

Utility of T-wave alternans during night time as a predictor for ventricular fibrillation in patients with Brugada syndrome

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Abstract The prognostic value of T-wave alternans (TWA) during the night time in patients with Brugada syndrome (Br-S) remains unknown. We assessed TWA for risk stratification using 24-h multichannel Holter electrocardiogram (24-M-ECG) in Br-S. We enrolled 129 patients with Br-S [grouped according to histories of ventricular fibrillation (VF), $n = 16$; syncope, $n = 10$; or no symptoms (asymptomatic), $n = 103$] and 11 controls. Precordial electrodes were attached to the third (3L-V1, 3L-V2) and fourth (4L-V1, 4L-V2 and 4L-V5) intercostal spaces. We measured the values of maximum TWA (max-TWA) during the night time (12 a.m.–6 a.m.) and the day time (12 p.m.–6 p.m.) and calculated parameters of heart rate variability. Compared to the asymptomatic and control groups, the VF and syncope groups showed significantly greater 3L-V2 max-TWA during the night time. The cut-off value for the 3L-V2 max-TWA during the night time was determined as 20 μV (sensitivity 94 % and specificity 48 %; $p = 0.01$). Multivariate analysis revealed that 3L-V2 max-TWA during the night time $\geq 20 \mu\text{V}$ and previous VF episodes were independent predictors of future VF episodes. During a mean follow-up period of 68 ± 37 months, 16 patients experienced VF episodes. The incidence of VF episodes was the highest during the night time ($p < 0.001$). The 3L-V2 max-TWA during the night time may be a useful predictor for VF episodes in patients with Br-S.

Keywords T-wave alternans · Heart rate variability · Holter electrocardiogram · Brugada syndrome

Abbreviations

ECG	Electrocardiogram
VF	Ventricular fibrillation
Br-S	Brugada syndrome,
F-QRS	Fragmented QRS
TWA	T-wave alternans
HRV	Heart rate variability
24-M-ECG	24-Hour multichannel Holter electrocardiogram
max-TWA	Maximum T-wave alternans
MMA	Modified moving average
Bpm	Beats per minute
ICD	Implantable cardioverter defibrillator
RVOT	Right ventricular outflow tract

Introduction

Brugada syndrome (Br-S) is characterized by abnormal ST-segment electrocardiogram (ECG) patterns in leads V1–V3, and is associated with a high risk of sudden cardiac death due to ventricular fibrillation (VF) [1–3]. Previous experimental studies have reported that the pathogenesis of the Br-S are late potentials and fractionated electrocardiogram activity in epicardium of the right ventricular outflow tract (RVOT), setting in repolarization [4]. The PRELUDE registry indicated some clinical factors that are useful to identify candidates for implantable cardioverter defibrillators (ICDs) [5]. However, the mechanism responsible for the arrhythmogenesis and the syndrome's risk stratification are not fully established.

T-wave alternans (TWA) is the beat-to-beat fluctuation in T-wave amplitude and reflects repolarization heterogeneity. Many clinical studies have shown that TWA predicts

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life-threatening arrhythmias or sudden cardiac death in patients, including those with chronic heart failure and cardiovascular disease [6–8]. Heart rate variability (HRV) is a suitable approach for the analysis of the autonomic balance [9, 10]. The Br-S is also influenced by the autonomic system. Previous reports showed that vagal tone activity was increasing rapidly before arrhythmic events in Br-S [11] and sleep has been reported to be associated with an increased incidence of VF in patients with Br-S [12].

However, the prognostic value of TWA and HRV in patients with Br-S has not been fully evaluated during the night time and the day time. In the present study, we simultaneously recorded TWA and HRV using 24-h multichannel Holter electrocardiograms (24-M-ECGs) in patients with Br-S and compared TWA and HRV during the night time with those during the day time. We evaluated the utility of TWA and HRV as predictors for VF occurrence.

Patients and methods

Study population

We enrolled 129 patients with Br-S (122 men; mean age, 52 ± 12 years) and 11 control subjects (11 men; mean age, 50 ± 9 years) in this study. All patients met the following criteria: (1) either spontaneous or pilsicainide-induced type-1 Brugada ECG [1] at the standard position and/or higher intercostal space of 12-lead ECGs during sinus rhythm; (2) normal findings on physical examination; (3) not taking antiarrhythmic drugs; and (4) no abnormality in either the right or left ventricular morphology and/or function as demonstrated by chest radiography and echocardiography. We performed ICD implantation according

to the Class I or IIa indication in current Japanese guideline: (1) documented episodes of VF or aborted sudden cardiac death; or (2) experienced unknown syncope with type-1 Brugada ECG (either spontaneous or pilsicainide-induced) and induced sustained ventricular tachyarrhythmias during electrophysiological study [13]. The patients with Br-S were divided into 3 groups according to their histories of documented VF episodes (VF group, $n = 16$), unknown syncope (syncope group, $n = 10$), and no symptoms (asymptomatic group, $n = 103$) (Table 1). The ethics review board of Osaka City University Graduate School of Medicine approved this study. A written informed consent was obtained from all patients and control subjects.

Study protocol

12-Lead electrocardiogram

12-Lead ECG was examined in all subjects before attachment of 24-h multichannel Holter ECG. We analyzed fragmented QRS (F-QRS) and J-wave. F-QRS and J-wave were defined as reported previously [14, 15].

T-wave alternans

All individuals underwent 24-M-ECG using a SEER 12 Holter monitor (GE Healthcare, Tokyo, Japan) between April 2012 and September 2013. The record of precordial electrodes was based on unipolar mode like 12-lead ECG. For 24-M-ECG, precordial electrodes were attached at the standard positions for leads V1 (4L-V1), V2 (4L-V2) and V5 (4L-V5), and at the third intercostal space for lead V1 (3L-V1) and V2 (3L-V2) [we attached the precordial electrodes at the third intercostal space for leads V1 and

Table 1 Comparison of the clinical profile of individuals among VF, Syncope, Asymptomatic with Brugada ECG and control groups

	VF group ($n = 16$)	Syncope group ($n = 10$)	Asymptomatic group ($n = 103$)	control group ($n = 11$)	<i>p</i> value
Age (year)	53 ± 11	54 ± 13	51 ± 12	50 ± 9	0.8
Male <i>n</i> (%)	15 (94 %)	9 (90 %)	98 (95 %)	11 (100 %)	0.7
Spontaneous type-1 ECG <i>n</i> (%)	12 (75 %)	6 (60 %)	60 (58 %)	–	0.4
Family history of sudden death <i>n</i> (%)	5 (31 %)	5 (50 %)	16 (16 %)	–	0.03*
ICD implantation <i>n</i> (%)	16 (100 %)	10 (100 %)	10 (10 %)	–	0.001*
Arrhythmic events during follow-up <i>n</i> (%)	12 (75 %)	3 (30 %)	1 (1 %)	–	0.001*
Incidence of F-QRS <i>n</i> (%)	2 (13 %)	0 (0 %)	7 (7 %)	–	0.4
Incidence of J-wave <i>n</i> (%)	2 (13 %)	2 (20 %)	23 (23 %)	–	0.6

ECG electrocardiogram, VF ventricular fibrillation, ICD implantable cardioverter defibrillator, F-QRS fragmented QRS

* $p < 0.05$

V2, instead of the standard positions for leads V3 and V4]. Using the MARS PC Holter Monitoring and Review System software (version 8, GE Healthcare), TWA values were calculated using the time-domain, modified moving average (MMA) method [16]. The MMA method employs the noise-rejection principle of recursive averaging. The algorithm continuously streamed odd and even beats into separate bins, and the morphology of the beats in each bin was averaged over 8 beats. The average morphologies of the odd and even beats were continuously updated to every new, incoming beat using a weighting factor of 1–8, reflecting the differences between the ongoing average and the new incoming beats. T-wave values were calculated as the maximum amplitude differences between the odd and even beat average from the J-point to the end of the T-wave for every 15 beats. TWA-values at heart rates ≥ 120 bpm and those with noise levels ≥ 20 μV , which are not displayed automatically in this software, were excluded, as reported previously [7]. We indicate representative case in Fig. 1a, b. Sleep has been also reported to be associated with an increased incidence of VF in patients with Br-S [12]. Previous study showed that circadian distribution of VF episodes in Br-S was highest during the night time [17]. Therefore, we measured the maximum TWA (max-TWA) value for the two time periods (12 a.m.–6 a.m. and 12 p.m.–6 p.m.), that were consistent with sleeping time and the day time for activity, respectively. Additionally, we analyzed the incidence of type-1 Brugada ECG at 4L-V1, 4L-V2, 3L-V1 and 3L-V2 during the night time and the day time.

Heart rate variability

Frequency domain variables of the HRV were obtained using Fourier transformation of the 24-M-ECG recordings. We computed the power spectral density for the two time periods (12 a.m.–6 a.m. and 12 p.m.–6 p.m.) and calculated the power within 2 frequency bands. The 2 frequency bands were low frequency power (LF) band, 0.04–0.15 Hz; and high frequency power (HF) band, 0.15–0.4 Hz. The ratio of the low- to high-frequency power (LF/HF) was also calculated, and the HF and LF/HF were compared among the 4 groups.

Follow-up

All patients with Br-S were assessed for clinical events every 4 months, and the incidence of arrhythmic events was determined. Arrhythmic events during follow-up were defined as the occurrence of VF or interventions involving appropriate ICDs.

Statistical analysis

Variables are expressed as mean \pm standard deviation. Differences between groups were assessed using the Wilcoxon signed-rank test, and logistic regression analysis was performed to identify the independent factor(s) for the occurrence of VF. Pearson's r coefficient was calculated for relation between max-TWA values at 3L-V2 during the night time and the existence of type-1 Brugada ECG. Analysis

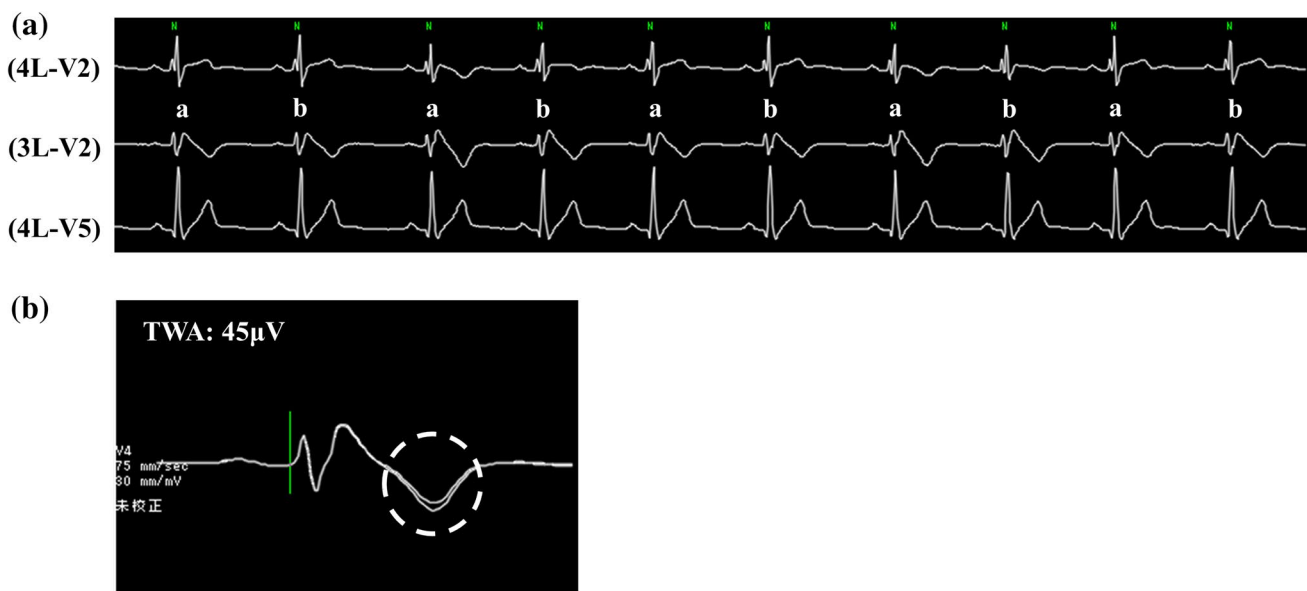


Fig. 1 Representative case of T-wave alternans (TWA) at leads 4L-V2, 4L-V5, and 3L-V2 in a patient with the past history of ventricular fibrillation. **a** The sample of algorithm continuously streamed

odd (*a*) and even (*b*) beats into separate bins. **b** A high-resolution template of QRS-aligned complexes at the time of peak TWA. The value of TWA was 45 μV

of receiver operating characteristic curves (ROCs) was performed to determine the predictive parameters and cut-off value. Additionally, inter- and intra-observer variabilities were assessed according to the Bland and Altman method. All data were analyzed using JMP software, version 10 (SAS, Cary, NC, USA); p values <0.05 were considered significant.

Results

Clinical characteristics of patients with Br-S and controls

The clinical profiles of patients with Br-S and controls are shown in Table 1. There were no significant differences in age, gender, or the prevalence of spontaneous type-1 Brugada ECG patterns among the 4 groups. The prevalence of a family history of sudden death was significantly higher in the VF and syncope groups than in the asymptomatic group. The number of the patients requiring implanted ICDs was also significantly higher in the VF and syncope groups than in asymptomatic group. Sixteen patients experienced VF episodes during a mean follow-up period of 68 ± 37 months (VF group, $n = 12$, syncope group, $n = 3$, and asymptomatic group, $n = 1$). All the number of ICD therapies were 155 times. The appropriate therapies were 140 times according to VF, and the remaining 15 times were inappropriate shocks accompanying atrial fibrillation or sinus tachycardia. There was no cardiac death in our study during follow-up period. The incidence of VF episodes (appropriate shocks) during follow-up was the highest among patients in the VF group. VF episodes were recorded by the patients' ICDs and shown in Fig. 2 according to the time zones during entire day. The circadian distribution of the VF episodes was statistically different among the 4 time zones. The incidence of VF episodes was the highest during the night time (12 a.m.–6 a.m.) ($p < 0.001$).

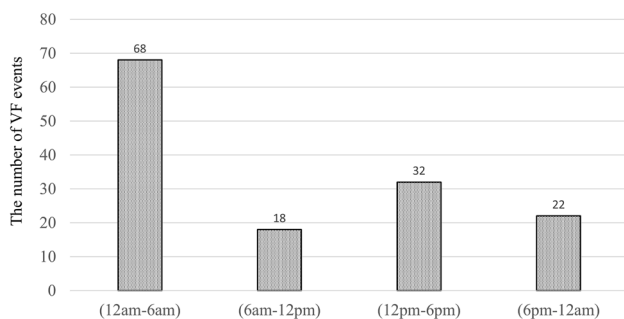


Fig. 2 Circadian distribution of the ventricular fibrillation (VF) episodes recorded by ICDs during follow-up. The occurrence of VF was significantly more frequent in the night time (12 a.m.–6 a.m.)

12-Lead electrocardiogram findings

There were no significant differences in the incidence of F-QRS (VF group 13 % vs. syncope group 0 % vs. asymptomatic group 7 %, $p = 0.4$) and J-wave (VF group 13 % vs. syncope group 20 % vs. asymptomatic group 23 %, $p = 0.6$), among the 3 groups (Table 1).

TWA findings

The circadian patterns of TWA at 4L-V1, 4L-V2, 4L-V5, 3L-V1, and 3L-V2 among the 4 groups are shown in Fig. 3. The VF and syncope groups had significantly greater max-TWA values at 3L-V2 during the night time than did the asymptomatic group and control group (12 a.m.–6 a.m.: VF group: 31 ± 13 , syncope group: 30 ± 13 , asymptomatic group: 21 ± 12 , and control group: 20 ± 4 μV , $p = 0.004$, 12 p.m.–6 p.m.: VF group: 39 ± 16 , syncope group: 32 ± 9 , asymptomatic group: 33 ± 21 , and control group: 29 ± 11 μV , $p = 0.6$) (Fig. 3d). There were no significant differences in the 3L-V1 max-TWA values (12 a.m.–6 a.m.: VF group: 26 ± 13 , Syncope group: 24 ± 10 , asymptomatic group: 20 ± 10 , and control group: 22 ± 7 μV , $p = 0.07$, 12 p.m.–6 p.m.: VF group: 31 ± 17 , syncope group: 26 ± 6 , asymptomatic group: 28 ± 13 , and control group: 27 ± 6 μV , $p = 0.7$) (Fig. 3c), 4L-V1 max-TWA values (12 a.m.–6 a.m.: VF group: 26 ± 13 , Syncope group: 25 ± 11 , asymptomatic group: 25 ± 11 , and control group: 24 ± 9 μV , $p = 0.9$, 12 p.m.–6 p.m.: VF group: 31 ± 12 , syncope group: 34 ± 10 , asymptomatic group: 32 ± 13 , and control group: 29 ± 6 μV , $p = 0.8$) (Fig. 3a), 4L-V2 max-TWA values (12 a.m.–6 a.m.: VF group: 34 ± 17 , Syncope group: 38 ± 11 , asymptomatic group: 35 ± 16 , and control group: 27 ± 10 μV , $p = 0.4$, 12 p.m.–6 p.m.: VF group: 45 ± 30 , syncope group: 42 ± 20 , asymptomatic group: 39 ± 22 , and control group: 46 ± 15 μV , $p = 0.7$) (Fig. 3b) and 4L-V5 max-TWA values (12 a.m.–6 a.m.: VF group: 26 ± 18 , syncope group: 21 ± 10 , asymptomatic group: 19 ± 13 and control group: 22 ± 8 μV , $p = 0.2$, 12 p.m.–6 p.m.: VF group: 26 ± 13 , syncope group: 23 ± 12 , asymptomatic group: 24 ± 16 , and control group: 30 ± 14 μV , $p = 0.7$) (Fig. 3e), during any of the time periods among the 4 groups. There were not significant differences of 3L-V1 max-TWA values among 4 groups during the night time. However, 3L-V1 max-TWA values in VF and syncope groups tended to be higher than those in asymptomatic and control groups.

The mean values of heart rate are shown in Fig. 3f. In all groups, the mean values of heart rate during the day time (VF group: 87 ± 13 , syncope group: 83 ± 17 , asymptomatic group: 80 ± 15 , and control group: 82 ± 11 bpm) tended to be higher than those during the night time (VF group: 68 ± 15 , syncope group: 63 ± 9 ,

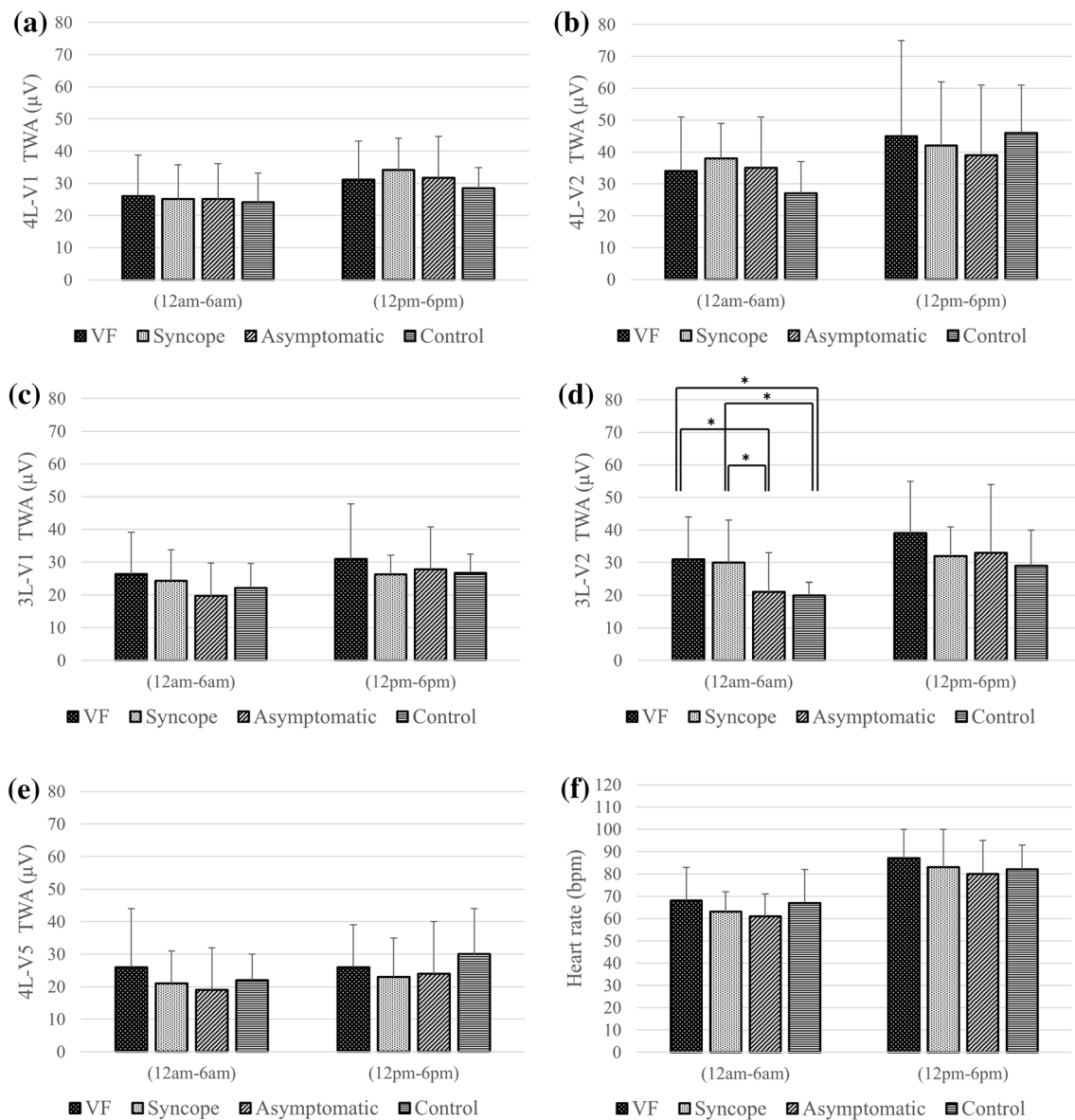


Fig. 3 The circadian pattern of T-wave alternans (TWA) at 4L-V1 **a**, 4L-V2 **b**, 3L-V1 **c**, 3L-V2 **d**, and 4L-V5 **e**, and of heart rate **f** during 24-h multichannel Holter ECGs among the 4 groups. **a–c** and **e** There were no significant differences in the maximum TWA at 4L-V1, 4L-V2, 3L-V1, and 4L-V5 during the night and the day time periods among the 4 groups. **d** The VF and syncope groups had significantly

larger maximum TWA values at 3L-V2 during the night time than did the asymptomatic with Brugada-type ECG and control group. **f** There were no significant differences in the mean values of heart rate during any of time periods among the 4 groups. However, the mean values of heart rate during the day time tended to be higher than those during the night time in all groups. * $p < 0.05$, bpm beat per minute

asymptomatic group: 61 ± 10 , and control group: 67 ± 15 bpm). However, there were no significant differences in heart rate during both the day and night times among the 4 groups. Additionally, the incidence of type-1 Brugada ECG according to the each precordial leads were shown in Fig. 4. The incidence of type-1 Brugada ECG at 3L-V2 was higher than that of 3L-V1, 4L-V1, and 4L-V2 during the night time (4L-V1: 11 %, 4L-V2:

15 %, 3L-V1: 19 %, and 3L-V2: 39 %, $p = 0.0001$) and the day time (4L-V1: 13 %, 4L-V2: 11 %, 3L-V1: 17 %, and 3L-V2: 28 %, $p = 0.002$). The incidence of type-1 Brugada ECG at 3L-V2 during the night time tended to be higher than that during the day time. However, the 3L-V2 max-TWA values during the night time was not correlated with existence of type-1 Brugada ECG at 3L-V2 ($r^2 = 0.15$, $p = 0.08$).

Fig. 4 The incidence of type-1 Brugada ECG at 4L-V1, 4L-V2, 3L-V1 and 3L-V2 during the night time and the day time in patients with Brugada syndrome. The incidence of type-1 Brugada ECG at 3L-V2 was significantly higher than that of 3L-V1, 4L-V1, and 4L-V2 during the night time and the day time

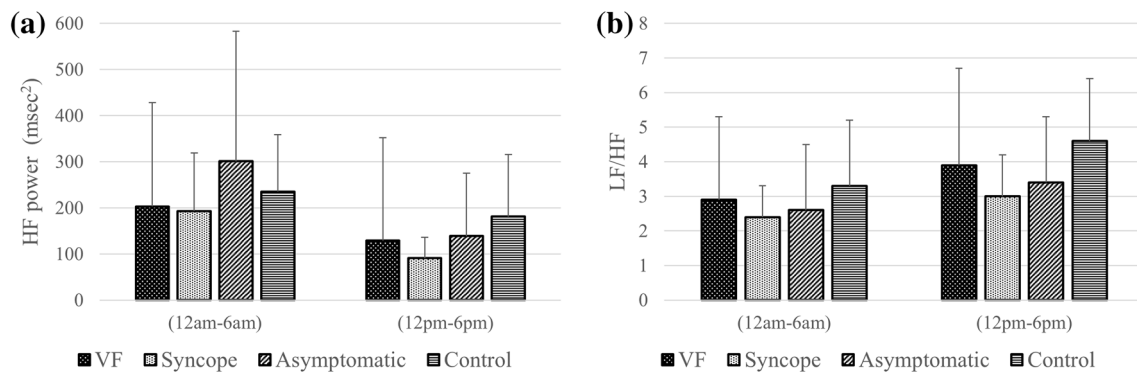
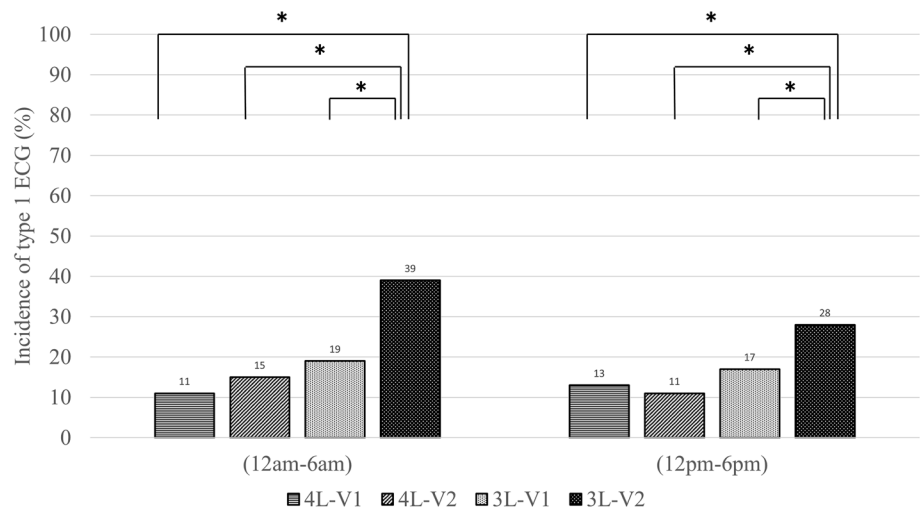


Fig. 5 The circadian variation in high frequency power (HF) and the low frequency/high frequency power (LF/HF) ratio among the 4 groups. The HF (a) and LF/HF ratio (b) were not significantly different among the 4 groups during both time periods

HRV findings

A comparison of HF and LF/HF among the 4 groups is shown in Fig. 5a, b. There were no differences in the HF and LF/HF among the 4 groups. Similarly, there were no differences in the HF and LF/HF among the 4 groups during the night time and the day time.

Risk assessment for VF episodes during follow-up

We performed a univariate analysis to investigate the factors that predict VF episodes during follow-up. The 3L-V1 max-TWA and 3L-V2 max-TWA during the night time and a history of documented VF were associated with VF episodes during follow-up (Table 2). Using a ROC curve analysis, cutoff values for the 3L-V1 and 3L-V2 max-TWA values during the night time were determined as 26 μ V [area under the ROC curve (AUC) = 0.69; sensitivity 56 %; specificity 76 %; $p = 0.003$], and 20 μ V [area under the ROC curve (AUC) = 0.74;

sensitivity 94 %; specificity 48 %; $p = 0.01$], respectively. The incidence of VF episodes was significantly higher in patients with 3L-V1 max-TWA value greater than or equal to the cutoff values ($\geq 26 \mu$ V) and 3L-V2 max-TWA value greater than or equal to the cutoff values ($\geq 20 \mu$ V) for the night time as determined by univariate analysis. Multivariate analysis revealed that the 3L-V2 max-TWA $\geq 20 \mu$ V during the night time (OR 9; 95 % CI 1.1–232; $p = 0.04$) and a history of documented VF episodes (OR 68; 95 % CI 15–440; $p = 0.001$) were independent predictors of future VF episodes. However, the 3L-V1 max-TWA $\geq 26 \mu$ V during the night time (OR 3; 95 % CI 0.6–18; $p = 0.17$) was not associated with VF episodes (Table 3). Additionally, age, gender, the prevalence of spontaneous type-1 Brugada ECG, family histories of sudden death, incidence of F-QRS, incidence of J-wave, previous episodes of syncope, and the existence of type-1 Brugada ECG at any precordial electrodes during the night and the day time, were not associated with VF episodes during follow-up.

Table 2 Univariate analysis for VF episodes during follow-up

	Univariate analysis		
	Odds ratio	95 % CI	<i>p</i> value
Age	0.97	0.98–1.08	0.25
Male	1.2	0.06–7.72	0.87
Family history of sudden death	2.82	0.87–8.53	0.08
Spontaneous type-1 ECG	2.18	0.71–8.12	0.18
Previous syncope	3.49	0.69–14.5	0.12
Previous VF	82	20–434	0.001*
Incidence of F-QRS	1.14	0.19–22	0.9
Incidence of J-wave	1.17	0.34–5.39	0.8
Max-TWA			
4L-V1 (12 a.m.–6 a.m.)	1.04	0.92–1.01	0.08
4L-V1 (12 p.m.–6 p.m.)	1.01	0.95–1.03	0.56
4L-V2 (12 a.m.–6 a.m.)	1.0	0.97–1.03	0.84
4L-V2 (12 p.m.–6 p.m.)	1.01	0.97–1.01	0.17
3L-V1 (12 a.m.–6 a.m.)	1.07	0.88–0.98	0.003*
3L-V1 (12 p.m.–6 p.m.)	1.03	0.94–1.01	0.14
3L-V2 (12 a.m.–6 a.m.)	1.05	0.91–0.99	0.01*
3L-V2 (12 p.m.–6 p.m.)	1.02	0.96–1.01	0.09
4L-V5 (12 a.m.–6 a.m.)	1.03	0.94–1.01	0.07
4L-V5 (12 p.m.–6 p.m.)	1.00	0.97–1.04	0.96
HF (12 a.m.–6 a.m.)	1.00	0.99–1.01	0.13
HF (12 p.m.–6 p.m.)	1.00	0.99–1.01	0.99
LF/HF (12 a.m.–6 a.m.)	0.99	0.78–1.39	0.94
LF/HF (12 p.m.–6 p.m.)	0.89	0.71–1.15	0.36
Existence of type-1 ECG			
4L-V1 (12 a.m.–6 a.m.)	1.94	0.41–7.15	0.37
4L-V1 (12 p.m.–6 p.m.)	2.56	0.65–8.09	0.17
4L-V2 (12 a.m.–6 a.m.)	1.3	0.28–4.59	0.71
4L-V2 (12 p.m.–6 p.m.)	1.2	0.18–5.05	0.82
3L-V1 (12 a.m.–6 a.m.)	1.55	0.4–4.99	0.5
3L-V1 (12 p.m.–6 p.m.)	1.14	0.25–3.97	0.85
3L-V2 (12 a.m.–6 a.m.)	1.06	0.37–3.31	0.91
3L-V2 (12 p.m.–6 p.m.)	1.2	0.36–3.6	0.75

CI confidence interval, *TWA* T-wave-alternans, *ECG* electrocardiogram *VF* ventricular fibrillation, *HF* high-frequency power, *LF/HF* the ratio of the low- to high-frequency power, *F-QRS* fragmented QRS

* $p < 0.05$

Discussion

The results of this study indicated that (1) patients with Br-S had a significant circadian peak during the night time in terms of the recurrence of VF ($p < 0.001$), (2) VF and syncope groups had significantly greater 3L-V2 max-TWA during the night time than did the asymptomatic group and control group, (3) the incidence of type-1 Brugada ECG at 3L-V2 was higher than that at any other precordial electrodes during the night time and the day time, and (4) in multivariate analysis, documented VF episodes and 3L-V2 max-TWA value $\geq 20 \mu\text{V}$ for the night time were independent predictors of VF episodes during follow-up.

Previous studies have reported that an MMA analysis of TWA, using 24-h Holter monitoring, is useful for predicting arrhythmic death among patients with post-myocardial infarction and non-ischemic cardiomyopathy [8, 18]. For the patients with Br-S, two studies used an MMA analysis of TWA to evaluate the predictive role of life-threatening arrhythmias. Yalin et al. showed that an MMA analysis of TWA during exercise testing and recovery time could not identify high-risk patients with Br-S [19]. In the present study, we focused on the MMA analysis of TWA using 24-M-ECG, without conducting stress tests. On the other hand, Uchimura et al. [20] showed that max-TWA at the standard lead position (4L-V2) during the entire day was useful for risk stratification of VF episodes. We investigated the max-TWA values in more patients in detail. We compared the max-TWA values at the standard lead positions (4L-V1, 4L-V2 and 4L-V5) and at the third intercostal space (3L-V1 and 3L-V2) between the night and the day times. We showed that the VF and syncope groups had significantly greater 3L-V2 max-TWA value during the night time than did the asymptomatic group and control group. To the best of our knowledge, this is the first report to evaluate the max-TWA values during night time in patients with Br-S. Previous reports to evaluate TWA values during the night time are very rare. Nemati et al. [21] showed that in the healthy population during sleep, there was a nadir in TWA values around 60–70 bpm, and the value of TWA tended to increase with heart rate. Our data also showed

Table 3 Univariate and multivariate analysis for VF episodes during follow-up

	Univariate analysis			Multivariate analysis		
	Odds ratio	95 % CI	<i>p</i> value	Odds ratio	95 % CI	<i>p</i> value
Max-TWA 3L-V1 (12 a.m.–6 a.m.) $\geq 26 \mu\text{V}$	4	1.3–12	0.02*	3	0.6–18	0.17
Max-TWA 3L-V2 (12 a.m.–6 a.m.) $\geq 20 \mu\text{V}$	14	2.6–252	0.0005*	9	1.1–232	0.04*
Previous VF	82	20–430	0.0001*	68	15–440	0.001*

TWA T-wave-alternans, *CI* confidence interval, *VF* ventricular fibrillation

* $p < 0.05$

heart rates during the night time were around 60–70 bpm (Fig. 3f) in all 4 groups and the max-TWA values during the night time were lower than those during the day time, even in control subjects. One report indicated that patients with congestive heart failure showed low values of TWA during the night time, compared with those during the day time [7]. Therefore, our cutoff value of max-TWA values during the night time (20 μ V) was a lower amplitude, compared with that in previous reports assessing the max-TWA values during entire day. We believe that our cutoff value of max-TWA values during the night time is acceptable. There were no differences in the max-TWA values for 4L-V1, 4L-V2, 3L-V1, and 4L-V5 among the 4 groups. The discrepancy of the results from the previous report [20] may be related to the number of the patients and a regional specificity of TWA [22]. Morita et al. demonstrated that phase 2 reentry and TWA were more likely to originate from RVOT epicardium than from the RVOT endocardium or the RV inferoanterior site in tissue models of Brugada syndrome. Additionally, they reported that the instability and heterogeneity of repolarization within the RVOT epicardium was associated with arrhythmogenesis in Brugada syndrome [23]. Shimeno et al. [24] reported that Holter-ECG recording at the third intercostal space was more sensitive for detecting spontaneous type-1 ECG patterns than that at the standard positions. Another group reported that the electrophysiological mechanism in patients with Br-S involved delayed depolarization over the anterior aspect of the RVOT epicardium, and that catheter ablation over the abnormal depolarization was effective for eliminating VF and normalizing the Brugada ECG pattern [25]. We think that the recorded position of 3L-V2 is the closest to the RVOT site, and that TWAs at 3L-V2 can detect the repolarization abnormality within the RVOT more sensitively than those at the other recorded positions.

In Br-S, unlike in other structural heart diseases, VF occurs mainly during the night time. Takigawa et al. [26] reported that the episodes of VF occurred most frequently during the night in patients with Br-S. In the present study, the incidence of VF recurrence was also the highest during the night time. The ST-segment elevation observed in patients with Br-S, is reported to diminish during exercise and isoproterenol infusion. Kaufman et al. [27] indicated that the amplitude of TWA is depended on heart rate and increased at higher rate in any subjects. Ikeda et al. [28] reported that microvolt TWA during a 80–110 bpm heart rate was not a marker for identifying high-risk patients with Br-S. In this study, we analyzed the mean heart rate during the 2 time periods and found that it was higher during the day time than during the night time (Fig. 3f). In all groups, mean heart rate was between 80 and 90 bpm during the day time. The 3L-V2 max-TWA during the day time was higher than that during the night time in all the 4 groups.

There were statistically no significant differences of the 3L-V2 max-TWA during the day time among the 4 groups. However, in VF and syncope groups with Br-S, variation of TWA tended to be fewer than in asymptomatic and control groups. As previous reports [19, 28] the values of max-TWA at higher heart rate may not be associated with cardiac events in patients with Br-S.

An imbalance between the sympathetic and parasympathetic tones in patients with Br-S has been discussed in previous studies. Some authors showed that the HF, determined by analysis of HRV using Holter ECG recording, was significantly higher among symptomatic patients than among asymptomatic patients [29]. In contrast, many reports showed that the mean HF and LF/HF ratio values during the day time and the night time were similar between patients with and without previously documented VF [30–32]. In our study, the HF and LF/HF values were similar among the 4 groups. The reason for the discrepant results among these studies remains unclear, although day-to-day variation in autonomic tone may account for the difference.

In the present study, 3L-V2 max-TWA above the cutoff value (≥ 20 μ V) during the night time was useful marker of VF occurrences during the follow-up period by the multivariate analysis. To the best of our knowledge, this is the first report showing the utility of max-TWA during the night time at 3L-V2, using MMA analysis of TWA by 24-M-ECG, which has provided useful information for distinguishing between high- and low-risk patients with Br-S.

This study has several limitations. First, the number of VF episodes during the follow-up period was relatively small, and a much larger patient population is regarded to improve the TWA cutoff value and to clarify the identification of high-risk patients with Br-S. Second, we evaluated TWA and HRV in a single study using 24-M-ECG. We were unable to evaluate daily fluctuations or seasonal variability in TWAs and autonomic tone. Third, the LF and HF powers were used to represent autonomic tone. However, similar magnitudes for each of those powers might not indicate similar autonomic tone among other individuals. Fifth, the rate of VF episodes in our study was higher than that of a previous report, which showed the occurrence of VF was 17 % in patients with history of VF during follow-up period (34 ± 44 months) [33]. On the other hand, another study reported higher recurrence of VF (62 %) in patients with history of VF during longer follow-up periods (54 ± 54 months) [34]. Follow-up periods (68 ± 37 months) in our study are longer than those in the most previous reports, which might account for the differences. As another reason of the differences, we might include some more severe cases in this study. Sixth, we did not perform genetic test and electrophysiologic study in all the subjects, and we could not evaluate these factors in this

study. Finally, a large-scale prospective study is required to better understand the efficacy of TWA for predicting VF episodes.

In conclusion, maximum TWA during the night time may be useful for risk stratification of patients with Br-S. The 3L-V2 max-TWA values $\geq 20 \mu\text{V}$ during the night time (12 a.m.–6 a.m.) was an independent predictor of VF episodes in patients with Br-S.

Conflict of interest The authors declare that they have no conflict of interest.

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