in an early phase. Patients with ischemic stroke or TIA within 7 days after symptom onset were enrolled in the study.

Patients were enrolled from 201 sites in which 171 sites voluntarily participated in the CNSR-III biomarker subgroup study and collected blood samples after admission.

Baseline information

The trained site investigators collected baseline data for each participant through face-to-face interviews or related medical records with a standard operating protocol. Baseline information included age, gender, weight, height, current smoking status, drinking status, medical history of hypertension, diabetes, hypercholesterolemia, ischemic stroke, coronary heart disease and thrombolysis treatment. Body mass index (BMI) was calculated as body weight (kg) divided by the square of height (m^2). Pre-stroke modified Rankin scale (mRS)score and National Institutes of Health Stroke Scale (NIHSS) score were measured on admission. According to the Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification standard, ischemic stroke was centrally classified as different etiologies[15].

HRV measurement

Participants received a 24-hour ECG Holter examination during hospitalization. The measuring time of participants was between 18 and 24 hours. The simplest time-domain measure of HRV testing was performed in a quiet, temperature-controlled room after a rest period of at least 15 min in the supine position. HRV indicators, including the standard deviation of all N-N intervals (SDNN) and the square root of the mean of the sum of the squares of differences between adjacent N-N intervals (RMSSD), were automatically measured and reported in units of ms[6, 16]. Reflecting total variability, SDNN was thought to mirror the

contribution of both the sympathetic nervous system (SNS) and parasympathetic nervous system (PNS). RMSSD was considered as the primary index of vagally-mediated HRV and mainly affected the PNS.[9]

Inflammatory markers measurement

We collected fasting blood samples and extracting serum and plasma samples within seven days after admission (median time,53 hours [interquartile range, 26–96 hours]) after index event onset. The extracting serum and plasma samples were transported from the local hospitals to the clinical biobank in Beijing Tiantan Hospital in two layers of anti-contamination packaging and monitored by a cold chain all the time. All specimens were stored in -80°C refrigerators until tests were performed centrally and blindly[13]. High-sensitive C-reactive protein (hs-CRP) was detected on Roche Cobas C701 analyzer. Interleukin-6 (IL-6), chitinase-3-like protein 1 (YKL-40), lipoprotein-associated phospholipase A2 activity (Lp-PLA2-A) were measured by using enzyme-linked immunosorbent assay kits (catalogue number: PHS600C for IL-6, DPLG70 for Lp-PLA2 and DC3L10 for YKL-40, R&D Systems, Inc, Minneapolis, MN, USA).

Follow-up and outcome

Participants were followed up at one year by trained site investigators. The primary outcome was an ischemic stroke defined as either severe primary neurological impairment (an increase of 4 or more points on the NIHSS score) resulted from cerebral ischemia or new neurological impairment lasting more than 24 hours[17]. Secondary outcomes included new stroke, composite vascular events, all-cause death and disability. New stroke included ischemic stroke and hemorrhagic stroke. All-cause death was defined as death from any cause. The composite vascular event included ischemic stroke, hemorrhagic stroke, myocardial infarction and vascular death. Disability was defined as with an mRS of 3 to 6. The events were collected by site researchers and finally determined by the independent endpoint determination committee. Case fatality was either confirmed on a death certificate from the attended hospital or the local civil registry[17].

Statistical method

SDNN and RMSSD were categorized into four groups by quartiles. Continuous variables were presented as mean with standard deviation (SD) or median with the interquartile range, and categorical variables were reported as frequencies with percentages. Demographic, medical history, and imaging parameters were compared among patients in different HRV subgroups by $\chi 2$ test or the Fisher exact method for categorical variables and one-way ANOVA or Kruskal Wallis test for continuous variables. Logistic regression was used for the outcome of disability (mRS scores 3–6). Cox proportional hazard model was used to evaluate the relationship between HRV and other stroke outcomes. The proportional hazards assumption for the Cox models was examined by the Schoenfeld residual method. We calculated adjusted hazard ratios (HRs) or odds ratios (ORs) with their 95% confidence intervals (CIs) using the first quartile as the reference by three multivariable Cox/logistic methods. In model 1, we adjusted for age, sex. In model 2, we further adjusted for current smoking, heavy drinking, systolic blood pressure, medical histories of coronary heart disease, ischemic stroke, diabetes, hypertension and hypercholesterolemia, baseline NIHSS score, antidiabetic agent and anticoagulation treatment during hospitalization, index event at discharge and TOAST subtype. To reduce selection bias due to missing values, model 3 adjusted for covariates in model 2 using the inverse-

probability-of-treatment-weighted(IPTW) approach to account for missing data for HRV and inflammatory markers[18]. IPTW was then defined as the inverse of the estimated propensity score for the included patients.

The potential mediating role of inflammatory markers between HRV and stroke outcome was assessed by using the mediation analysis model. SDNN and RMSSD were set as the independent variables in the mediation model, stroke outcomes as the dependent variables, and inflammatory markers as the mediating variables. It is essential to measure 3 pathways to perform mediation analysis.

Step 1, the association of HRV with outcomes (pathway c);

Step 2, the association of HRV with inflammatory markers (pathway a);

Step 3, the association of inflammatory markers with outcomes, after controlling for HRV (pathway b).

If steps 1 to 3 were satisfied, we further performed mediation analysis and measured the total effect (TE), natural direct effect (NDE) and natural indirect effect (NIE). Mediation (indirect effect) can be established through estimation of the direct causal relationship (pathway c'). The mediation effect is full when c' is 0 and partial when it is unequal to 0[4]. HRV and inflammatory markers variables were standardized with a mean score of 0 and SD of 1, and then compared the differences under the same scale[19]. The estimate of pathway a was reported as β and that of all other pathways were reported as HRs/ORs. Mediation percentage was estimated by dividing log HRs/ORs of the indirect effect (pathways ab) by the log HRs/ORs of the total effect (pathway c)[4, 20, 21]. We used SAS macro "*%mediation*" to carry out the mediation analysis[21].

A 2-sided P value < 0.05 was considered statistically significant. All analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC).

Data availability

Data related to the analysis are available to researchers on request to reproduce the results or replicate the procedure by directly contacting the corresponding author.

Results

Study participants and characteristics

Among all the patients with ischemic stroke or TIA enrolled in the CNSR-III study, 11,261 patients underwent baseline blood samples collection. After excluding 851 patients with atrial fibrillation or atrial flutter, 5,818 patients with missing data of laboratory tests or HRV, a total of 4,592 patients were included in this analysis (Additional file1: Figure S1). There were 2.2% patients lost to follow-up at one year.

The patients included in and those excluded from this study were well-balanced (Additional file1: Table S1). The mean age of participants included in this analysis was 61.4 ± 11.0 years and 3133 (68.2%) patients were male. The median level of SDNN was 104.0 ms (interquartile range 85.1–125.0 ms). Patients with lower SDNN were more likely to have elder age and higher proportions of history of diabetes mellitus and coronary artery disease, and had a lower proportion of history of current smoking and heavy drinking (Table 1). The

median level of RMSSD was 27.0 ms (interquartile range 20.0-37.9 ms). Participants with lower RMSSD were more likely to have elder age and higher proportions of history of diabetes mellitus and had a lower proportion of history of current smoking (Additional file1: Table S2).

Table 1
Baseline characteristics of participants according to quartiles of SDNN.

Characteristic	Total	SDNN, ms				P value
		Quartile 1 (< 85.1)	Quartile 2 (85.1–104.0)	Quartile 3 (104.0-125.0)	Quartile 4 (≥ 125.0)	
Participants, n	4592	1114	1149	1177	1152	
Age (yr), mean ± SD	61.4 ± 11.0	63.5 ± 11.2	62.0 ± 10.5	60.3 ± 10.6	59.8 ± 11.2	< 0.001
Male, No. (%)	3133(68.2)	669(60.1)	750(65.3)	845(71.8)	869(75.4)	< 0.001
BMI, kg/m2, mean ± SD	24.8 ± 3.3	24.7 ± 3.5	24.8 ± 3.5	24.9 ± 3.2	24.8 ± 3.2	0.45
Current smoking, No. (%)	1523(33.2)	304(27.3)	373(32.5)	419(35.6)	427(37.1)	< 0.001
Heavy drinking, No. (%)	689(15.0)	141(12.7)	163(14.2)	182(15.5)	203(17.6)	0.008
Diastolic blood pressure, mm Hg	87(80–96)	88(80–97)	86(79–95)	87(79–96)	87(80–95)	0.11
Systolic blood pressure, mm Hg	150(135–165)	150(138–167)	150(135–165)	150(135–167)	147(134–163)	0.002
Medical history, No. (%)						
Stroke	1007(21.9)	275(24.7)	236(20.5)	250(21.2)	246(21.4)	0.08
TIA	111(2.4)	24(2.2)	37(3.2)	24(2.0)	26(2.3)	0.23
Hypertension	2881(62.7)	712(63.9)	733(63.8)	739(62.8)	697(60.5)	0.30
Diabetes	1118(24.4)	356(32.0)	267(23.2)	248(21.1)	247(21.4)	< 0.001
Hypercholesterolemia	385(8.4)	82(7.4)	103(9.0)	98(8.3)	102(8.9)	0.50
Coronary artery disease	483(10.5)	168(13.5)	136(11.5)	119(9.2)	110(8.8)	< 0.001
Heart failure	16(0.4)	6(0.5)	3(0.3)	5(0.4)	2(0.2)	0.45
Peripheral artery disease	32(0.7)	11(1.0)	6(0.5)	5(0.4)	10(0.9)	0.31
NIHSS at admission	3(1–6)	4(2–6)	3(1–6)	3(1–5)	3(1–5)	< 0.001

SDNN indicates the standard deviation of all N-N intervals; BMI, Body mass index; mRS, modified Rankin Scale; TIA, transient ischemic attack; NIHSS, National Institutes of Health Stroke Scale; TOAST, Trial of Org 10172 in Acute Stroke Treatment.

Values are presented as median (interquartile range) or number (%) unless otherwise indicated.

Characteristic	Total	SDNN, ms				P value
		Quartile 1 (< 85.1)	Quartile 2 (85.1–104.0)	Quartile 3 (104.0-125.0)	Quartile 4 (≥ 125.0)	
Thrombolysis treatment	400(8.71)	95(8.53)	112(9.75)	111(9.43)	82(7.12)	0.11
Prestroke mRS 2–5, No. (%)	174(3.8)	53(4.8)	44(3.8)	38(3.2)	39(3.4)	0.22
Index event, No. (%)						< 0.001
Ischemic stroke	4244(92.4)	1061(95.2)	1073(93.4)	1077(91.5)	1033(89.7)	
TIA	348(7.6)	53(4.8)	76(6.6)	100(8.5)	119(10.3)	
TOAST, No. (%)						0.005
Large-artery atherosclerosis	1282(27.9)	351(31.5)	338(29.4)	283(24.0)	310(26.9)	
Cardiogenic embolism	77(1.7)	19(1.7)	19(1.7)	20(1.7)	19(1.7)	
Small artery occlusion	1022(22.3)	228(20.5)	253(22.0)	277(23.5)	264(22.9)	
Other cause	57(1.2)	23(2.1)	12(1.0)	13(1.1)	9(0.8)	
Undetermined cause	2154(46.9)	493(44.3)	527(45.9)	584(49.6)	550(47.7)	
Medication during hospitalization, No. (%)						
Antiplatelet	4467(97.3)	1077(96.7)	1116(97.1)	1147(97.5)	1127(97.8)	0.38
Anticoagulation	319(7.0)	92(8.3)	90(7.8)	77(6.5)	60(5.2)	0.02
Lipid-lowering	4431(97.5)	1065(96.9)	1114(98.0)	1136(97.3)	1116(97.7)	0.39
Antidiabetic	1230(26.8)	395(35.5)	314(27.3)	265(22.5)	256(22.2)	< 0.001
Antihypertensive	2143(46.7)	553(49.6)	516(44.9)	557(47.3)	517(44.9)	0.07

SDNN indicates the standard deviation of all N-N intervals; BMI, Body mass index; mRS, modified Rankin Scale; TIA, transient ischemic attack; NIHSS, National Institutes of Health Stroke Scale; TOAST, Trial of Org 10172 in Acute Stroke Treatment.

Values are presented as median (interquartile range) or number (%) unless otherwise indicated.

HRV and stroke outcomes

Among all included patients, a total of 435 (9.5%) patients had new stroke (398 [8.7%] ischemic stroke and 43 [0.9%] hemorrhagic stroke), 455 (9.9%) composite vascular events, 106 (2.3%) died, and 511 (11.4%) had

disability (mRS 3–6) at one year. All proportional hazards assumptions were met (P > 0.05). Patients with higher SDNN quartile were associated with a lower risk of a new stroke, ischemic stroke, all-cause death, composite vascular events and disability within one year after adjusting for all covariates. The adjusted HR for the fourth vs. the first quartile of SDNN was 0.57 (95% CI 0.42–0.77) for ischemic stroke at one year. We observed the consistent results when the missing values were processed using the inverse weighted probability model. Similar results were observed for new stroke, all-cause death, composite vascular events and disability within one year (Table 2). However, no significant association between RMSSD and stroke outcome was observed in the present study (Additional file1: Table S3).

Table 2
Associations of SDNN with Stroke Outcomes at 1 Year.

SDNN, ms	Events (%)	Model 1†		Model 2‡		Model 3§	
		HR/OR(95%CI)¶	P value	HR/OR (95%CI)	P value	HR/OR (95%CI)	P value
Primary outcome							
Ischemic stroke							
Quartile 1 (< 85.1)	134(12.0)	Reference		Reference		Reference	
Quartile 2 (85.1- 103.9)	104(9.1)	0.75(0.58–0.98)	0.03	0.81(0.63–1.05)	0.12	0.73(0.57–0.95)	0.02
Quartile 3 (104.0- 124.9)	91(7.7)	0.65(0.50–0.86)	0.002	0.73(0.56–0.96)	0.03	0.62(0.48–0.81)	0.001
Quartile 4 (≥ 125.0)	69(6.0)	0.50(0.38–0.67)	< 0.001	0.57(0.42–0.77)	< 0.001	0.48(0.36–0.64)	< 0.001
Secondary outcome							
New stroke							
Quartile 1 (< 85.1)	143(12.8)	Reference		Reference		Reference	
Quartile 2 (85.1- 103.9)	117(10.2)	0.79(0.62–1.01)	0.06	0.85(0.66–1.08)	0.18	0.78(0.61–0.999)	0.0498
Quartile 3 (104.0- 124.9)	100(8.5)	0.66(0.51–0.86)	0.002	0.74(0.57–0.96)	0.02	0.65(0.50–0.84)	< 0.001
Quartile 4 (≥ 125.0)	75(6.5)	0.50(0.38–0.67)	< 0.001	0.57(0.43–0.76)	< 0.001	0.49(0.37–0.65)	< 0.001

SDNN indicates the standard deviation of all N-N intervals; HR, hazard ratio; CI, confidence interval.

†Model 1: adjusted for age, gender.

‡Model 2: model 1 with further adjustment for current smoking, heavy drinking, systolic blood pressure, medical histories of coronary heart disease, ischemic stroke, diabetes, hypertension and hypercholesterolemia, baseline NIHSS score, antidiabetic agent, anticoagulation treatment and thrombolytic treatment during hospitalization, Index event at discharge and TOAST (Trial of Org 10172 in Acute Stroke Treatment) subtype.

§Model 3: model2 using the inverse-probability-of-treatment-weighted (IPTW) approach to account for missing data for HRV and inflammatory markers.

¶Odd ratios were used for the disability and hazard ratios for the other outcomes.

SDNN, ms	Events (%)	Model 1†		Model 2‡		Model 3§	
		HR/OR(95%CI)¶	P value	HR/OR (95%CI)	P value	HR/OR (95%CI)	P value
Composite vascular events							
Quartile 1 (< 85.1)	147(13.2)	Reference		Reference		Reference	
Quartile 2 (85.1- 103.9)	125(10.9)	0.82(0.65–1.04)	0.10	0.88(0.69–1.12)	0.30	0.81(0.64–1.03)	0.09
Quartile 3 (104.0- 124.9)	105(8.9)	0.68(0.53–0.87)	0.003	0.76(0.59–0.98)	0.03	0.66(0.51–0.85)	0.001
Quartile 4 (≥ 125.0)	78(6.8)	0.51(0.39–0.67)	< 0.001	0.57(0.43–0.76)	< 0.001	0.49(0.37–0.65)	< 0.001
All-cause mortality							
Quartile 1 (< 85.1)	42(3.8)	Reference		Reference		Reference	
Quartile 2 (85.1- 103.9)	27(2.4)	0.67(0.41–1.08)	0.10	0.80(0.49–1.31)	0.38	0.62(0.39–1.01)	0.057
Quartile 3 (104.0- 124.9)	21(1.8)	0.55(0.32–0.93)	0.025	0.69(0.40–1.18)	0.18	0.47(0.28–0.79)	0.005
Quartile 4 (≥ 125.0)	16(1.4)	0.43(0.24–0.76)	0.004	0.53(0.30–0.96)	0.04	0.37(0.21–0.66)	0.001
Disability							
Quartile 1 (< 85.1)	204(18.8)	Reference		Reference		Reference	
Quartile 2 (85.1- 103.9)	119(10.6)	0.55(0.43–0.71)	< 0.001	0.64(0.49–0.84)	0.001	0.52(0.43–0.62)	< 0.001

SDNN indicates the standard deviation of all N-N intervals; HR, hazard ratio; CI, confidence interval.

†Model 1: adjusted for age, gender.

‡Model 2: model 1 with further adjustment for current smoking, heavy drinking, systolic blood pressure, medical histories of coronary heart disease, ischemic stroke, diabetes, hypertension and hypercholesterolemia, baseline NIHSS score, antidiabetic agent, anticoagulation treatment and thrombolytic treatment during hospitalization, Index event at discharge and TOAST (Trial of Org 10172 in Acute Stroke Treatment) subtype.

§Model 3: model2 using the inverse-probability-of-treatment-weighted (IPTW) approach to account for missing data for HRV and inflammatory markers.

¶Odd ratios were used for the disability and hazard ratios for the other outcomes.

SDNN, ms	Events (%)	Model 1†		Model 2‡		Model 3§	
		HR/OR(95%CI)¶	P value	HR/OR (95%CI)	P value	HR/OR (95%CI)	P value
Quartile 3 (104.0- 124.9)	106(9.2)	0.51(0.40–0.66)	< 0.001	0.63(0.48–0.84)	0.001	0.44(0.36–0.53)	< 0.001
Quartile 4 (≥ 125.0)	82(7.3)	0.40(0.30–0.53)	< 0.001	0.52(0.38–0.70)	< 0.001	0.34(0.28–0.42)	< 0.001
SDNN indicates the standard deviation of all N-N intervals; HR, hazard ratio; CI, confidence interval.							
†Model 1: adjusted for age, gender.							
‡Model 2: model 1 with further adjustment for current smoking, heavy drinking, systolic blood pressure, medical histories of coronary heart disease, ischemic stroke, diabetes, hypertension and hypercholesterolemia, baseline NIHSS score, antidiabetic agent, anticoagulation treatment and thrombolytic treatment during hospitalization, Index event at discharge and TOAST (Trial of Org 10172 in Acute Stroke Treatment) subtype.							
§Model 3: model2 using the inverse-probability-of-treatment-weighted (IPTW) approach to account for missing data for HRV and inflammatory markers.							
¶Odd ratios were used for the disability and hazard ratios for the other outcomes.							

HRV and Inflammatory markers

The relationship between HRV and inflammatory markers is displayed in Additional file1 (Table S4 and Table S5). Comparing with the first quartile, the fourth quartile of SDNN was associated with a lower level of hs-CRP (β -5.27; 95% CI -7.09, -3.45), IL-6 (β -1.82; 95% CI -2.16, -1.47) and YKL-40 (β -9.71; 95% CI -14.59, -4.84) after adjusting for all potential covariates. No significant association was observed between SDNN and Lp-PLA2-A (Additional file1: Table S4). RMSSD was not associated with the other three inflammatory markers either in continuous or categorical variables, except for YKL-40 (Additional file1: Table S5).

Mediation effect analysis

Based on the above analyses, we found that SDNN (rather than RMSSD) were associated with the level of hs-CRP, IL-6 and YKL-40 and stroke outcomes. We further performed mediation analyses to evaluate the mediation effects of inflammatory markers on the associations of SDNN with stroke outcomes at and one year.

Figure 1 and Table 3 show the mediation effect of hs-CRP between SDNN and stroke outcomes after adjustment for potential confounders. The results showed that hs-CRP mediated SDNN and ischemic stroke, composite vascular events, all-cause mortality and disability at one year. The proportions of the mediation effect of hs-CRP for SDNN and outcomes including ischemic stroke, composite vascular events, all-cause death and disability within one year were 3%, 4%, 7%, and 5%, respectively.

Table 3
Mediation Analysis of the Relationship Between SDNN and Stroke Outcome at 1 Year by hs-CRP.

Outcome	Total Effect (TE)		Natural Direct Effect (NDE)		Natural Indirect Effect (NIE)		Percentage Mediated (PM)
	HR/OR (95% CI)†	P value	HR/OR (95% CI)	P value	HR/OR (95% CI)	P value	%
Primary outcome							
Ischemic stroke	0.81(0.72, 0.90)	< 0.001	0.81(0.72, 0.91)	< 0.001	0.993(0.987, 0.998)	0.04	3%
Secondary outcome							
New stroke	0.80(0.72, 0.89)	< 0.001	0.81(0.72, 0.90)	< 0.001	0.994(0.988, 0.999)	0.07	-
Composite vascular events	0.81(0.72, 0.89)	< 0.001	0.81(0.73, 0.90)	< 0.001	0.992(0.986, 0.998)	0.02	4%
All-cause mortality	0.77(0.63, 0.96)	0.02	0.79(0.64, 0.98)	0.03	0.982(0.973, 0.991)	< 0.001	7%
Disability	0.78(0.70, 0.87)	< 0.001	0.79(0.71, 0.88)	< 0.001	0.988(0.980, 0.995)	0.001	5%

SDNN indicates the standard deviation of all N-N intervals; hs-CRP, high-sensitive C-reactive protein; CI, confidence interval.

All models were adjusted for potential confounders. SDNN and hs-CRP were centrally standardized.

†Odd ratios were used for the disability and hazard ratios for the other outcomes.

The proportions of the mediation effect of IL-6 for outcomes including ischemic stroke, composite vascular events, all-cause death and disability within one year were 8%, 7%, 14%, and 13%, respectively (Table 4; Fig. 2). Meanwhile, YKL-40 mediated the relationship between SDNN and all-cause mortality at one year (Additional file1: Table S6).

Table 4
Mediation Analysis of the Relationship Between SDNN and Outcome at 1 Year by IL-6.

Outcome	Total Effect (TE)		Natural Direct Effect (NDE)		Natural Indirect Effect (NIE)		Percentage Mediated (PM)
	HR/OR (95% CI)†	P value	HR/OR (95% CI)	P value	HR/OR (95% CI)	P value	%
Primary outcome							
Ischemic stroke	0.81(0.72, 0.90)	< 0.001	0.82(0.73, 0.92)	< 0.001	0.983(0.969, 0.996)	0.013	8%
Secondary outcome							
New stroke	0.80(0.72, 0.90)	< 0.001	0.81(0.73, 0.90)	< 0.001	0.987(0.974, 1.001)	0.051	-
Composite vascular events	0.81(0.72, 0.90)	< 0.001	0.82(0.73, 0.91)	< 0.001	0.986(0.973, 0.999)	0.031	7%
All-cause mortality	0.78(0.63, 0.96)	0.02	0.81(0.65, 0.999)	0.0497	0.967(0.946, 0.988)	0.003	14%
Disability	0.78(0.70, 0.88)	< 0.001	0.81(0.73, 0.90)	< 0.001	0.969(0.956, 0.983)	< 0.001	13%
SDNN indicates the standard deviation of all N-N intervals; IL-6, interleukin-6; CI, confidence interval.							
All models were adjusted for potential confounders. SDNN and IL-6 were centrally standardized.							
†Odd ratios were used for the disability and hazard ratios for the other outcomes.							

Discussion

In the prospective national stroke registry, we found SDNN, a sensitive index of HRV, was negatively associated with both the inflammatory markers and stroke outcomes in patients with acute ischemic stroke or TIA. Two biomarkers of systematic inflammation, hs-CRP and IL-6, partially mediated the association between SDNN and stroke outcomes. In contrast, RMSSD, another index of HRV, was not associated with inflammatory markers and stroke outcomes.

The relationship between autonomic nervous function and stroke has been evaluated by several studies[2, 22, 23]. In line with our findings, a previous study proposed that ANS dysfunction was associated with an increased risk of stroke recurrence and death[22]. Meanwhile, SDNN was also found to be associated with risk of stroke and all-cause death[2, 23]. The atherosclerosis risk in communities' study with longer follow-up showed lower HRV (measured by time-domain and frequency-domain) was associated with a higher risk of incident stroke, and SDNN and RMSSD were both the good predictive factors for stroke risk[3]. However, the present study found that only SDNN, rather than RMSSD, was associated with stroke outcomes. The potential explanation of positive relationship for SDNN, rather than RMSSD, might be that RMSSD reflects

parasympathetic nerve, which changes are transient and variable, whereas SDNN reflects the excitability of both sympathetic and parasympathetic nerves and the sympathetic excitation period is longer after ischemic stroke. Difference in outcome (recurrent stroke vs incident stroke), time of follow-up (one year vs. longer up to 10 years) and HRV measurement method (time-domain vs. frequency-domain) may also account for the different findings between our study and previous studies[3, 22–24].

Many studies have confirmed a continuous cross-talk between both sympathetic and parasympathetic branches of the autonomic nervous system and inflammatory response in different clinical scenarios[9, 25, 26]. The study about vagal nerve pointed that HRV could predict inflammation-mediated death in patients with pancreatic cancer, which suggested that inflammation may be a mediator of some diseases[27]. A meta-analysis on HRV and inflammation showed the importance of the vagus nerve in regulating and controlling the inflammatory response, and there was an overall negative relationship between HRV and markers of inflammation[9]. Previous studies, including the Heart and Soul Study, showed that SDNN, rather than RMSSD, was negatively related to hs-CRP and IL-6, which was consistent with our findings[25, 26]. Furthermore, three previous studies have shown that changes in HRV often occurred before abnormal changes in inflammatory marker indicators, which added evidences that HRV might influence stroke outcome by regulating inflammation[11, 12, 27]. Our study further added evidence supporting that the association between HRV, especially SDNN, and stroke outcome was partially mediated by inflammatory marker.

The possible reason why IL-6 and hs-CRP, rather than YKL-40 and LP-PLA2-A, showed mediation effects might be that IL-6 and hs-CRP are markers of systemic inflammation. Whereas, LP-PLA2 was related to the plaque inflammation/endothelial dysfunction, and YKL-40 was an inflammatory biomarker involved in regulating glial activation and neuroinflammation[28–30]. Moreover, we also found that although hs-CRP and IL-6 mediated the relationship between HRV and stroke recurrence, the mediating percentage of IL-6 was larger than that of hs-CRP. The cytokine of hs-CRP was considered a downstream biomarker in the IL-1, IL-6, hs-CRP pathway; whereas, IL-6 was an upstream signaling cytokine, which has become a major target for immune regulation and thrombosis protection in atherosclerosis[31]. Therefore, as the indicator of inflammation, IL-6 may have higher sensitivity and accuracy. Nevertheless, it should be acknowledged that the mediating percentage of IL-6 was still somewhat small, suggesting that inflammation might only be a partial mediating factor. There might be other pathways or mechanisms involved in mediating the association between autonomic nervous system and stroke outcome which warranted further research.

Inflammation is closely related to atherosclerotic disease, and inflammatory regulation can prevent clinical complications of atherosclerosis[32]. Previous study demonstrated that inflammation was associated with the residual risk of stroke recurrence for ischemic stroke with adherence to guideline-based secondary stroke prevention[13]. Inflammatory response was controlled, in part, by the neural circuitry of the autonomic nervous system[5]. The nervous system, via the anti-inflammatory pathway, could inhibit cytokine synthesis and protect against inflammatory cytokine-mediated diseases[33]. Our study demonstrated that inflammatory markers partially mediated the relationship between HRV and stroke outcomes. This implicates that reducing inflammation by regulating HRV might improve stroke prognosis. However, this needs more rational research in future.

Limitations

This study has several limitations. First, the mediating proportion of our study is relatively low, so inflammatory markers may not be the most important mediating and influencing factors. Second, although we used the inverse weighted probability method to deal with the missing value, selection bias caused by missing data still might exist. Third, since Cox proportional hazard model was rarely used in mediation analysis, the evidence on these models is limited, leaving room for unknown bias. Fourth, HRV and related blood sample data are both collected during hospitalization. We can't accurately judge the successive relationship between them in time. Furthermore, inflammatory markers and HRV might be affected by the stress function in acute ischemic stroke patients, reflecting only be temporarily high or low rather than steady-state data. Fifth, hs-CRP and IL-6 were not the most upstream inflammatory markers and couldn't represent all inflammatory response. Sixth, we did not verify the validity of the prediction model in other external cohorts. Therefore, the accurate mediation effect of inflammation for the association between ANS and the stroke outcomes might need more investigation in large-scale prospective studies with more rigorous protocols or sophisticated instruments.

Conclusion

SDNN, an indicator of HRV, was a predictor for stroke outcomes and had a negative relationship with inflammatory markers. This study demonstrated that inflammatory markers, especially IL-6 and hs-CRP, might partially mediate the relationship between HRV and stroke outcomes at one year. However, the specific role of inflammation in HRV and stroke outcomes needs to be verified by more studies and our results may only provide clues and implications for future studies.

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Ethics Committee of the Beijing Tiantan Hospital and all participating centers. All participants or their representatives provided the written informed consent before participating in the study.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

MW and CL made substantial contributions to study design, data analysis and manuscript writing. WX and AJ made substantial contributions to data analysis. XM, JJL, and XJL made contributions to data collection. HL, YP and YW made substantial contributions to study design and intellectual direction. All authors read and approved the final manuscript.

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Figures

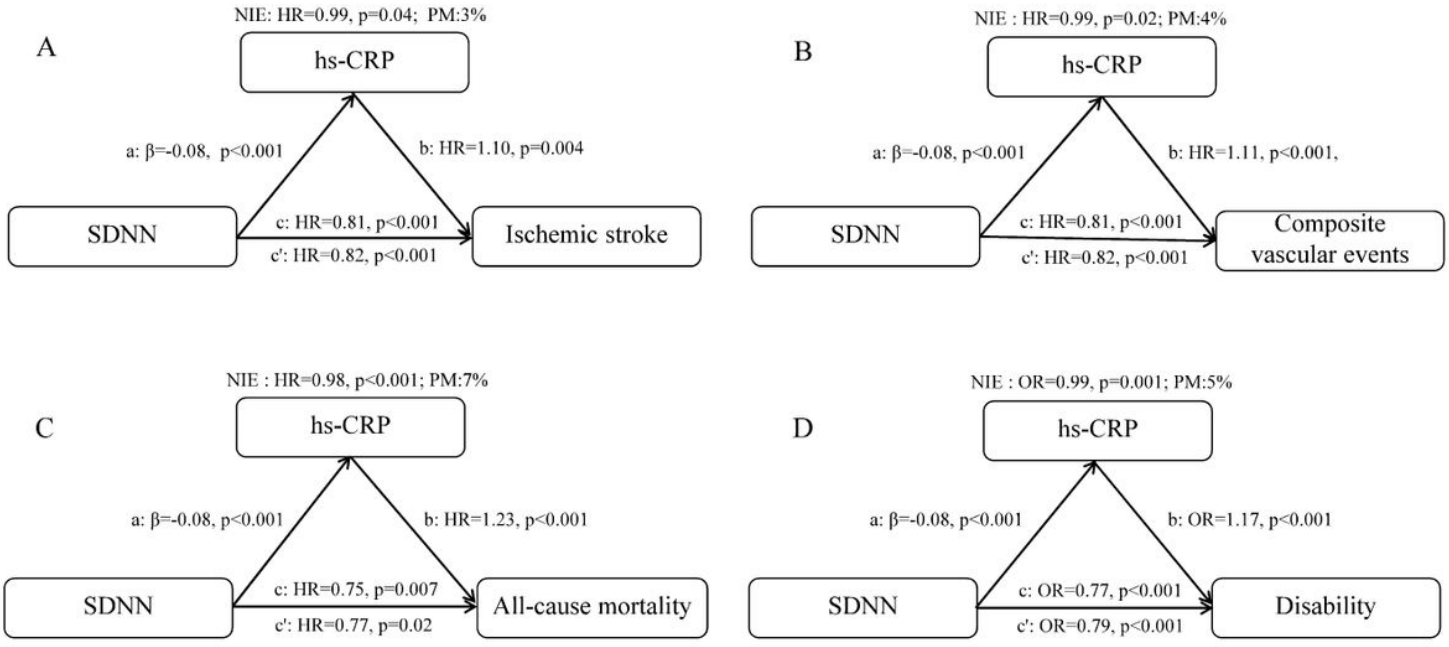

Figure 1

The mediation effect of hs-CRP on the association between SDNN and ischemic stroke (A), composite vascular events (B), all-cause death (C) and disability (D) at one year. Beta values represent unstandardized regression coefficients.

Hs-CRP indicates high-sensitive C-reactive protein; SDNN, the standard deviation of all N-N intervals; NIE, natural indirect effect; OR, odd ratios; HR, hazard ratios; PM, Percentage mediated.

Total effect (c) = direct effect (c') + indirect effect (ab).

All models were adjusted for potential confounders. SDNN and hs-CRP were centrally standardized.

Hazard ratio was used for the outcome of stroke, ischemic stroke, composite vascular events and all-cause death and odd ratios for the disability.

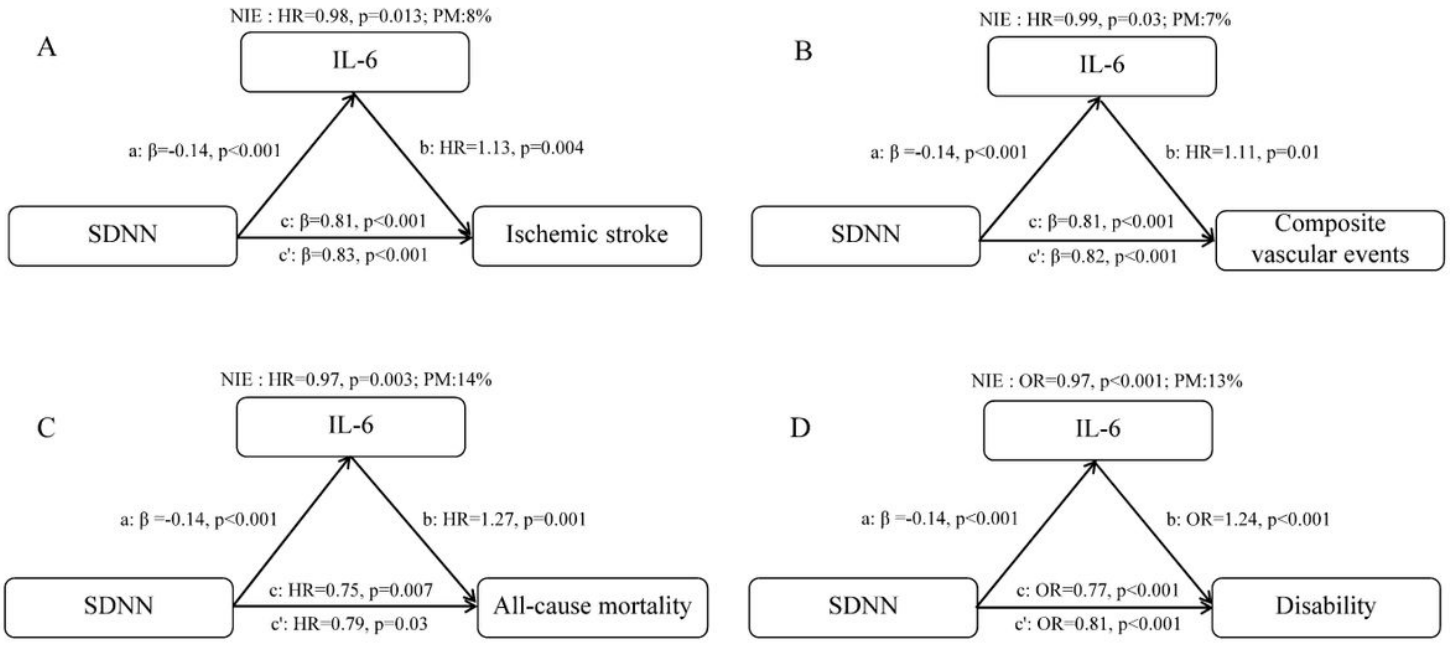

Figure 2

The mediation effect of IL-6 on the association between SDNN and ischemic stroke (A), composite vascular events (B), all-cause death (C) and disability (D) at one year.

IL-6 indicates interleukin-6; SDNN, the standard deviation of all N-N intervals; NIE, natural indirect effect; OR, odd ratios; HR, hazard ratios; PM, Percentage mediated.

Total effect (c) = direct effect (c') + indirect effect (ab).

All models were adjusted for potential confounders. SDNN and IL-6 were centrally standardized.

Hazard ratio was used for the outcome of stroke, ischemic stroke, composite vascular events and all-cause death and odd ratios for the disability.

Supplementary Files

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- Additionalfile1.pdf