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Abstract: *Background and Objectives*: Holter-based late potentials (LPs) are useful for predicting lethal arrhythmias in organic cardiac diseases. Although Holter-based LPs exhibit diurnal variation, no studies have evaluated the optimal timing of LP measurement over 24 h for predicting lethal arrhythmia that leads to sudden cardiac death. Thus, this study aimed to validate the most effective timing for Holter-based LP testing and to explore factors influencing diurnal variability of LP parameters. *Materials and Methods*: We retrospectively analyzed 126 patients with post-myocardial infarction (MI) status and 60 control participants who underwent high-resolution Holter electrocardiography. Among the 126 post-MI patients, 23 developed sustained ventricular tachycardia (VT) (the MI-VT group), while 103 did not (the MI-non-VT group) during the observation period. Holter-based LPs were measured at 0:00, 4:00, 8:00, 12:00, 16:00, and 20:00, and heart rate variability analysis was simultaneously performed to investigate factors influencing diurnal variability of LP parameters. *Results*: Holter-based LP parameters showed diurnal variation with significant deterioration at night and improvement during the day. Assessment at the time with the longest duration of low-amplitude signals <40 μV in the filtered QRS complex terminus (LAS40) gave the highest receiver operating characteristics curve (area under the curve, 0.659) and the highest odds ratio (3.75; 95% confidence interval, 1.45–9.71; p=0.006) for predicting VT. In the multiple regression analysis, heart rate and noise were significant factors affecting LP parameters in the MI-VT and control groups. In the non-VT group, LP parameters were significantly influenced by noise and parasympathetic heart rate variability parameters such as logPNN50. *Conclusions*: For Holter-based LP measurements, test accuracy was higher when LP was measured at the time of the highest or worst value of LAS40. Changes in autonomic nervous system activity, including heart rate and noise levels, were factors influencing diurnal variability.

Keywords: late potentials; signal-averaged electrocardiography; heart rate variability; fatal arrhythmia; sudden cardiac death

1. Introduction

Since the 1990s, late potentials (LPs) detected using signal-averaged electrocardiography (SAECG) have been reported to be useful for predicting sudden cardiac death (SCD) and fatal arrhythmic events in patients with post-myocardial infarction (MI) status [1,2], dilated

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cardiomyopathy [3], arrhythmogenic right ventricular cardiomyopathy [4], cardiac sarcoidosis [5], and other organic heart diseases.

Recently, Holter electrocardiography (ECG) has also been used to measure LPs [6], and reports have emerged on the usefulness of Holter-based LPs for predicting SCD/lethal arrhythmia in patients with organic heart disease and chronic kidney disease [7–9]. Holter-based LP measurement is expected to become a mainstream test because it is performed simultaneously with routine Holter ECG, saving time for both patients and medical professionals compared to the conventional real-time LP measurement method. However, Holter-based LPs exhibit diurnal variation in post-MI patients [8], patients with Brugada syndrome [10,11], and healthy participants [12]. Given that 24 consecutive hours of LP data are obtained from Holter electrocardiographs, it is important to determine which method of LP data collection is most useful for stratifying patients by SCD/lethal arrhythmia risk. In previous studies, LP parameters such as filtered QRS duration (fQRS) and root mean square voltage of the terminal 40 ms in the filtered QRS complex (RMS40) captured at the time of the most abnormal or worst (lowest) RMS40 in 24 h [8,9,13] or the most abnormal or worst (highest) fQRS in 24 h [14] were used as representative Holter-based LP values. However, the timing of collecting SAECG testing data yielding LP parameters most useful for predicting lethal arrhythmias under ordinary daily conditions has not been completely validated. Moreover, factors influencing diurnal variation of LP parameters are not completely understood in patients with post-MI status.

Therefore, this study aimed to identify the optimal timing for LP testing for stratifying risk of post-MI patients and to investigate factors influencing LP diurnal variation in post-MI patients and control participants.

2. Materials and Methods

2.1. Study Design and Ethics

In this retrospective cohort study, we initially enrolled 150 patients with post-MI status and 66 control participants, all of whom underwent high-resolution Holter electrocardiography (H-ECG) from March 2012 to December 2022 (Figure S1). Among the 150 patients with post-MI status, 33 had clinically sustained ventricular tachycardia (VT) as of March 2021 and were assigned to the MI-VT group, while 117 did not have sustained VT and were assigned to the MI-non-VT group; sustained VT was defined as ≥30 s of consecutive ventricular complexes at a rate of >100 bpm. The control group included 66 participants who underwent outpatient H-ECG for close examination of chest symptoms and ultimately showed no sign of cardiac disease.

The exclusion criteria were as follows: 1) cardiomyopathy or arrhythmogenic right ventricular cardiomyopathy, 2) persistent atrial fibrillation or flutter, 3) right or left bundle branch block and intraventricular conduction delay, 4) permanent pacing with pacemaker or implantable cardioverter defibrillator, 5) atrioventricular block II–III degree, and 6) channelopathies such as long QT syndrome, Brugada syndrome, and early repolarization syndrome; 24 patients were excluded for these reasons. Finally, 126 patients with post-MI status (MI-VT group, n=23; MI-non-VT group, n=103) and 60 control participants were included in the study (Figure S1, Table 1).

Table 1. Baseline characteristics of the study participants by group.

Demographics	MI-VT group (n=23)	MI-non-VT group (n=103)	p value	Control group (n=60)
Age (years)	66.9±12.4	66.9±13.1	0.994	56.7±20.5
Sex: male, n (%)	22 (96)	83 (81)	0.195	33 (55)
Hypertension, n (%)	18 (23)	87 (84)	0.758	—
Dyslipidemia, n (%)	14 (61)	68 (66)	0.831	—
Diabetes mellitus, n (%)	17 (74)	41 (40)	0.002	—
Coronary culprit lesion				
RCA	3 (13)	39 (38)	0.023	—
LAD	17 (73)	43 (42)	0.04	—

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Cx	2 (13)	10 (20)	0.562	—
Echocardiographic data				
LVEF (%)	48.5±16.0	58.4±11.9	<0.001	70.8±6.5
LVDd (mm)	57.1±11.6	50.1±7.4	<0.001	44.4±4.6
Renal function				
Estimate GFR (mL/min per 1.73 m^2)	46.9 [34.7, 68.5]	61.3 [37.7, 76.1]	0.146	78.5±18.2
Creatine (mg/dL)	1.1 [0.8, 1.5]	0.93 [0.7, 1.2]	0.152	0.69 [0.63, 0.79]
Therapy				
β-Blocker (%)	19 (83)	77 (75)	0.424	—
RAS inhibitor (%)	14 (61)	66 (64)	0.729	—
CCB (%)	11(48)	32 (31)	0.125	—
Diuretic (%)	12 (52)	42 (41)	0.581	—
Amiodarone (%)	8 (34)	6 (6)	<0.001	—
Ib (%)	1 (4.3)	5 (4.8)	0.918	—
Ic (%)	0 (0)	0 (0)	—	—

Data are given as n (%) or means±SDs. CCB, calcium channel blockers; Cx, circumflex branch; LAD, left anterior descending; LVEF, left ventricular ejection fraction; LVDd, left ventricular dimension diameter; MI-non-VT, myocardial infarction without ventricular tachycardia; MI-VT, myocardial infarction with ventricular tachycardia; RAS, renin–angiotensin system; RCA, right coronary artery.

The study protocol conformed to the Declaration of Helsinki and was approved by the Medical Ethics Committee of the National Defense Medical College Hospital (approval no. 4692), Saitama, Japan, and the Nihon University School of Medicine, Itabashi Hospital, Tokyo, Japan (approval no. MF-2302-0063). Written informed consent was obtained from all patients.

2.2. Ambulatory ECG Recordings

Patients underwent H-ECG recording during ordinary daily activities at least 3 weeks after MI onset to avoid acute phase electrical instability. Data obtained from the H-ECG system (SpiderView; ELA Medical, Paris, France) were analyzed for routine arrhythmic events. The length of the H-ECG recording conducted for each patient was 24 h.

2.3. Measurement of Holter-Based LPs

LPs were recorded for all patients and control participants using the H-ECG system. ECG data were obtained at a sampling rate of 1000 Hz using 16-bit A/D conversion. For LP measurement, ECG data were filtered and ranged from 40 to 250 Hz. Then, the LP signals of 250 complexes were averaged (default setting). Orthogonal X, Y, and Z bipolar leads with silver-silver chloride electrodes (Blue SENSOR®; METS, Tokyo, Japan) were used for all LP recordings. LP parameters were automatically measured by the software during the 24-h time period; parameters assessments were manually edited by expert electrophysiological investigators using Syne Scope (SORIN GROUP, Milano, Italy). The expert electrophysiological investigators were blinded to patient outcomes. LP parameters were assessed independently by two expert electrophysiological investigators, and disagreements were resolved by consensus.

Three LP parameters were evaluated in the 24-h records of the MI-VT, MI-non-VT, and control groups: filtered QRS duration (fQRS) (ms), root mean square voltage of the terminal 40 ms in the filtered QRS complex (RMS40) (μV), and duration of low-amplitude signals <40 μV in the filtered QRS complex terminus (LAS40) (ms). We evaluated each of the LP parameters every 4 h at 6 time points: 0:00, 4:00, 8:00, 12:00, 16:00, and 20:00. To adjust the LP signal for noise level, a Holter-based LP measured at 0.8 μV or less was used; however, if no part of the LP measurement had a noise level below 0.8 μV, the LP parameters were measured over a wider range of up to 2 h before and after the time period to find such a portion, and noise reduction was performed. Then, the signals at times corresponding to the (a) fQRS worst, (b) fQRS best, (c) RMS40 worst, (d) RMS40 best, (e) LAS40 worst, and (f) LAS40 best values relative to the presence of LPs and (g) the 24-h mean values of each of the

three LP parameters were selected for evaluation. The averaged signal obtained at each selected time was judged to be positive or negative for the presence of LPs (Figure 1). LPs were considered to be present when any two of the following three criteria were met: fQRS >114 ms, RMS40 <20 μV, and LAS40>38 ms [15]. Furthermore, the periodical times (0:00, 4:00, 8:00, 12:00, 16:00 and 20:00) were evaluated as representative times for assessing the presence or absence of LPs. The LP parameters of the signals at these times were judged as LP positive or negative. The body position at the time of LP measurement was estimated manually from the behavior record card.

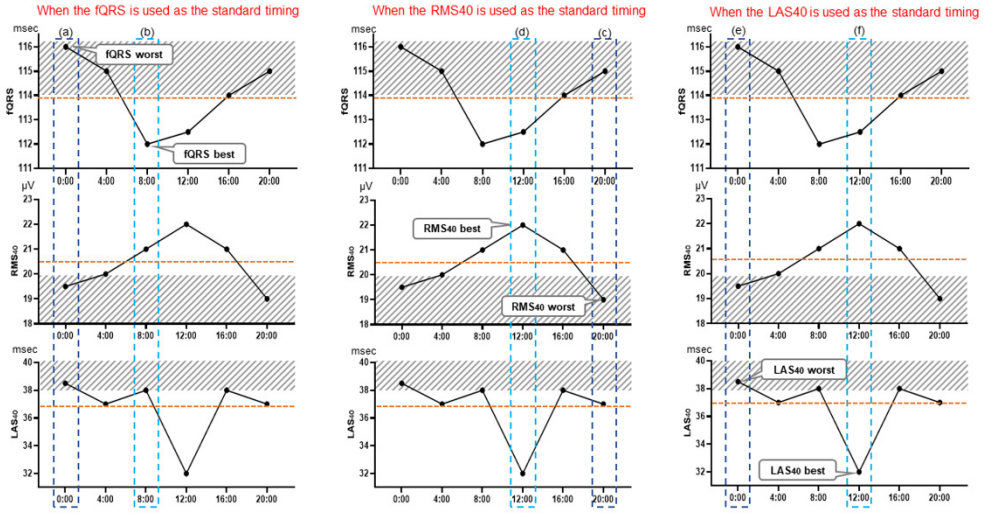

Figure 1. Holter-based late potential measurement point examples. These graphs illustrate the selection of measurement points: at the time of the fQRS worst point, where fQRS=116 ms, RMS40=19.5 μV, and LAS40=38.5 ms (**a**), and at the time of the fQRS best point, where fQRS=112 ms, RMS40=21 μV, and LAS40=38 ms (**b**). Similarly, LP determination was performed using the values of each parameter at the time of the worst (**c**) and best (**d**) RMS40 point, and of the worst (**e**) and best (**f**) LAS40 points. LP measurement was also performed using the mean value of each parameter, which were fQRS=113.8 ms, RMS40=20.5 μV, and LAS40=36.8 ms. fQRS, filtered QRS duration; RMS40 root mean square voltage of the terminal 40 ms of the filtered QRS complex (μV); LAS40, duration of low-amplitude signals <40 μV in the filtered QRS complex terminus (ms).

2.4. Heart Rate Variability Analysis

Heart rate (HR) variability (HRV) analysis was also performed to evaluate autonomic nervous activity using the SpiderView (Ela, Paris, France) at the same time when Holter-based LPs were measured. HRV time- and frequency-domain analyses were conducted at 5-min intervals. For frequency-domain analysis, the RR interval was calculated using fast Fourier transformation. Time-domain analysis included the percent difference between adjacent normal NN intervals greater than 50 ms (PNN50), root mean squared successive differences of NN intervals (RMSSD), mean of 5-min standard deviations of NN intervals (ASDNN) (ms), and standard deviation of the average NN interval for each 5-min segment (SDANN) (ms). For frequency-domain analysis, the power in the low-frequency area (LF), power in the high-frequency area (HF), and power in the low-frequency area/power in the high-frequency area (LF/HF) ratio were also analyzed every 5 min. The power spectra of frequency-domain analysis were defined as follows: total power (TP), <0.4 Hz; power in the very low-frequency range (VLF), 0.0033–0.04 Hz; power in the low-frequency range (LF), 0.04–0.15 Hz; and power in the HF, 0.15–0.40 Hz. Based on a previously published report [16], LF normalized unit (LFnu) and HF normalized unit (HFnu) were calculated using the following formulas: LFnu = [LF/(TP-VLF)] × 100 and HFnu = [HF/(TP-VLF)] × 100. HRV parameters were evaluated simultaneously with LP parameters whenever the signal had an acceptable noise level of <0.8 μV.

2.5. Statistical Analyses

Data are presented as means±standard deviations for normally distributed continuous variables and as medians (interquartile range: 25th–75th percentile) for non-normally distributed variables. Patient characteristics were compared using the $\chi 2$ test for categorical variables, Student's t-test for continuous and parametric data, and Mann–Whitney U test for nonparametric data. The distribution of continuous variables was evaluated for normality by the Shapiro–Wilk test. Friedman's analysis of variance (ANOVA) on rank was used to compare LP parameters (fQRS, RMS40, and LAS40) for each LP measurement time. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated using standard formulas. A receiver operating characteristic (ROC) curve was generated, and the area under the curve (AUC) was calculated to determine the LP measurement timing that best predicted VT. Logistic regression analysis was performed to correlate the occurrence of VT with each time of LP measurement (when fQRS, RMS40, andLAS40 were worst; when they were best; and at 0:00, 4:00, 8:00, 12:00, 16:00, and 20:00). Cochran's Q test was performed to compare LP positivity rates at each time point (0:00, 4:00, 8:00, 12:00, 16:00, and 20:00). Multivariate regression analysis was performed to determine the intensity of diurnal variation of LP parameters and to theoretically consider important factors such as the HR and HRV indices. Because HRV indices (PNN50, RMSSD, ASDNN, SDANN, PNN50, LFnu, VLF, HFnu, and LF/HF) showed skewed distributions, they were natural log-transformed before multiple regression analysis to explore factors influencing the diurnal variation of Holter-based LPs was performed.

Sample size calculation was performed based on the correlation among six repeated measures ANOVA using the R (4.2.3.2 Ver.) (R Foundation for Statistical Computing, Vienna, Austria), a two-tailed hypothesis, an effect size of 0.40, an α error probability of 0.05 with a β level of 10%, between-group variance=5, within-group variance=30, and the desired power analysis of 90% (1-β error probability). This calculation showed that a total sample size of at least 20 participants with sustained ventricular tachycardia (SVT) was required to achieve the desired power. Consequently, a total of 150 patients with MI were included (120 MI-non-SVT participants) to enroll consecutive cases with at least 20 SVT. Ultimately, a total of 126 patients with MI were included based on the inclusion criteria (MI-VT, n=23; MI-non-VT, n=103). All statistical analyses, except for the sample size analysis, were performed using SPSS version 28 (IBM Corp., Armonk, NY, USA). T-tests were two-sided, and p values of <0.05 were considered statistically significant.

3. Results

3.1. Patient Demographics

The demographic data, including age, sex, comorbidities, echocardiographic data, renal function parameters, and medication therapy, were extracted from the electronic medical records of the patients. Table 1 shows the characteristics of patients and control participants included in the study. The number of patients with diabetes mellitus, culprit coronary lesions of the left atrial descending artery, left ventricular end-diastolic diameter, and amiodarone use post-MI were significantly higher in the MI-VT group than in the MI-non-VT group. Consequently, LVEF was significantly lower in the MI-VT group than in the MI-non-VT group.

3.2. Optimal Measurement Timing for Assessment of Holter-Based LPs

In the MI-VT, MI-non-VT, and control groups, Holter-based LPs showed significant diurnal variation for all three parameters (fQRS, RMS40, and LAS40) (Table 2). In all groups, LPs deteriorated during the nighttime (20:00–8:00) and improved during the daytime (8:00–20:00) (Table 2). LP-positivity rates of the three groups (MI-VT, MI-non-VT, and control groups) were significantly higher at night and lower during the daytime (p=0.002–0.009) (Table 3).

Table 2. Comparison of LP parameters among the six measurement times.

MI-VT group (n=23)

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		0:00	4:00	8:00	12:00	16:00	20:00	p value
fQRS (ms)	median [interquartile range]	115.0 [108.0,134.8]	116.0 [108.0,131.0]	116.0 [101.0,135.0]	116.0 [102.0,135.0]	114.0 [107.0,132.0]	118.0 [107.0,134.0]	0.005
RMS40 (μV)	median [interquartile range]	14.0 [10.3,54.8]	14.0 [10.0,43.0]	21.0 [11.0,55.0]	18.0 [8.0,57.0]	16.0 [9.0,43.0]	16.0 [6.6,52.0]	0.04
LAS40 (ms)	median [interquartile range]	43.5 [29.0,53.0]	41.0 [31.0,48.0]	37.0 [27.0,46.0]	40.0 [27.0,46.0]	40.0 [26.0,51.0]	39.0 [30.0,50.0]	0.02
MI-non-VT group (n=103)								
		0:00	**4:00**	**8:00**	**12:00**	**16:00**	**20:00**	**p value**
fQRS (ms)	median [interquartile range]	101.0 [93.0,115.0]	102.5 [94.0,113.5]	100.5 [91.8,112.3]	98.0 [93.0,114.0]	99.0 [90.0,110.5]	99.0 [94.0,113.5]	<0.001
RMS40 (μV)	median [interquartile range]	30.5 [16.0,45.8]	30.5 [16.0,45.8]	32.5 [20.0,48.3]	34.0 [18.5,47.0]	36.0 [19.5,50.5]	30.0 [20.8,48.5]	<0.001
LAS40 (ms)	median [interquartile range]	30.0 [24.0,41.5]	32.0 [24.0,39.5]	30.0 [24.0,36.5]	30.0 [24.0,36.5]	29.0 [24.5,36.0]	31.0 [25.0,36.0]	0.03
Control group (n=60)								
		0:00	**4:00**	**8:00**	**12:00**	**16:00**	**20:00**	**p value**
fQRS (ms)	median [interquartile range]	90.0 [86.0,95.3]	90.0 [87.0,96.0]	87.5 [83.0,93.3]	85.0 [83.8,90.0]	87.0 [83.0,91.0]	88.0 [83.0,93.0]	<0.001
RMS40 (μV)	median [interquartile range]	45.5 [29.5,64.0]	44.5 [28.8,65.8]	49.5 [31.0,81.8]	55.5 [33.0,81.5]	53.0 [38.3,78.8]	47.0 [33.0,79.6]	<0.001
LAS40 (ms)	median [interquartile range]	28.0 [23.0,32.0]	27.0 [24.0,31.3]	27.0 [21.0,33.0]	26.0 [20.0,30.3]	26.0 [21.0,29.0]	25.0 [22.0,31.3]	0.03

fQRS, filtered QRS duration; LAS40, duration of low-amplitude signals <40 μV in the filtered QRS complex terminus (ms); LP, late potential; MI-non-VT, myocardial infarction without ventricular tachycardia, MI-VT, myocardial infarction with ventricular tachycardia, RMS40, root mean square voltage of the terminal 40 ms in the filtered QRS complex (μV).

Table 3. Diurnal variation of the LP-positive rate in each group.

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MI-VT group (n=23)							
	0:00	**4:00**	**8:00**	**12:00**	**16:00**	**20:00**	**p value**
Number of patients (%)	13 (57)	13 (57)	10§ (43)	11# (48)	13 (57)	12 (52)	0.009
MI-non-VT group (n=103)							
	0:00	**4:00**	**8:00**	**12:00**	**16:00**	**20:00**	**p value**
Number of patients (%)	24 (23)	23 (22)	18§ (17)	19§ (18)	21 (20)	21 (20)	0.002
Control group (n=60)							
	0:00	**4:00**	**8:00**	**12:00**	**16:00**	**20:00**	**p value**
Number of participants (%)	7 (12)	4# (7)	4# (7)	3§ (5)	2§ (3)	3§ (5)	0.009

#=0.005 vs. 0:00; §≤0.001 vs. 0:00. MI-non-VT, myocardial infarction without ventricular tachycardia; MI-VT group, myocardial infarction with ventricular tachycardia; LP, late potential.

Table 4 shows the predictive values associated with Holter-based LPs for each parameter and time point. For each LP parameter, the NPV was not different among the LP parameters (85–89%). However, the PPV in the fQRS worst (61%), RMS40 worst (61%), and LAS40 worst (65%) tended to be better than the other PPVs of LP parameters (43–48%). for each time setting (Table 4, right). Although the NPV at 16:00 was the lowest, the PPV at 0:00, 4:00, 16:00, and 20:00 (57–61%) tended to be better than that during the daytime (8:00, 12:00) (43–52%).

Table 4. Predictive values associated with Holter-based LP measurement for each parameter at each time point.

	Sensitivity	**Specificity**	**PPV**	**NPV**		**Sensitivity**	**Specificity**	**PPV**	**NPV**
Parameter					**Time point**				
fQRS worst	61	67	61	89	0:00	57	74	57	88
fQRS best	43	80	43	86	4:00	57	75	57	89
RMS40 worst	61	65	61	88	8:00	43	75	43	86
RMS40 best	43	85	43	87	12:00	52	57	52	76
LAS40 worst	65	63	65	87	16:00	61	78	61	90
LAS40 best	43	84	43	87	20:00	57	80	57	90
Mean values of 3 LP parameters	48	78	48	85					

fQRS, filtered QRS duration; LAS40, duration of low-amplitude signals <40 μV in the filtered QRS complex terminus; LP, late potential; NPV, negative predictive value; PPV, positive predictive value; RMS40, root mean square voltage of the terminal 40 ms in the filtered QRS complex.

In the ROC curve for each LP parameter, when the timing of the worst LAS40 reading was selected as the standard, the AUC was higher (0.659) and the test accuracy was the highest (Figure 2a). However, in the ROC curve for each time period (0:00, 4:00, 8:00, 12:00, 16:00, and 20:00), when

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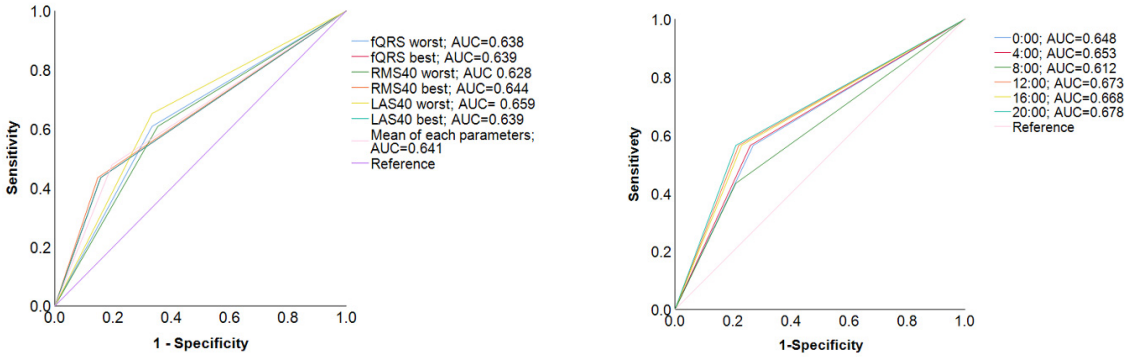
20:00 was selected as the standard for LP measurement timing, the AUC was the highest (AUC=0.678) and the SAECG test was highly accurate (Figure 2b). In the logistic multivariate regression analysis, the highest odds ratio was observed when LAS40 worst timing was used as the standard (odds ratio=3.75, 95% confidence interval [CI]=1.45–9.71, p=0.006) (Table 5. In each LP parameter). In contrast, the highest odds ratio was observed when 20:00 was selected as the standard for LP measurement timing (odds ratio=4.89, 95% CI=1.88-12.7, p=0.006) (Table 5. In each time zone).



(**a**) (**b**)

Figure 2. Receiver operating characteristic curves. (**a**) In the ROC curve for each parameter, when the LP parameter value was at the point with the worst LAS40, the AUC was higher (AUC=0.659) and the test accuracy was lower; (**b**) in contrast, in the ROC curve for each time period, when the LP parameter value was at the 20:00 time point, the AUC was the highest (AUC=0.678) and the test was highly accurate.

Table 5. Relationship between LP measurement timing and lethal arrhythmia.

For each LP parameter	**Univariate**			**Multivariate**			**Multivariate (stepwise)**		
	OR	**95% CI**	**p**	**OR**	**95% CI**	**p**	**OR**	**95% CI**	**p**
fQRS worst	3.11	1.22–7.91	<0.001	1.00	0.87–11.56	0.998			
fQRS best	4.13	1.55–11.03	<0.001						
RMS40 worst	2.85	1.12–7.23	<0.001	0.332	0.021–5.36	0.437			
RMS40 best	4.46	1.66–12.0	<0.001						
LAS40 worst	3.75	1.45–9.71	0.006	10.41	0.58–185.46	0.111	3.75	1.45–9.71	0.006
LAS40 best	4.14	1.55–11.04	<0.001						
Mean values of three LP parameters	3.76	1.45–9.75	<0.001						
For each time point	**Univariate**			**Multivariate**			**Multivariate (stepwise)**		
	OR	**95% CI**	**p**	**OR**	**95% CI**	**p**	**OR**	**95% CI**	**p**
0:00	3.61	1.42–9.19	0.007	0.66	0.75–5.81	0.710			
4:00	3.80	1.49–9.70	<0.001	0.93	0.084–10.27	0.953			
8:00	2.97	1.14–7.70	<0.001	0.21	0.024–1.75	0.148			

12:00	4.67	1.80–12.07	<0.001	3.16	0.29–33.91	0.342			
16:00	4.41	1.11–11.36	<0.001	2.74	0.39–19.26	0.310			
20:00	5.00	1.93–13.02	<0.001	4.40	0.52–37.25	0.174	4.89	1.88–12.7	0.001

fQRS, filtered QRS duration; LAS40, duration of low-amplitude signals <40 μV in the filtered QRS complex terminus; LP, late potential; RMS40, root mean square voltage of the terminal 40 ms in the filtered QRS complex; CI, confidence interval; OR, odds ratio.

3.3. Factors Influencing Diurnal Variability in Holter-Based LP

In the multiple regression analysis, HR was the factor most influencing diurnal variation of LP parameters in the MI-VT group (fQRS, β=0.180, p=0.037; RMS40, β=0.305, p=0.003; LAS40, β=-0.261, p=0.011) (Table 6a. MI-VT group). In the MI-non-VT group, LP parameters were significantly influenced by noise or parasympathetic nervous activity parameters of HRV, such as logPNN50 or logLF/HF, which are considered a balance of sympathetic, nervous, and parasympathetic nervous activities (Table 6b. MI-non-VT group). These results suggest the involvement of the autonomic nervous system in both the MI-VT and MI-non-VT groups. In addition, logVLF and logASDNN were significant factors in the MI-non-VT group. In contrast, in the control group, diurnal variability of LP parameters was significantly influenced by noise or HR (Table 6c. Control group).

Table 6. (**a**) Factors influencing diurnal variation in LP parameters (MI-VT group). (**b**) Factors influencing diurnal variation in LP parameters (MI-non-VT group). (**c**) Factors influencing diurnal variation in LP parameters (control group).

(**a**)

fQRS	**R=0.490**			**R=0.448[a]**		
	β	**p**	**VIF**	**β**	**p**	**VIF**
body position	0.031	0.770	1.527			
log Noise (μV)	0.081	0.484	1.812			
log HR (bpm)	-0.188	0.085	1.599	-0.180	0.037	1.016
log PNN50 (%)	0.256	0.270	7.296	0.433	<0.001	1.016
log RMSSD (ms)	0.212	0.417	9.246			
log ASDNN (ms)	-0.180	0.382	5.771			
log SDANN (ms)	-0.021	0.832	1.325			
log VLF (ms²)	0.187	0207	2.977			
log HFnu (TP)	0.183	0.140	2.070			
log LF/HF	0.086	0.400	1.399			
RMS40	**R=0.500**			**R=0.305[a]**		
	β	**p**	**VIF**	**β**	**p**	**VIF**
body position	-0.092	0.417	1.397			
log Noise (μV)	-0.018	0.881	1.550			
log HR (bpm)	0.422	0.000	1.441	0.305	0.003	1.000
log PNN50 (%)	-0.230	0.336	6.211			
log RMSSD (ms)	-0.066	0.790	6.619			
log ASDNN (ms)	0.180	0.415	5.264			
log SDANN (ms)	0.077	0.509	1.483			

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log VLF (ms²)	0.076	0.648	2.971			
log HFnu (TP)	0.796	0.002	7.084			
log LF/HF	0.733	0.007	7.566			
LAS40	**R=0.392**			**R=0.292[a]**		
	β	***p**	**VIF**	**β**	**p**	**VIF**
body position	0.013	0.916	1.492			
log Noise (μV)	-0.010	0.942	1.788			
log HR (bpm)	-0.330	0.010	1.524	-0.261	0.011	1.000
log PNN50 (%)	0.081	0.749	6.233			
log RMSSD (ms)	0.148	0.568	6.483			
log ASDNN (ms)	-0.032	0.890	5.196			
log SDANN (ms)	-0.008	0.950	1.475			
log VLF (ms²)	-0.134	0.448	2.970			
log HFnu (TP)	-0.525	0.057	7.175			
log LF/HF	-0.402	0.154	7.582			

log ASDNN=logarithm of mean of the standard deviations of all NN intervals for all 5-min segments in 24-h HF; log HR=logarithm of heart rate; log HFnu=logarithm of power in the high-frequency area normalized unit; log LF/HF=logarithm of power in the low-frequency/power in the high-frequency ratio; log pNN50=logarithm of percent of difference between adjacent normal RR intervals greater than 50 ms; log RMSSD=logarithm of root mean square successive difference; log SDANN=logarithm of standard deviation of 5-min average NN intervals; VIF=variance inflation factor; log VLF=logarithm of low frequency area. a=variables by multiple linear regression with stepwise selection.

(**b**)

fQRS	**R=0.366**			**R=0.353[a]**		
	β	**p**	**VIF**	**β**	**p**	**VIF**
body position	-0.054	0.348	1.287			
log Noise (μV)	-0.036	0.529	1.308			
log HR (bpm)	-0.021	0.725	1.436			
log PNN50 (%)	0.305	0.001	3.092	0.298	0.001	2.945
log ASDNN (ms)	-0.235	0.028	4.480	-0.222	0.029	4.047
log SDANN (ms)	0.005	0.934	1.406			
log VLF (ms²)	-0.184	0.037	3.027	-0.180	0.030	2.684
log HFnu (TP)	-0.038	0.692	3.680			
log LF/HF	0.190	0.071	4.291	0.209	0.002	1.822
RMS40	**R=0.367**			**R=0.327[a]**		
	β	**p**	**VIF**	**β**	**p**	**VIF**
body position	-0.039	0.493	1.287			
log Noise (μV)	0.155	0.007	1.308	0.156	0.002	1.000
log HR (bpm)	0.046	0.446	1.436			
log PNN50 (%)	-0.241	0.007	3.092	-0.208	0.003	1.903
log ASDNN (ms)	0.136	0.203	4.480	0.206	0.003	1.902

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log SDANN (ms)	0.075	0.209	1.406			
log VLF (ms²)	0.119	0.175	3.027			
log HFnu (TP)	-0.027	0.777	3.680			
log LF/HF	-0.157	0.134	4.291			
LAS40	**R=0.344**			**R=0.314[a]**		
	β	**p**	**VIF**	**β**	**p**	**VIF**
body position	0.029	0.617	1.287			
log Noise (μV)	-0.119	0.041	1.308	-0.122	0.017	1.000
log HR (bpm)	-0.008	0.890	1.436			
log PNN50 (%)	0.265	0.003	3.092	0.219	0.002	1.903
log ASDNN (ms)	-0.221	0.041	4.480	-0.224	0.001	1.902
log SDANN (ms)	-0.086	0.154	1.406			
log VLF (ms²)	-0.008	0.929	3.027			
log HFnu (TP)	0.070	0.472	3.680			
log LF/HF	0.155	0.142	4.291			

Abbreviations as in Table 6 (MI-VT group). a=Variables identified by multiple linear regression with stepwise selection. ※Logarithm of root mean square successive difference (log RMSSD) was removed from analysis because of multicollinearity.

(c)

fQRS	**R=0.458**			**R=0.452[a]**		
	β	**p**	**VIF**	**β**	**p**	**VIF**
body position	-0.035	0.556	1.352			
log Noise (μV)	-0.473	<0.001	1.271	-0.484	<0.001	1.179
log HR (bpm)	0.139	0.050	1.948	0.141	0.022	1.473
log PNN50 (%)	-0.048	0.631	3.860			
log ASDNN (ms)	0.118	0.332	5.753			
log SDANN (ms)	-0.024	0.705	1.530			
log VLF (ms²)	-0.105	0.298	3.985			
log HFnu (TP)	-0.150	0.319	8.789	-0.129	0.028	1.356
log LF/HF	0.004	0.982	9.626			
RMS40	**R=0.396**			**R=0.356[a]**		
	β	**p**	**VIF**	**β**	**p**	**VIF**
body position	0.112	0.078	1.385			
log Noise (μV)	0.138	0.042	1.588	0.147	0.008	1.049
log HR (bpm)	-0.081	0.265	1.840			
log PNN50 (%)	0.123	0.249	3.925	0.094	0.089	1.049
log ASDNN (ms)	-0.013	0.911	4.489			
log SDANN (ms)	0.035	0.552	1.227			
log VLF (ms²)	-0.075	0.407	2.837			
log HFnu (TP)	-0.027	0.768	2.795			

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log LF/HF	0.001	0.987	1.523			
LAS40	**R=0.575**			**R=0.563[a]**		
	β	**p**	**VIF**	**β**	**p**	**VIF**
body position	0.032	0.558	1.352			
log Noise (μV)	-0.633	<0.001	1.271	-0.609	<0.001	1.169
log HR (bpm)	0.240	<0.001	1.948	0.245	<0.001	1.169
log PNN50 (%)	0.100	0.278	3.860			
log ASDNN (ms)	-0.008	0.946	5.753			
log SDANN (ms)	0.035	0.548	1.530			
log VLF (ms^2)	-0.026	0.781	3.985			
log HFnu (TP)	0.051	0.715	8.789			
log LF/HF	0.152	0.295	9.626			

Abbreviations as in Table 6 (MI-VT group). a=Variables by multiple linear regression with stepwise selection. ※ Logarithm of root mean square successive difference (log RMSSD) was removed because of multicollinearity.

4. Discussion

When the time of the worst LAS40 reading or nighttime (20:00) was used as the standard time of LP measurement over 24 h, the odds ratio for VT and the accuracy of the SAECG test were higher than for other Holter-based LP measurement times. The times of the worst fQRS and RMS40 readings were also candidates for the standard test times, although they were inferior in terms of odds ratio but not in terms of sensitivity, specificity, NPV, and PPV. Regarding factors influencing diurnal variation of LP parameters, multiple regression analysis revealed HR to be the factor that influenced diurnal variation of LP parameters in the MI-VT group the most. In the MI-non-VT group, LP parameters were significantly influenced by noise and by HRV markers of parasympathetic nervous activity such as logPNN50 and logLF/HF. In the control group, LP parameters were significantly influenced by noise and HR.

4.1. Optimal Measurement Timing of LP for Predicting VT

Amino et al. [14] reported the usefulness of Holter-based LP assessment as a predictor of rehospitalization in patients with post-MI status. They reported that a positive LP at the time of the worst fQRS value was significantly predictive of rehospitalization. In contrast, in the multicenter collaborative study (Janan Noninvasive Risk Stratification [JANIES] study [8], when a comparison was made between the predictive value of the readings at the times of the RMS40 worst and best levels, it was concluded that the LP's measured at the time of the worst RMS40 was more useful, with a higher risk hazard of 8.2 (p=0.003) for fatal arrhythmias in patients with MI. Our study compared the usefulness of all possible patterns for the first time. In direct terms, we compared the usefulness of readings at the times of the worst and best values of fQRS, RMS40, and LAS40, and of all mean values (Figure 1).

There have also been no comparative studies of optimal LP measurements according to the time of day. In general, the times of the worst fQRS, RMS40, and LAS40 readings often coincided; however, this was not always the case. The determination that the optimal timing for Holter-based LP measurement was when LAS40 was at its worst point was a novel finding. Although LP is an automatic measurement for some electrocardiography models, others require manual editing for Holter-based LP measurements,. in which case fQRS and RMS40 measurements can be influenced by the manual editing of onset and offset settings at the time of LP measurement, and this may cause

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bias among examiners. However, LAS40 assessment was not affected by manual editing of onset or offset settings. Therefore, using LAS40 to measure LP could help avoid bias among examiners.

4.2. Diurnal Variation of LP and Factors Influencing LP Values

It has been reported that LP varies diurnally in patients with MI [8,17,18] and healthy participants [12]. In both MI and healthy patients, the late potentials worsened at night and improved during the daytime. An important finding in our study is that each LP parameter in the MI-VT group showed diurnal variation around the cutoff values of the SAECG diagnostic criteria [16] (i.e., the mean values of fQRS, RMS40 and LAS40 were near 114 ms, 20 μV, and 38 ms, respectively) (Table 2). Therefore, it is key to consider diurnal variation as it relates to positive/negative LP determination. Factors reported to influence diurnal variation include HR [19], autonomic nervous system activity [20], body position [21], and physical activity [17,22]. Goldberger et al. [20] studied the effects of tilt, epinephrine, isoproterenol, beta-blockers, beta-blockers +atropine, and phenylephrine on LP in 14 healthy participants. The results showed that LP parameters improved with tilt-up and isoproterenol and worsened with epinephrine. Atrial pacing and atropine did not significantly change the LP parameters compared with those observed in healthy participants. In these results, the response to tilt is of interest. The results are consistent with our findings, suggesting that endogenous sympathetic tone and decreased parasympathetic activity may contribute to changes in LP parameters throughout the day. LPs were also influenced by HR, especially in the MI-VT and control groups after 24 h. Among LP parameters, fQRS and LAS40 are considered to be more influenced by HR than by RMS40 because bradycardia directly prolongs fQRS and LAS40. Yoshioka et al. [21] examined patients with Brugada syndrome and healthy participants and reported that LP parameters were influenced by body position. In our results, body position was not a significant influential parameter for LP parameters in any of the groups in the multivariate analysis (Table 6). However, this is the first demonstration of diurnal variations of LP parameters, including related to body position. Our results indicate that body position was not a significant factor influencing LP parameter values compared with autonomic activity, HR, or noise level in natural daily activities. Further investigations are required for other cardiac diseases.

4.3. Clinical Implications

Holter-based LP analysis requires the extraction of useful data from a large dataset. Therefore, its use to predict risk requires manpower and imposes a large time burden on cardiologists and technicians. Therefore, artificial intelligence (AI) support will be essential for future applications.

Recently, with AI technology advances, the accuracy of ECG analysis at the μV level has improved [23]. Based on data from the present study, it is possible to develop AI-supported clinical practices. We believe that this will lead to the widespread use of Holter-based LP assessment.

4.4. Limitations

This study had some limitations. First, the H-ECG recorder used (SpiderView®) did not have an accelerometer. Although consistency of body position was confirmed to some extent using the activity record card, this assessment was not precise, and information on body position in this study may not necessarily be accurate. Second, the study results only provide data for risk stratification of fatal arrhythmias in patients with post-MI status. Therefore, it should be applied clinically with caution as it may not be indicated in other cardiac diseases such as Brugada syndrome, ARVC, dilated cardiomyopathy, or heart failure.

5. Conclusions

In the Holter-based LP measurement, when the time of the LAS40 worst value or nighttime (20:00) was used as the standard value for predicting VT, both the odds ratio and accuracy of the SAECG test were the highest in patients with MI. In contrast, time points taken when the fQRS and RMS40 were at their worst were also candidates as LP measurement time points. The involvement of

the autonomic nervous system, including the HR or noise level, has been suggested as a factor influencing patients with post-MI status and healthy control participants.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org. Figure S1: Study population.

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