

## ORIGINAL ARTICLE

# Autonomic dysfunction in cardiac amyloidosis assessed by heart rate variability and heart rate turbulence

Shinya Yamada MD<sup>1</sup>  | Akiomi Yoshihisa MD<sup>1,2</sup> | Naoko Hijioka MD<sup>1</sup> |  
Masashi Kamioka MD<sup>1</sup> | Takashi Kaneshiro MD<sup>1,3</sup> | Tetsuro Yokokawa MD<sup>1</sup> |  
Tomofumi Misaka MD<sup>1,2</sup> | Takafumi Ishida MD<sup>1</sup> | Yasuchika Takeishi MD<sup>1</sup>

<sup>1</sup>Department of Cardiovascular Medicine, Fukushima Medical University, Fukushima, Japan

<sup>2</sup>Department of Advanced Cardiac Therapeutics, Fukushima Medical University, Fukushima, Japan

<sup>3</sup>Department of Arrhythmia and Cardiac Pacing, Fukushima Medical University, Fukushima, Japan

## Correspondence

Shinya Yamada, MD, Department of Cardiovascular Medicine, Fukushima Medical University, 1 Hikarigaoka, Fukushima 960-1295, Japan.  
Email: smyyamada0124@yahoo.co.jp

## Abstract

**Background:** Cardiac amyloidosis (CA) is characterized by left ventricular hypertrophy (LVH) and autonomic nervous imbalance due to amyloid infiltration. However, autonomic dysfunction is often seen in heart failure (HF) with LVH from other etiologies. We aimed to characterize autonomic dysfunction in CA from other etiologies of LVH.

**Methods:** Fifty-five HF patients with LVH (35 males, mean age  $65 \pm 16$  years) were enrolled. LVH was defined as left ventricular mass index measured by echocardiography  $>95 \text{ g/m}^2$  in women and  $115 \text{ g/m}^2$  in men. The etiology was as follows: amyloid light chain (AL)-CA,  $n = 14$ ; hypertrophic cardiomyopathy,  $n = 21$ ; and aortic stenosis (AS),  $n = 20$ . With the patient in a clinically stable condition, heart rate variability (HRV) and heart rate turbulence (HRT), which reflect autonomic dysfunction, were measured using Holter monitoring and compared among the three groups.

**Results:** Brain natriuretic peptide levels, LVH severity, left ventricular ejection fraction, and tissue Doppler index  $E/e'$  did not differ among the three groups. However, severe abnormalities of HRV and HRT were obtained in AL-CA. In the ROC analysis to identify AL-CA in HF with LVH, the best cutoff value for standard deviation of all R-R intervals, standard deviation of the 5-min mean R-R intervals, turbulence onset, and turbulence slope were 68.5 ms (AUC: 0.865), 58.5 ms (AUC: 0.834), 0.25% (AUC: 0.813), and 1.00 ms/RR (AUC 0.736), respectively.

**Conclusion:** Autonomic dysfunction is a hallmark of AL-CA, and its noninvasive assessment by Holter monitoring may be a useful tool for differential diagnosis of HF with LVH.

## KEYWORDS

autonomic dysfunction, cardiac amyloidosis, heart rate turbulence, heart rate variability, left ventricular hypertrophy

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2020 The Authors. *Annals of Noninvasive Electrocardiology* published by Wiley Periodicals, LLC

## 1 | INTRODUCTION

The prognosis of amyloidosis is poor, and its most common outcome is cardiac death. Depending on the types of amyloidosis, great differences in the manner of cardiac amyloid deposition and disease progression have been reported (Hongo et al., 2000). In particular, it has been suggested that cardiac amyloidosis (CA) in patients with amyloid light-chain (AL) amyloidosis (AL-CA) rapidly develop heart failure (HF) or cardiac arrhythmia, resulting in adverse prognosis (Grogan & Dispenzieri, 2015). AL-CA is generally characterized by left ventricular hypertrophy (LVH) and autonomic dysfunction due to amyloid infiltration in the myocardium and autonomic nervous system (Cueto-Garcia et al., 1985; Reyners, Hazenberg, Reitsma, & Smit, 2002). Although both LVH and autonomic dysfunction are commonly considered risk factors of adverse outcomes in HF patients from various etiologies (Bauer et al., 2008; Kleiger, Miller, Bigger, & Moss, 1987; Mathew et al., 2001; Nolan et al., 1998), it remains unclear whether direct amyloid deposition into the autonomic nervous system can lead to severe autonomic dysfunction, compared to HF patients with LVH from other etiologies.

Clinically important information on the management of HF patients is provided via Holter monitoring (Kleiger et al., 1987; Nolan et al., 1998; Reyners et al., 2002); heart rate variability (HRV) and heart rate turbulence (HRT) are considered to be suitable parameters to assess autonomic function. There is some evidence that abnormal HRV and impaired HRT are related to the development of HF and cardiac death in HF patients (Bauer et al., 2008; Kleiger et al., 1987; Nolan et al., 1998); however, their relationships have not yet been fully investigated in HF patients with LVH from different etiologies. Thus, in the current study, we aimed to (a) assess autonomic dysfunction of AL-CA using Holter monitoring (HRV and HRT) and (b) examine the utility of Holter monitoring for the detection of AL-CA from other etiologies of LVH.

## 2 | METHODS

### 2.1 | Study population and protocol

The current study's participants consisted of 55 HF patients with LVH detected using echocardiography, who were admitted to Fukushima Medical University Hospital for HF treatment and underwent Holter monitoring between 2015 and 2018. The diagnosis of HF was defined based on the Framingham criteria (Ho, Anderson, Kannel, Grossman, & Levy, 1993). LVH was defined as left ventricular mass indexed to body surface area (LVMI) measured by echocardiography greater than 95 g/m<sup>2</sup> in women and 115 g/m<sup>2</sup> in men (Lang et al., 2015). The etiologies of LVH were AL-CA (*n* = 14), hypertrophic cardiomyopathy (HCM, *n* = 21), and aortic stenosis (AS, *n* = 20). All patients with AL-CA were evidenced by endomyocardial biopsy, followed by microscopic analysis with Congo red staining and apple-green birefringence examination (Getz, 2004). The signal of the Congo red staining maintained after oxidation with

permanganate solution was observed to exclude the amyloid A amyloidosis (Bély & Apáthy, 2000). The diagnosis of AL amyloidosis was confirmed based on the histologic evidence of systemic amyloidosis, which was related with plasma cell dyscrasia and/or identification of an immunoglobulin light chain in the serum or urine (Leung, Nasr, & Sethi, 2012; Schonland et al., 2012). In the AL-CA group, multiple myeloma (*n* = 8, 57.1%), monoclonal gammopathy of undetermined significance (*n* = 3, 21.4%), and waldenstrom macroglobulinemia (*n* = 1, 7.1%) were accompanied by AL-CA. An immunoglobulin light chain was identified in the serum (*n* = 2, 14.2%). When transthyretin-associated hereditary amyloidosis was determined by checking the family history for amyloidosis, polyneuropathy, transthyretin-staining, and/or <sup>99m</sup>Tc pyrophosphate cardiac scintigraphy, the patients were excluded. The diagnosis of HCM was made on the basis of echocardiographic demonstration of LVH with end-diastolic wall thickness ≥ 15 mm in more than one segment but without any cardiac or systemic disorder that causes LVH (Authors & members et al., 2014). The patients with HCM underwent magnetic resonance imaging and/or endomyocardial biopsy for the differentiation of CA (Takeda et al., 2013). The severity of AS was assessed by echocardiographic indices of maximum transaortic velocity and the Doppler-derived mean pressure gradient (Nishimura et al., 2014). Patients with moderate to severe AS were enrolled in the present study. Blood samples (brain natriuretic peptide) were collected, and echocardiography, 12-lead electrocardiogram, and 24-hr Holter monitoring were performed when the patients were in a clinically stable condition. In addition, these parameters were compared among the AL-CA, HCM, and AS groups. Patients who had a history of myocardial infarction and/or were receiving hemodialysis were excluded. Additionally, patients with atrial fibrillation and paced rhythm were also excluded due to restrictions for reliable data analysis. Written informed consent was obtained from all study subjects, and the study protocol was approved by the ethics committee of Fukushima Medical University.

### 2.2 | Echocardiography

Echocardiography was performed using standard techniques by experienced echocardiographers at our hospital (ACUSON Sequoia, Siemens Medical Solutions USA, Inc., Mountain View, CA, USA). Echocardiographic parameters included left ventricular ejection fraction (LVEF), interventricular septum (IVST), left ventricular end-diastolic diameter (LVDd), left ventricular end-systolic diameter (LVDs), posterior wall (PWT), LVMI, the E/A ratio and deceleration time of transmitral flow velocity, and the ratio of early transmitral flow velocity to mitral annular velocity (mitral valve E/e'). LVEF was calculated using Simpson's method, and LVMI was calculated based on the following formula:  $\{0.8 \times (1.04[(LVDd + PWT + IVST)^3 - (LVDd)^3]) + 0.6\} / \text{body surface area}$  (Lang et al., 2015). Mitral valve E/e' was calculated by transmitral Doppler flow and tissue Doppler imaging. Tissue Doppler imaging was obtained from the average of septal annulus velocities.

## 2.3 | Electrocardiogram

We used standard 12-lead electrocardiogram tracing at 25-mm/s paper speed and 10-mm/mV amplitude (FCP-7541; Fukuda Denshi, Tokyo, Japan), as described previously (Kimishima et al., 2019). Heart rate, PR interval, QRS duration,  $RV_5$  and  $SV_1$  voltages, and axis in the peripheral leads were assessed on the electrocardiogram recording. The QT interval was manually measured and corrected using Bazett's formula (Bazett, 1920). Low-voltage QRS complexes were defined as a QRS amplitude of less than 5 mm in all limb leads. Poor R wave progression in the precordial leads was defined as no R waves in leads  $V_1$  to  $V_3$ . ST-T change was defined as ST-T elevation or depression by 1 mm or more from baseline. Additionally, the presence of a right bundle branch block (RBBB), left bundle branch block (LBBB), and left axis deviation was investigated in all 12 leads.

## 2.4 | Holter monitoring

Holter monitoring was performed when the patients were in a clinically stable condition during the 24-hr recording period (Yamada et al., 2018). In HRV analysis, standard deviation of all R-R intervals (SDNN) and standard deviation of the 5-min mean R-R intervals (SDANN) were calculated using the time-domain analysis. In the power spectral analysis, low-frequency component, high-frequency component and low-frequency to high-frequency ratio were measured. Spectral measures were computed using the fast-Fourier transform method, as described previously (Montano et al., 1994; Yamada et al., 2013). Spectral powers were expressed in  $ms^2$ . A component in the frequency band from 0.04 to 0.15 Hz was considered low-frequency power. A component in the frequency band from 0.15 to 0.4 Hz was considered high-frequency power. HRT parameters included turbulence onset (TO) and turbulence slope (TS), which were determined as previously described (Schmidt et al., 1999). TO was defined as the difference between the mean of the first two sinus R-R intervals preceding the premature ventricular complex (PVC) and the mean of the subsequent two sinus R-R intervals, expressed as a percentage. TS was defined as the maximum positive value of the slope of a regression line assessed over any sequence of five subsequent sinus-rhythm R-R intervals within the first 20 sinus-rhythm intervals after PVC. The number of premature atrial complex (PAC), PVC, and nonsustained ventricular tachycardia (NSVT) was also estimated. NSVT was defined as three or more consecutive beats arising below the atrioventricular node, with a mean R-R interval of  $\leq 600$  ms lasting less than 30 s. In addition, T-wave alternans (TWA) was analyzed by the time-domain modified moving average method (Nearing & Verrier, 2002). TWA at heart rates higher than 120 beats/min or those with noise levels greater than 20  $\mu V$  were excluded from the analysis. TWA was analyzed in the modified  $V_1$  and  $V_5$  leads. Of the two leads, the one with the higher TWA was categorized as the higher lead. The Holter monitoring analyses were performed using the MARS PC Holter Monitoring and Review System (version 7; GE Healthcare, Milwaukee, WI, USA).

## 2.5 | Comorbidities

The comorbidities, such as hypertension, diabetes, dyslipidemia, chronic kidney disease, and anemia, were investigated as previously described (Yamada et al., 2018). Hypertension was defined as the recent use of antihypertensive drugs, systolic blood pressure  $\geq 140$  mmHg, and/or diastolic blood pressure  $> 90$  mmHg. Diabetes was defined as the recent use of insulin or antidiabetic drugs, a fasting blood glucose value of  $>126$  mg/dl, and/or a hemoglobin  $A_{1c}$  value of  $>6.5\%$ . Dyslipidemia was defined as the recent use of cholesterol-lowering drugs, a triglyceride value of  $>150$  mg/dl, a low-density lipoprotein cholesterol value of  $>140$  mg/dl, and/or a high-density lipoprotein cholesterol value of  $<40$  mg/dl. The estimated glomerular filtration rate (GFR) was measured by the Modification of Diet in Renal Disease formula (Levey et al., 2006). Chronic kidney disease was defined as an estimated GFR of  $<60$  ml/min/1.73 m (Levey et al., 2006; McMurray et al., 2012), and anemia was defined as hemoglobin of  $<12.0$  g/dl in females and  $<13.0$  g/dl in males (McMurray et al., 2012).

## 2.6 | Statistical analysis

The data are presented as the mean  $\pm$  SD for normally distributed continuous variables and proportions for categorical variables. The differences between normally distributed continuous values were assessed using one-way analysis of variance (ANOVA) with post hoc Bonferroni's correction. The differences between nominal variables were compared by chi-square test. A  $p$  value of  $<.05$  was considered statistically significant. Receiver-operating characteristic (ROC) analysis for identification of AL-CA in HF patients with LVH was performed to calculate sensitivity, specificity, areas under the ROC curve, and the optimal cutoff value. The analysis was performed by a biostatistician using SPSS statistical software (version 25.0; SPSS Institute, Chicago, IL, USA).

# 3 | RESULTS

## 3.1 | Baseline characteristics

The baseline patient characteristics are shown in Table 1. Age and prevalence of hypertension were highest in the AS group. The AL-CA and AS groups had a higher prevalence of chronic kidney disease and anemia compared to the HCM group. However, there were no significant differences in male gender, prevalence of NYHA class III/IV, diabetes, dyslipidemia, brain natriuretic peptide levels, and intake of medications for HF and amiodarone among the three groups.

In echocardiography, IVST was thicker, and LVDd and LVDs were smaller, in the HCM group compared with the AS group. However, there were no significant differences in LVEF, PWT, LVMI, E/A, deceleration time, and  $E/e'$  among the three groups. In the AS group, maximum transaortic velocity was  $4.12 \pm 1.00$  m/sec, and the

|                               | AL-CA (n = 14) | HCM (n = 21)  | AS (n = 20)                | P Value |
|-------------------------------|----------------|---------------|----------------------------|---------|
| Age                           | 64.9 ± 9.6*    | 53.8 ± 16.8   | 79.0 ± 8.8 <sup>#,**</sup> | <.001   |
| Male (n, %)                   | 10 (71.4%)     | 15 (71.4%)    | 10 (50.0%)                 | .283    |
| NYHA class III/IV (n, %)      | 5 (35.7%)      | 1 (4.7%)      | 6 (30.0%)                  | .051    |
| Comorbidity                   |                |               |                            |         |
| Hypertension (n, %)           | 3 (21.4%)      | 12 (57.1%)    | 16 (80.0%)                 | .003    |
| Diabetes (n, %)               | 2 (14.2%)      | 4 (19.0%)     | 5 (25.0%)                  | .737    |
| Dyslipidemia (n, %)           | 8 (57.1%)      | 12 (57.1%)    | 13 (65.0%)                 | .849    |
| Chronic kidney disease (n, %) | 10 (71.4%)     | 7 (33.3%)     | 15 (75.0%)                 | .013    |
| Anemia (n, %)                 | 9 (64.2%)      | 5 (23.8%)     | 16 (80%)                   | .001    |
| BNP (pg/ml)                   | 603.0 ± 516.0  | 320.3 ± 232.1 | 781.9 ± 1,024.3            | .115    |
| Echocardiography              |                |               |                            |         |
| LVEF (%)                      | 51.8 ± 12.3    | 56.8 ± 10.8   | 51.4 ± 15.9                | .378    |
| IVST (mm)                     | 15.2 ± 2.5     | 16.8 ± 3.2    | 13.0 ± 1.5**               | <.001   |
| LVDd (mm)                     | 41.6 ± 6.3     | 41.2 ± 8.1    | 47.6 ± 8.6*                | .029    |
| LVDs (mm)                     | 31.3 ± 7.1     | 26.0 ± 8.4    | 34.1 ± 11.9*               | .034    |
| PWT (mm)                      | 14.4 ± 1.7     | 14.0 ± 3.0    | 13.1 ± 1.3                 | .228    |
| LVMI (g/m <sup>2</sup> )      | 150.1 ± 17.2   | 152.7 ± 64.8  | 160.3 ± 42.9               | .650    |
| E/A                           | 1.72 ± 0.70    | 1.24 ± 0.80   | 1.20 ± 0.97                | .211    |
| Deceleration time (ms)        | 162.4 ± 36.1   | 209.7 ± 68.2  | 233.4 ± 123.3              | .076    |
| E/e'                          | 17.5 ± 6.4     | 14.8 ± 8.0    | 18.0 ± 7.3                 | .386    |
| Medication                    |                |               |                            |         |
| β blockers (n, %)             | 9 (64.2%)      | 19 (90.4%)    | 13 (65.0%)                 | .103    |
| ACE-Inhibitors/ARBs (n, %)    | 7 (50.0%)      | 12 (57.1%)    | 11 (55.0%)                 | .916    |
| Amiodarone (n, %)             | 3 (21.4%)      | 5 (19.6%)     | 1 (5.0%)                   | .223    |

**TABLE 1** Comparison of clinical characteristics among the three groups

Abbreviations: ACE-Inhibitors, angiotensin-converting enzyme-Inhibitors; AL-CA, amyloid light-chain cardiac amyloidosis; ARBs, angiotensin II receptor blockers; AS, aortic stenosis; BNP, brain natriuretic peptide; HCM, hypertrophic cardiomyopathy; IVST, interventricular septum thickness; LVDd, left ventricular end-diastolic diameter; LVDs, left ventricular end-systolic diameter; LVEF, left ventricular ejection fraction; LVMI, left ventricular mass index; NYHA, New York Heart Association; PWT, posterior wall thickness.

<sup>#</sup>*p* < .05 vs. CA

\**p* < .05 vs. HCM

\*\**p* < .01 vs. HCM

Doppler-derived mean pressure gradient was 44.0 ± 20.3 mmHg. In the AL-CA group, a granular sparkling sign was observed in nine patients (64.2%).

### 3.2 | Electrocardiogram and Holter monitoring

The parameters of electrocardiogram and Holter monitoring are shown in Table 2. In the 12-lead electrocardiogram, the AL-CA group had the lowest voltage of R wave in V<sub>5</sub> + S wave in V<sub>1</sub>, a higher prevalence of low-voltage QRS complexes, and poor R

progression, compared with the HCM and AS groups. However, there were no significant differences in heart rate, PR interval, QRS duration, QTc interval, axis in peripheral leads, and prevalence of bundle branch block, left axis deviation, and ST-T change among the three groups.

In the Holter monitoring, there were no significant differences in the frequency of PAC or PVC, prevalence of NSVT, high-frequency power and the maximum voltage of TWA among the three group. However, low-frequency power was significantly lower in the AL-CA group compared with the HCM group. SDNN, SDANN, low-frequency to high-frequency ratio, and TS were significantly lower in

**TABLE 2** Comparison of parameters from a 12-lead electrocardiogram and 24-hr Holter monitoring among the three groups

|   | AL-CA (n = 14)            | HCM (n = 21)              | AS (n = 20)               | p value |
|---|---------------------------|---------------------------|---------------------------|---------|
| <b>Electrocardiogram</b>                |                           |                           |                           |         |
| Heart rate (bpm)                        | 69.4 ± 9.5                | 64.8 ± 9.4                | 72.1 ± 10.1               | .062    |
| PR interval (ms)                        | 183.3 ± 28.7              | 172.4 ± 26.5              | 198.2 ± 50.0              | .103    |
| QRS duration (ms)                       | 108.2 ± 19.3              | 116.2 ± 25.1              | 110.3 ± 20.9              | .534    |
| BBB (n, %)                              | 1 (7.1%)                  | 4 (19.0%)                 | 2 (10.0%)                 | .527    |
| LBBB (n, %)                             | 1 (7.1%)                  | 0 (0%)                    | 0 (0%)                    |         |
| RBBB (n, %)                             | 0 (0%)                    | 4 (19.0%)                 | 2 (10.0%)                 |         |
| QTc interval (ms)                       | 449.7 ± 32.4              | 450.1 ± 29.5              | 432.9 ± 35.9              | .189    |
| Axis (degree)                           | -2.8 ± 67.8               | 36.7 ± 41.5               | 28.4 ± 36.9               | .058    |
| Left axis deviation (n, %)              | 6 (42.8%)                 | 5 (23.8%)                 | 3 (15.0%)                 | .181    |
| Low-voltage QRS complexes (n, %)        | 9 (64.2%)                 | 1 (4.7%)                  | 0 (0%)                    | <.001   |
| RV <sub>5</sub> + SV <sub>1</sub> (mV)  | 1.93 ± 0.81               | 4.23 ± 2.06 <sup>##</sup> | 4.38 ± 1.46 <sup>##</sup> | <.001   |
| Poor R progression (n, %)               | 11 (78.5%)                | 4 (19.0%)                 | 1 (5%)                    | <.001   |
| ST-T change (n, %)                      | 7 (50.0%)                 | 18 (85.7%)                | 15 (75.0%)                | .064    |
| <b>Holter monitoring</b>                |                           |                           |                           |         |
| PAC (beats/day)                         | 401.5 ± 659.9             | 975.7 ± 3,510.4           | 2,178.2 ± 3,516.0         | .232    |
| PVC (beats/day)                         | 1,077.0 ± 2,409.3         | 673.1 ± 1,571.4           | 1,113.1 ± 2,106.8         | .747    |
| NSVT (n, %)                             | 3 (21.4%)                 | 7 (33.3%)                 | 6 (30.0%)                 | .745    |
| SDNN (ms)                               | 59.2 ± 15.3 <sup>**</sup> | 130.3 ± 57.2              | 83.7 ± 31.6 <sup>**</sup> | <.001   |
| SDANN (ms)                              | 52.5 ± 13.3 <sup>**</sup> | 110.6 ± 50.3              | 73.4 ± 29.2 <sup>*</sup>  | <.001   |
| Low-frequency power (ms <sup>2</sup> )  | 41.4 ± 50.0 <sup>*</sup>  | 267.4 ± 303.0             | 137.0 ± 168.0             | .013    |
| High-frequency power (ms <sup>2</sup> ) | 42.2 ± 34.5               | 132.6 ± 131.0             | 95.9 ± 135.3              | .100    |
| Low-frequency to high-frequency ratio   | 0.98 ± 0.47 <sup>**</sup> | 2.52 ± 1.68               | 1.52 ± 1.04 <sup>*</sup>  | .002    |
| TO (%)                                  | 0.97 ± 1.46 <sup>**</sup> | -1.35 ± 1.48              | 0.14 ± 0.94 <sup>**</sup> | <.001   |
| TS (ms/RR)                              | 1.62 ± 2.04 <sup>**</sup> | 8.01 ± 5.27               | 1.54 ± 1.07 <sup>**</sup> | <.001   |
| T-wave alternans (μV)                   | 50.4 ± 20.5               | 65.0 ± 22.6               | 61.7 ± 17.9               | .119    |

Abbreviations: BBB, bundle branch block; NSVT, nonsustained ventricular tachycardia; PAC, premature atrial complex; PVC, premature ventricular complex; SDANN, standard deviation of the 5-min mean R-R intervals; SDNN, standard deviation of all R-R intervals; TO, turbulence onset; TS, turbulence slope. The other abbreviations are as in Table 1.

<sup>##</sup>*p* < .05 vs. CA

<sup>\*</sup>*p* < .05 vs. HCM

<sup>\*\*</sup>*p* < .01 vs. HCM.

the AL-CA and AS groups compared with the HCM group. In addition, TO was significantly higher in the AL-CA and AS groups compared to the HCM group. The parameters of HRV and HRT did not differ between the AL-CA and AS groups.

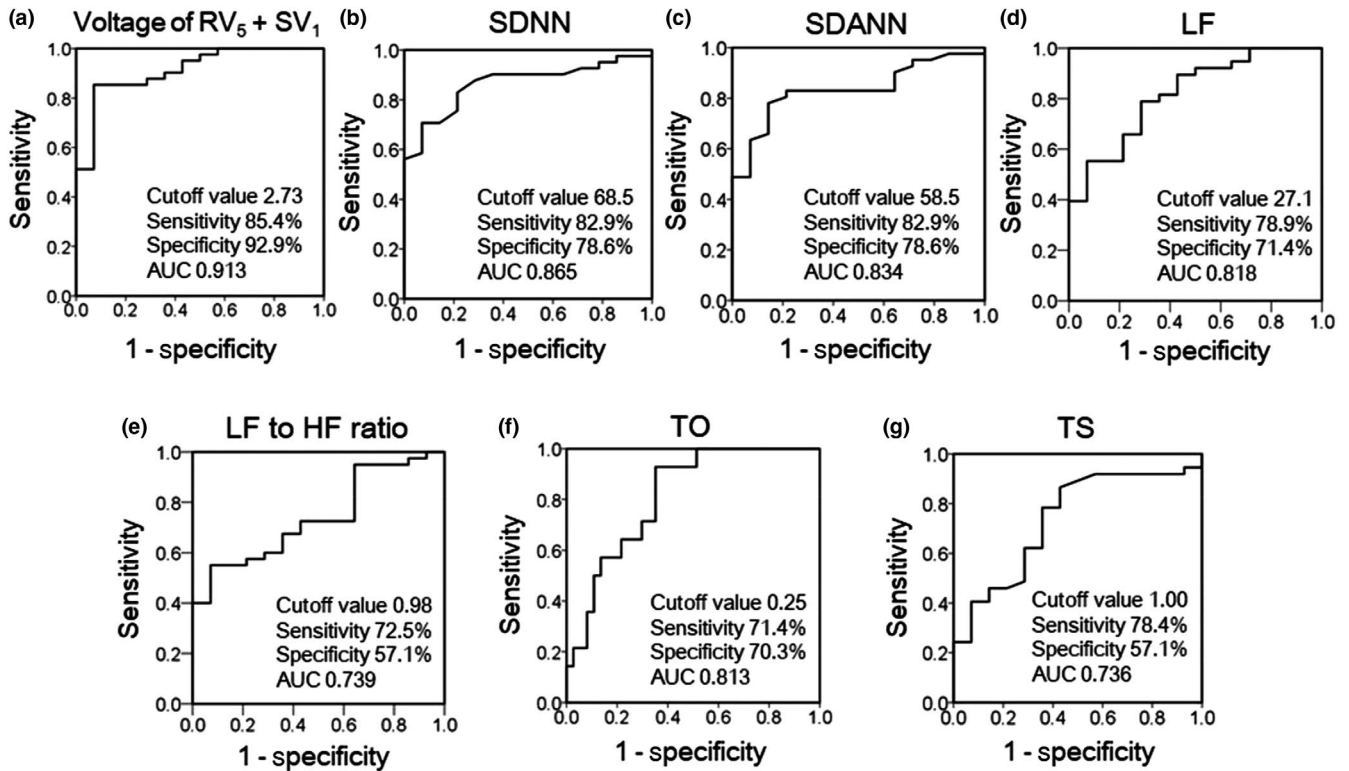
In the ROC analysis for the detection of AL-CA in HF patients with LVH, the best cutoff value for voltage of R wave in V<sub>5</sub> + S wave in V<sub>1</sub>, SDNN, SDANN, low-frequency power, low-frequency to high-frequency ratio, TO, and TS were 2.73 mV (AUC: 0.913), 68.5 ms (AUC: 0.865), 58.5 ms (AUC: 0.834), 27.1 ms<sup>2</sup> (AUC: 0.818), 0.98 (AUC: 0.739), 0.25% (AUC: 0.813), and 1.00 ms/RR (AUC: 0.736), respectively (Figure 1).

Figure 2 shows a representative case of AL-CA, in which low-voltage QRS complexes and poor R progression were not clearly seen in the 12-lead electrocardiogram (Figure 2a), and a granular sparkling sign was not observed on echocardiography

(Figure 2b). On the other hand, the voltage of R wave in V<sub>5</sub> + S wave in V<sub>1</sub>, SDNN, SDANN, low-frequency power, low-frequency to high-frequency ratio, TO, and TS were 1.26 mV (<2.73 mV), 52 ms (<68.5 ms), 46 ms (<58.5 ms), 9.61 ms<sup>2</sup> (<27.1 ms<sup>2</sup>), 0.44 (<0.98), 0.74% (>0.25%), and 0.73 ms/RR (<1.00 ms/RR), respectively. The data of HRV and HRT indicated marked abnormalities. The diagnosis of AL-CA was confirmed by endomyocardial biopsy (Figure 2c).

## 4 | DISCUSSION

There were electrocardiographic differences between the AL-CA group and the HCM and AS groups in the current study. First, in the 12-lead electrocardiogram, the voltage of R wave in V<sub>5</sub> + S



**FIGURE 1** Receiver-operator characteristic (ROC) analysis for the identification of amyloid light-chain cardiac amyloidosis in heart failure patients with left ventricular hypertrophy. (a) ROC analysis of the voltage of R wave in  $V_5$  + S wave in  $V_1$ . (b) ROC analysis of standard deviation of all R-R intervals (SDNN). (c) ROC analysis of standard deviation of the 5-min mean R-R intervals (SDANN). (d) ROC analysis of low-frequency (LF) power. (e) ROC analysis of LF to high-frequency (HF) ratio. (f) ROC analysis of turbulence onset (TO). (g) ROC analysis of turbulence slope (TS). AUC, area under the curve

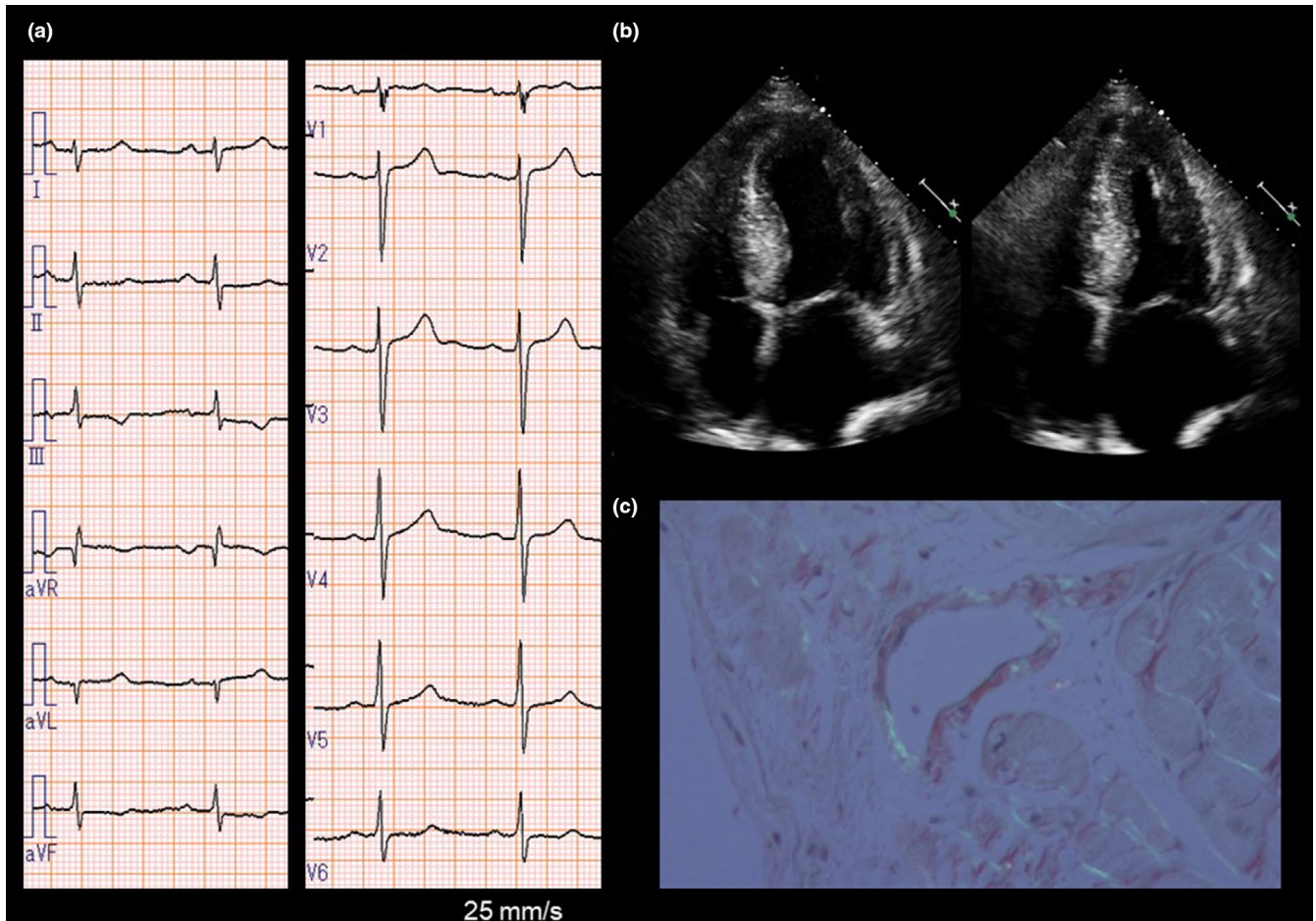
wave in  $V_1$  was significantly lower in the AL-CA group. Second, in the Holter monitoring, lower SDNN, SDANN, and TS and higher TO were observed in the AL-CA and AS groups compared with the HCM group. Third, in the ROC analysis, the severe abnormalities of HRV and HRT, which reflect autonomic dysfunction, were characterized in the patients with AL-CA from other etiologies in HF with LVH.

#### 4.1 | Features of AL-CA in the 12-lead electrocardiogram

Although invasive assessment by endomyocardial biopsy is an essential requirement for confirming amyloid deposition in the heart, it is sometimes difficult to perform biopsy due to the unstable condition of CA patients with rapidly developed HF. Therefore, combinations of clinical features, electrocardiogram, echocardiography, and biomarkers are often helpful for diagnosing of CA. Nevertheless, in some patients, it is difficult to differentiate HF patients with CA from HF patients with LVH of an origin other than CA. Indeed, in the present study, there were no significant differences in brain natriuretic peptide levels, LVH severity, LVEF, E/A, deceleration time, and  $E/e'$  (Table 1). To validate the electrocardiographic differences of CA from other etiologies,

we performed noninvasive assessment by electrocardiogram and Holter monitoring. It is well known that LVH causes electrocardiographic changes. The diagnostic electrocardiogram criteria for LVH have been described in the recommendations for the standardization and interpretation of the electrocardiogram (Surawicz et al., ). The measurement of QRS voltages is most commonly used for the diagnosis of LVH; the Sokolow and Lyon criteria (the sum of R wave in  $V_5$  or  $V_6$  and S wave in  $V_1$  > 3.5 mV) are widely used (Sokolow & Lyon, 1949). In addition, (a) increased QRS duration, (b) prevalence of bundle branch block, (c) prolonged QT interval, (d) left axis deviation, and (e) ST-T abnormality are also used as supporting criteria (Surawicz et al., ). On the contrary, in the electrocardiographic features of CA, low-voltage complexes (56%) (Yusuf et al., 2014) and poor R progression (44%) (Di Bella et al., 2015) have been reported although LVH is typically observed in echocardiography. In the present study, a higher prevalence of low-voltage complexes and poor R progression were seen in the AL-CA group as previously reported. In addition, in the ROC analysis for the characterization of the patients with AL-CA in HF with LVH, the AUC of the voltage of R wave in  $V_5$  + S wave in  $V_1$  was 0.913. However, QRS duration, QTc interval, and the prevalence of bundle branch block, left axis deviation, and ST-T change, were all comparable among the HF patients with LVH of various etiologies.





**FIGURE 2** An example of amyloid light-chain cardiac amyloidosis. (a) The voltage of R wave in  $V_5$  + S wave in  $V_1$  is 1.26 mV in the 12-lead electrocardiogram. However, the prevalence of low voltage in the limb leads, poor R progression, bundle branch block, left axis deviation, and ST-T change is not clearly seen. (b) The apical four-chamber views at the end of diastole (left panel) and systole (right panel). Left ventricular systolic function is normal, and left ventricular hypertrophy is present. Interventricular septum and posterior wall thickness are 13 mm and 12 mm, respectively. However, a granular sparkling sign is not clearly present. (c) Apple-green birefringence of Congo red stained preparations under polarized light is present in the perivascular region ( $\times 200$ )

#### 4.2 | Features of AL-CA in 24-hr Holter monitoring

Abnormal HRV and impaired HRT have been reported to be associated with cardiac autonomic dysfunction and an increase of risk for worsening HF and cardiac death (Bauer et al., 2008; Kleiger et al., 1987; Nolan et al., 1998). The assessment of SDNN and SDANN, widely used time-domain HRV parameters, carries important prognostic information in HF patients (Karcz, Chojnowska, Zareba, & Ruzyłło, 2003). It was previously reported that  $SDNN < 80$  ms was associated with poor prognosis in HF patients (Karcz et al., 2003). In CA, time-domain HRV analysis was also useful for predicting adverse outcomes, and  $SDNN \leq 50$  ms was significantly associated with mortality in AL-CA (Reyners et al., 2002). In the present study, the mean SDNNs in the AL-CA, HCM, and AS groups were 59.2 ms, 130.3 ms, and 83.7 ms, respectively. In the ROC analysis for the characterization of AL-CA in HF patients with LVH, the best cutoff value for SDNN was 68.5 ms (AUC: 0.865). Our findings indicate a severe abnormality of HRV

in the AL-CA group as previously reported, which seems to reflect significant autonomic dysfunction. However, in HCM, knowledge on the prognostic significance of HRV parameters remains limited and controversial. Previous studies have reported increased (Ajiki et al., 1993) and decreased (Fei et al., 1995) sympathetic nerve activity in HCM using HRV parameters. In the current study, decreased HRV parameters were not seen in the HCM group. On the other hand, in the AS group, the HRV parameters were decreased. Possible considerations for this result are as follows: (a) HF patients with moderate to severe AS had impaired sympathetic nerve activity and (b) the AS group primarily consisted of older patients. It has been reported that HRV parameters have significant negative correlations with aging (Umetani, Singer, McCraty, & Atkinson, 1998).

To our best knowledge, this is the first study to report HRT impairment in the patients with AL-CA. HRT is generally recognized as a vagally mediated phenomenon that reflects baroreflex sensitivity non-invasively and is considered to be a helpful marker of neurohormonal

activity in patients with HF (Schmidt et al., 1999). HRT parameters include TO and TS, and a higher TO ( $\geq 0\%$ ) and a lower TS ( $\leq 2.5$  ms/RR) may predict poor clinical outcomes in patients with HF (Miwa et al., 2009; Schmidt et al., 1999). In the present study, the mean TO and TS in the AL-CA group were 0.97% and 1.62 ms/RR, respectively. In the ROC analysis for the characterization of AL-CA in HF patients with LVH, the best cutoff values for TO and TS were 0.25% (AUC: 0.813) and 1.00 ms/RR (AUC 0.736), respectively. These findings indicated HRT impairment in AL-CA, which also reflects cardiac autonomic dysfunction. Therefore, the findings of the current study suggest that significant autonomic dysfunction was characterized in AL-CA from various etiologies. The assessment of HRV and HRT parameters may be useful for differential diagnosis for the etiology of LVH in HF patients.

### 4.3 | Limitations

There are some limitations in the present study. First, the results were obtained from a single institution using a relatively small number of subjects. Second, patients with atrial fibrillation and/or pacing were excluded. Third, other etiologies of LVH, including cardiac sarcoidosis and fabry disease, were not investigated. Finally, the patients in the AS group did not undergo myocardial biopsy. Thus, we were unable to completely deny the underdiagnosis of pathological CA in the AS group, which might have influenced the HRV and HRT findings. Nevertheless, severe abnormalities of HRV and HRT were observed in the AL-CA group. Further studies with larger sample sizes are necessary to validate the present findings.

## 5 | CONCLUSION

Our study demonstrated that low voltage of R wave in  $V_5 + S$  wave in  $V_1$ , abnormal HRV, and impaired HRT are characterized in AL-CA among various etiologies in HF with LVH. Autonomic dysfunction is a hallmark of CA, and its noninvasive assessment by Holter monitoring may be a useful tool for differential diagnosis of HF with LVH.

### CONFLICT OF INTEREST

None.

### AUTHORS' CONTRIBUTION

S.Y. was involved in concept/design, data collection, data analysis/interpretation, and drafting the article; A.Y., T.I. and Y.T. were involved in concept/design, data analysis/interpretation, and critical revision of the article; M.K. and T.K. were involved in data collection and critical revision of the article; N.H., T.Y. and T.M. were involved in data analysis and statistics.

### ETHICS

The study protocol was approved by the ethics committee of Fukushima Medical University (approval number: 1656).

### ORCID

Shinya Yamada  <https://orcid.org/0000-0001-5726-3926>

### REFERENCES

- Ajiki, K., Murakawa, Y., Yanagisawa-Miwa, A., Usui, M., Yamashita, T., Oikawa, N., & Inoue, H. (1993). Autonomic nervous system activity in idiopathic dilated cardiomyopathy and in hypertrophic cardiomyopathy. *American Journal of Cardiology*, 71, 1316–1320. [https://doi.org/10.1016/0002-9149\(93\)90547-P](https://doi.org/10.1016/0002-9149(93)90547-P)
- Authors/Task Force members, Elliott, P. M., Anastakis, A., Borger, M. A., Borggrefe, M., Cecchi, F., ... Watkins, H. (2014). ESC Guidelines on diagnosis and management of hypertrophic cardiomyopathy: The Task Force for the Diagnosis and Management of Hypertrophic Cardiomyopathy of the European Society of Cardiology (ESC). *European Heart Journal*, 2014(35), 2733–2779.
- Bauer, A., Malik, M., Schmidt, G., Barthel, P., Bonnemeier, H., Cygankiewicz, I., ... Zareba, W. (2008). Heart rate turbulence: Standards of measurement, physiological interpretation, and clinical use: International Society for Holter and Noninvasive Electrophysiology Consensus. *Journal of the American College of Cardiology*, 52, 1353–1365. <https://doi.org/10.1016/j.jacc.2008.07.041>
- Bazett, H. (1920). An analysis of the time-relations of electrocardiograms. *Heart*, 7, 353–370.
- Bély, M., & Apáthy, A. (2000). Histochemical and immunohistochemical differential diagnosis of amyloidosis—a brief illustrated essay and personal experience with Romhányi's method. *Amyloid*, 7, 212–217. <https://doi.org/10.3109/13506120009146836>
- Cueto-Garcia, L., Reeder, G. S., Kyle, R. A., Wood, D. L., Seward, J. B., Naessens, J., ... Tajik, A. J. (1985). Echocardiographic findings in systemic amyloidosis: Spectrum of cardiac involvement and relation to survival. *Journal of the American College of Cardiology*, 6, 737–743. [https://doi.org/10.1016/S0735-1097\(85\)80475-7](https://doi.org/10.1016/S0735-1097(85)80475-7)
- Di Bella, G., Minutoli, F., Piaggi, P., Casale, M., Mazzeo, A., Zito, C., ... Carerj, S. (2015). Usefulness of combining electrocardiographic and echocardiographic findings and brain natriuretic peptide in early detection of cardiac amyloidosis in subjects with transthyretin gene mutation. *American Journal of Cardiology*, 116, 1122–1127.
- Fei, L., Slade, A. K., Prasad, K., Malik, M., McKenna, W. J., & Camm, A. J. (1995). Is there increased sympathetic activity in patients with hypertrophic cardiomyopathy? *Journal of the American College of Cardiology*, 26, 472–480. [https://doi.org/10.1016/0735-1097\(95\)80025-C](https://doi.org/10.1016/0735-1097(95)80025-C)
- Getz, M. A. (2004). The classification and typing of amyloid deposits. *American Journal of Clinical Pathology*, 121, 787–789. <https://doi.org/10.1309/TR4LGLVRJAMV5QT>
- Grogan, M., & Dispenzieri, A. (2015). Natural history and therapy of AL cardiac amyloidosis. *Heart Failure Reviews*, 20, 155–162. <https://doi.org/10.1007/s10741-014-9464-5>
- Ho, K. K., Anderson, K. M., Kannel, W. B., Grossman, W., & Levy, D. (1993). Survival after the onset of congestive heart failure in Framingham Heart Study subjects. *Circulation*, 88, 107–115. <https://doi.org/10.1161/01.CIR.88.1.107>
- Hongo, M., Yamamoto, H., Kohda, T., Takeda, M., Kinoshita, O., Uchikawa, S., ... Kubo, K. (2000). Comparison of electrocardiographic findings in patients with AL (primary) amyloidosis and in familial amyloid polyneuropathy and anginal pain and their relation to histopathologic findings. *American Journal of Cardiology*, 85, 849–853. [https://doi.org/10.1016/S0002-9149\(99\)00879-6](https://doi.org/10.1016/S0002-9149(99)00879-6)
- Karcz, M., Chojnowska, L., Zareba, W., & Ruzyłło, W. (2003). Prognostic significance of heart rate variability in dilated cardiomyopathy. *International Journal of Cardiology*, 87, 75–81. [https://doi.org/10.1016/S0167-5273\(02\)00207-3](https://doi.org/10.1016/S0167-5273(02)00207-3)
- Kimishima, Y., Yoshihisa, A., Kiko, T., Yokokawa, T., Miyata-Tatsumi, M., Misaka, T., ... Takeishi, Y. (2019). Utility of B-type natriuretic peptide



- for detecting cardiac involvement in immunoglobulin amyloidosis. *International Heart Journal*, 60(5), 1106–1112.
- Kleiger, R. E., Miller, J. P., Bigger, J. T. Jr, & Moss, A. J. (1987). Decreased heart rate variability and its association with increased mortality after acute myocardial infarction. *American Journal of Cardiology*, 59, 256–262. [https://doi.org/10.1016/0002-9149\(87\)90795-8](https://doi.org/10.1016/0002-9149(87)90795-8)
- Lang, R. M., Badano, L. P., Mor-Avi, V., Afilalo, J., Armstrong, A., Ernande, L., ... Voigt, J. U. (2015). Recommendations for cardiac chamber quantification by echocardiography in adults: An update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *Journal of the American Society of Echocardiography*, 28, 1–39. <https://doi.org/10.1016/j.echo.2014.10.003>
- Leung, N., Nasr, S. H., & Sethi, S. (2012). How I treat amyloidosis: The importance of accurate diagnosis and amyloid typing. *Blood*, 120, 3206–3213. <https://doi.org/10.1182/blood-2012-03-413682>
- Levey, A. S., Coresh, J., Greene, T., Stevens, L. A., Zhang, Y. L., Hendriksen, S., ... Van Lente, F. (2006). Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. *Annals of Internal Medicine*, 145, 247–254. <https://doi.org/10.7326/0003-4819-145-4-200608150-00004>
- Mathew, J., Sleight, P., Lonn, E., Johnstone, D., Pogue, J., Yi, Q., ... Yusuf, S. (2001). Heart Outcomes Prevention Evaluation (HOPE) Investigators. Reduction of cardiovascular risk by regression of electrocardiographic markers of left ventricular hypertrophy by the angiotensin-converting enzyme inhibitor ramipril. *Circulation*, 104, 1615–1621. <https://doi.org/10.1161/hc3901.096700>
- McMurray, J. J., Adamopoulos, S., Anker, S. D., Auricchio, A., Bohm, M., Dickstein, K., ... ESC Committee for Practice Guidelines. (2012). ESC guidelines for the diagnosis and treatment of acute and chronic heart failure 2012: The Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure 2012 of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association (HFA) of the ESC. *European Journal of Heart Failure*, 14, 803–869.
- Miwa, Y., Ikeda, T., Sakaki, K., Miyakoshi, M., Ishiguro, H., Tsukada, T., ... Yoshino, H. (2009). Heart rate turbulence as a predictor of cardiac mortality and arrhythmic events in patients with dilated cardiomyopathy: A prospective study. *Journal of Cardiovascular Electrophysiology*, 20, 788–795. <https://doi.org/10.1111/j.1540-8167.2009.01438.x>
- Montano, N., Ruscone, T. G., Porta, A., Lombardi, F., Pagani, M., & Malliani, A. (1994). Power spectrum analysis of heart rate variability to assess the changes in sympathovagal balance during graded orthostatic tilt. *Circulation*, 90, 1826–1831. <https://doi.org/10.1161/01.CIR.90.4.1826>
- Nearing, B. D., & Verrier, R. L. (2002). Modified moving average analysis of T-wave alternans to predict ventricular fibrillation with high accuracy. *Journal of Applied Physiology*, 92, 541–549. <https://doi.org/10.1152/jappphysiol.00592.2001>
- Nishimura, R. A., Otto, C. M., Bonow, R. O., Carabello, B. A., Erwin, J. P. 3rd, & Guyton, R. A. ... American College of Cardiology/American Heart Association Task Force on Practice Guidelines. (2014). AHA/ACC guideline for the management of patients with valvular heart disease: A report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *Journal of the American College of Cardiology*, 63, 57–185. <https://doi.org/10.1161/CIR.0000000000000031>
- Nolan, J., Batin, P. D., Andrews, R., Lindsay, S. J., Brooksby, P., Mullen, M., ... Fox, K. A. (1998). Prospective study of heart rate variability and mortality in chronic heart failure: Results of the United Kingdom heart failure evaluation and assessment of risk trial (UK-heart). *Circulation*, 98, 1510–1516. <https://doi.org/10.1161/01.CIR.98.15.1510>
- Reyners, A. K., Hazenberg, B. P., Reitsma, W. D., & Smit, A. J. (2002). Heart rate variability as a predictor of mortality in patients with AA and AL amyloidosis. *European Heart Journal*, 23, 157–161. <https://doi.org/10.1053/euhj.2001.2972>
- Schmidt, G., Malik, M., Barthel, P., Schneider, R., Ulm, K., Rolnitzky, L., ... Schömig, A. (1999). Heart-rate turbulence after ventricular premature beats as a predictor of mortality after acute myocardial infarction. *Lancet*, 353, 1390–1396. [https://doi.org/10.1016/S0140-6736\(98\)08428-1](https://doi.org/10.1016/S0140-6736(98)08428-1)
- Schonland, S. O., Heigenbart, U., Bochtler, T., Mangatter, A., Hansberg, M., Ho, A. D., ... Röcken, C. (2012). Immunohistochemistry in the classification of systemic forms of amyloidosis: A systematic investigation of 117 patients. *Blood*, 119, 488–493. <https://doi.org/10.1182/blood-2011-06-358507>
- Sokolow, M., & Lyon, T. P. (1949). The ventricular complex in left ventricular hypertrophy as obtained by unipolar and limb leads. *American Heart Journal*, 37, 161–186.
- Surawicz, B., Childers, R., Deal, B. J., Gettes, L. S., Bailey, J. J., & Gorgels, A. ... Heart Rhythm Society. AHA/ACC/HRS recommendations for the standardization and interpretation of the electrocardiogram: part III: intraventricular conduction disturbances: a scientific statement from the American Heart Association Electrocardiography and Arrhythmias Committee, Council on Clinical Cardiology; the American College of Cardiology Foundation; and the Heart Rhythm Society: endorsed by the International Society for Computerized Electrocardiology. *Circulation*, 119, e235–e240.
- Takeda, M., Amano, Y., Tachi, M., Tani, H., Mizuno, K., & Kumita, S. (2013). MRI differentiation of cardiomyopathy showing left ventricular hypertrophy and heart failure: Differentiation between cardiac amyloidosis, hypertrophic cardiomyopathy, and hypertensive heart disease. *Japanese Journal of Radiology*, 31, 693–700. <https://doi.org/10.1007/s11604-013-0238-0>
- Umetani, K., Singer, D. H., McCraty, R., & Atkinson, M. (1998). Twenty-four hour time domain heart rate variability and heart rate: Relations to age and gender over nine decades. *Journal of the American College of Cardiology*, 31, 593–601. [https://doi.org/10.1016/S0735-1097\(97\)00554-8](https://doi.org/10.1016/S0735-1097(97)00554-8)
- Yamada, S., Suzuki, H., Kamioka, M., Suzuki, S., Kamiyama, Y., Yoshihisa, A., ... Takeishi, Y. (2013). Sleep-disordered breathing increases risk for fatal ventricular arrhythmias in patients with chronic heart failure. *Circulation Journal*, 77, 1466–1473. <https://doi.org/10.1253/circj.CJ-12-0836>
- Yamada, S., Yoshihisa, A., Sato, Y., Sato, T., Kamioka, M., Kaneshiro, T., ... Takeishi, Y. (2018). Utility of heart rate turbulence and T-wave alternans to assess risk for readmission and cardiac death in hospitalized heart failure patients. *Journal of Cardiovascular Electrophysiology*, 29, 1257–1264. <https://doi.org/10.1111/jce.13639>
- Yusuf, S. W., Solhpour, A., Banchs, J., Lopez-Mattei, J. C., Durand, J. B., Iliescu, C., ... Qazilbash, M. H. (2014). Cardiac amyloidosis. *Expert Review of Cardiovascular Therapy*, 12, 265–277. <https://doi.org/10.1586/14779072.2014.876363>

**How to cite this article:** Yamada S, Yoshihisa A, Hijioka N, et al. Autonomic dysfunction in cardiac amyloidosis assessed by heart rate variability and heart rate turbulence. *Ann Noninvasive Electrocardiol*. 2020;25:e12749. <https://doi.org/10.1111/anec.12749>