

Relation of Various Parameters Used to Estimate Cardiac Vagal Activity and Validity of pNN50 in Anesthetized Humans

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Objectives: Analysis of heart rate variability (HRV) has been used as a measure of cardiac autonomic function. According to the pNN50 statistic, the percentage of differences between successive normal RR intervals (RRI) that exceed 50 ms, has been known to reflect cardiac vagal modulation. Relatively little is known about the validity of pNN50 during general anesthesia (GA). Therefore, we evaluated the correlation of pNN50 with other variables such as HF, RMSSD, SD1 of HRV reflecting the vagal tone, and examined the validity of pNN50 in anesthetized patients. **Methods:** We assessed changes in RRI, pNN50, root mean square of successive differences of RRI (RMSSD), high frequency (HF) and standard deviation 1 (SD1) of Poincaré plots after GA using sevoflurane anesthesia. We also calculated the probability distributions for the family of pNNx statistics (x: 2-50 ms).

Results: All HRV variables were significantly decreased during GA. HF power was not correlated with pNN50 during GA ($r = 0.096$, $P = 0.392$). Less than pNN47 was shown to have a correlation with other variables.

Conclusions: These data suggest that pNN50 can not reflect the level of vagal tone during GA.

Key Words: General anesthesia, Heart rate variability, pNN50, Vagal tone

Heart rate variability (HRV) is widely used as a method of evaluating the effects of sympathetic and parasympathetic nerves in the sinoatrial node. Analyzing the information according to heart rate change helps determine the cardiovascular morbidity and prognosis in heart failure, myocardial infarction, diabetes cardiovascular diseases, as well as normal conditions.¹ Through frequency do-

main and time domain analysis, factors such as high frequency (HF), low frequency (LF), RMSSD (root mean square of successive differences of RR intervals), and pNN50 (proportion of successive RR intervals > 50 ms in relation to total RR intervals) can be obtained.¹⁻³ The clinical significance of each factor is as follows.

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(1) High-frequency band (HF)

The high-frequency band is a frequency band between 0.15 ~ 0.4 Hz. It is also referred to as respiratory arrhythmia associated with heart rate variability connected with the respiratory cycle. This is controlled by the activity of the parasympathetic nervous system.³³

(2) Low frequency (LF)

The low-frequency band is the frequency band between 0.04 Hz ~ 0.15 Hz and is controlled by both the sympathetic and parasympathetic nervous systems.³³

(3) RMSSD (Root mean square of successive differences of RR intervals)

This variable is a good indicator of short-term heart rate variability and indicates the degree of parasympathetic activity.^{31,32} Investigation of the actual correlation between each of these factors was conducted to a limited extent, and a correlation study under anesthesia is rare.

The time domain, pNN50, is the % of the total RRI in which the difference between two consecutive RR intervals (RRI) is greater than 50 ms, which reflects the activity of the vagus nerve and is also known to be correlated with HF in the frequency domain HF.^{1,4} Ewing et al. reported that⁵ pNN50 was the most sensitive among the various tests that could initially diagnose parasympathetic nerve damage in the heart.

However, some studies reported using thresholds other than 50 ms.⁶⁻⁸ Mietus et al.⁹ found that pNN50 is one of several factors that represent HRV and introduced the concept of pNNx (x < 50 ms) with consecutive RRI differences of less than 50 ms, which makes it possible to clarify the difference between normal or pathologic conditions more clearly than pNN50.

General anesthesia using inhalation anesthetics is known to suppress the autonomic nerves and reduce HRV in a dose-dependent manner.^{10,11} These HRVs are affected by anesthetic depth, medication administered, the operation of the surgical site, and body temperature of the patient. The HRV may also be used as an indicator of anesthesia depth.^{12,13} Therefore, the frequency of HRV analytical method use is gradually increasing as an aid for determining the function of the autonomic nervous system during the administration of general or regional anesthesia to the anesthesia site. Parasympathetic dysfunction, in particular, is considered to be the cause of death or ventricular arrhythmia independently in patients with heart failure.^{14,15} Therefore, it is important to evaluate the parasympathetic nervous dysfunction by inhalation anesthesia during anesthesia.

However, unlike other factors of HRV, few studies have examined whether pNN50 or pNNx reflect cardiac vagal activity during anesthesia and what changes are reported during general anesthesia by inhalation anesthesia.

In previous studies, HF, RMSSD, SD1, and pNN50 were found to be significantly reduced in patients compared to those who were awake during general anesthesia under isoflurane and sevoflurane 1 minimum alveolar concentration (MAC). pNN50, in particular, measured zero in both groups.¹⁶ Thus, it was considered necessary to study the usefulness of pNN50 during general anesthesia.

Therefore, studies on whether the correlation between pNN50 and other HRV measurements known to reflect cardiac vagal activity during general anesthesia were maintained. If this correlation was not maintained, further investigation was conducted to determine whether the introduction of the pNNx concept by Mietus et al. was necessary during anesthesia.

MATERIALS AND METHODS

Eighty patients with American Society of Anesthesiologists physical status classification ASA 1 and 2 were selected as target patients. Those with diseases that could cause abnormalities in the autonomic nervous system such as cardiovascular, diabetes, and nervous system were excluded from the study. The study was conducted after approval from the Hospital Ethics Committee. All patients fasted prior to surgery and were forbidden from consuming alcohol and caffeinated beverages for more than 24 hours. As

a preoperative treatment, 7.5 mg of midazolam was administered orally 90 minutes before inducing anesthesia.

After the patient entered the operating room and lay down in the supine position, the electrocardiogram was taken and connected to the monitor. A stable electrocardiogram was recorded for 5 minutes in the patient's spontaneous breathing state after 20 minutes to adapt to the operating room environment. Anesthesia was induced with 5 mg/kg thiopental, 1 μ g/kg fentanyl, and 0.1 mg/kg vecuronium for muscle relaxation. After endotracheal intubation, the mechanical ventilation changed the breathing frequency to 15 times/minute (0.25 Hz) and the ventilation was adapted to maintain the end-tidal CO₂ concentration to 35–40 mmHg. Maintenance anesthesia was performed with a sevoflurane and 50% oxygen-nitrous oxide mixture and muscle relaxation was performed with vecuronium. Prior to the operation, the end-tidal sevoflurane concentration was 1 MAC for a stabilization period of 10 minutes. Subsequently, an electrocardiogram of 5 minutes was obtained. Each beat of the electrocardiogram was digitized through an analog-to-digital converter (DI-720U, DATAQ Instruments, USA) and stored at 500 Hz using a computer-programmed signal conversion program (CODAS, DATAQ Instruments; DADiSP, DSP Development, USA).

RRI was determined as the difference between the peak R of each beat. The RRI obtained was

analyzed using an HRV analysis software (version 1.1, Biomedical signal analysis group, Finland).

The method of obtaining each measurement value was initiated with the calculation of the time domain HRV analysis, pNN50 (%), and RMSSD (root mean square of successive difference of RRI, millisecond, ms). NNx is defined as the difference between consecutive RRIs of x ms (x: 2 - 50 ms) or more, and pNNx is defined as the % of NNx for the total RRI. In the case of consciousness, pNN50 and RMSSD are known to be highly correlated with HF of HRV.¹⁷

Frequency-domain HRV analysis was conducted by using a power spectral analysis method using the Welch periodogram averaging algorithm. To summarize, a 300-second RRI is interpolated at 5 Hz to obtain equidistant time intervals, followed by obtaining 5 segments of 100 seconds overlapping every 50 seconds. Then detrended, Hanning filtered and fast Fourier transform are implemented to convert the frequency domain of HRV, which has a resolution of 0.01 Hz. The spectrum was obtained by averaging 5 periodograms. The spectrum was defined as low frequency (0.04-0.15 Hz) and high frequency (0.15-0.5 Hz) and its value was obtained. In addition, amongst the overall spectrum, the following were calculated: the LF fraction of nuLF (normalized unit of LF, $LF/[LF + HF]$), the HF fraction of nuHF (normalized unit of HF, $HF/[LF + HF]$), and the LF/HF ratio.

The Poincaré plot can measure the activity of the autonomic nerves of the heart by viewing the distribution of adjacent RRI. This facilitates the diagnosis of abnormal heartbeats and enables the quantitative analysis of HRV.^{18,19} Three measurements of the standard deviation (SD) of the instantaneous beat-to-beat RRI variability (SD1), the SD of the long-term RRI variability (SD2), and the axis ratio (SD1/SD2)¹⁹ can be obtained. As SD1, in particular, is known to reflect the cardiac vagal activity,²⁰ it was calculated in this study.

All values were a mean \pm standard deviation. To evaluate the effect of the HRV inhalation anesthesia, RMSSD, SD1, and LF HF, which did not follow the normal distribution except pNN50, were subjected to a paired test for changes before and after anesthesia after using natural logarithmic transformation. A correlation analysis was conducted using the Pearson method of each variable and $P < 0.05$ was considered statistically significant. Statistical analysis was performed using Sigma stat version 3.10 (Systat Software, USA).

RESULTS

Demographic data of patients who participated in the study are shown in Table 1. Table 2 shows changes in HRV variables due to general anesthesia and shows a significant decrease ($P < 0.001$) in pNN50, RMSSD, HF, and SD1 except nuHF by anesthesia, and RRI itself does not show any sig-

Table 1. Demographic Data

ASA score (1/2)	80 (48/32)
Age (yr)	45.7 ± 11.9
Sex (M/F)	80 (50/30)
Body weight (kg)	74.2 ± 30.5
Height (cm)	158.9 ± 28.9

Values are mean ± SD or number of patients.

Table 2. Descriptive Statistics of Indices Derived from Heart Rate Variability

Variables	Before GA	After GA
Time-domain		
RRI, ms	846.1 ± 135.8	818.8 ± 155.4
pNN50, %	11.42 ± 16.49	0.00 ± 0.03
Ln RMSSD, ms	3.18 ± 0.63	1.65 ± 0.54
Frequency-domain		
Ln LF, ms ₂	9.13 ± 0.89	5.01 ± 1.22
Ln HF, ms ₂	8.14 ± 1.26	4.82 ± 1.24
nuLF, (nu)	0.71 ± 0.15	0.54 ± 0.21*
nuHF, (nu)	0.29 ± 0.15	0.46 ± 0.21*
LF/HF ratio	3.32 ± 2.18	1.97 ± 2.22
Nonlinear method (Poincaré plot)		
Ln SD1(ms)	2.82 ± 0.62	1.31 ± 0.56

Values are mean ± SD. GA: general anesthesia, RRI: RR intervals, pNN50: proportion of successive RRI differences > 50 ms in relation to the total RRI, Ln RMSSD: natural logarithmic-transformed of root mean square of successive differences of RRI, Ln LF: natural logarithmic-transformed of low frequency, Ln HF: natural logarithmic-transformed of high frequency, nuLF: normalized unit of low frequency, nuHF: normalized unit of high frequency, LF/HF ratio: ratio between low frequency and high frequency, Ln SD1: natural logarithmic-transformed of standard deviation 1 from the Poincaré plots analysis. * $P < 0.05$, † $P < 0.001$ versus before state.

nificant change.

pNNx was significantly reduced by anesthesia (Fig. 1). Prior to anesthesia, NN50 was observed in 77% of patients and NN34 was observed in 90% of patients. By general anesthesia, however, NN50 was 0 in all patients except for one case, whose results were 26.8% in NN30, 41.8% in NN20 and 47.8% in NN15, respectively.

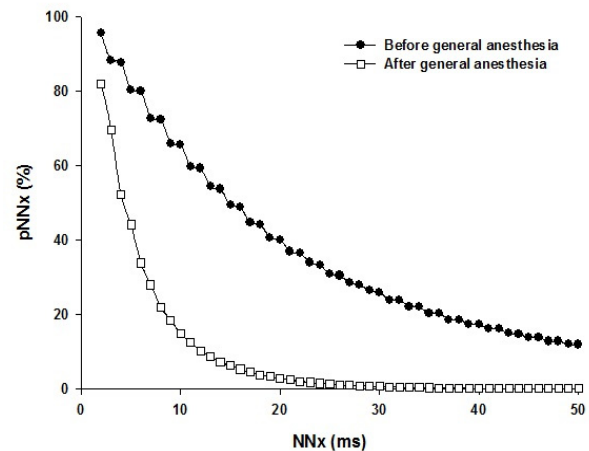


Fig. 1. Mean pNNx distributions. During general anesthesia, pNNx is significantly lower than before general anesthesia state and nearly zero at greater than 30 ms. pNNx: proportion of successive RR intervals differences > 2 – 50 ms in relation to total RR intervals ($x = 2 - 50$ ms).

Ln HF (Ln: natural logarithmic), Ln RMSSD, and Ln SD1 showed a significant correlation before and after anesthesia (before anesthesia: LnHF-Ln RMSSD; $r = 0.958$, $P < 0.001$, Ln HF-Ln SD1; $r = 0.966$, $P < 0.001$, after anesthesia: Ln HF-Ln RMSSD; $r = 0.945$, $P < 0.001$, Ln HF-Ln SD1, $r = 0.944$, $P < 0.001$). However, pNN50 was correlated with Ln HF before anesthesia ($r = 0.825$, $P < 0.001$), but the correlation disappeared when general anesthesia was administered (Fig. 2). The correlation between pNN50 and Ln RMSSD was $r = 0.861$, P

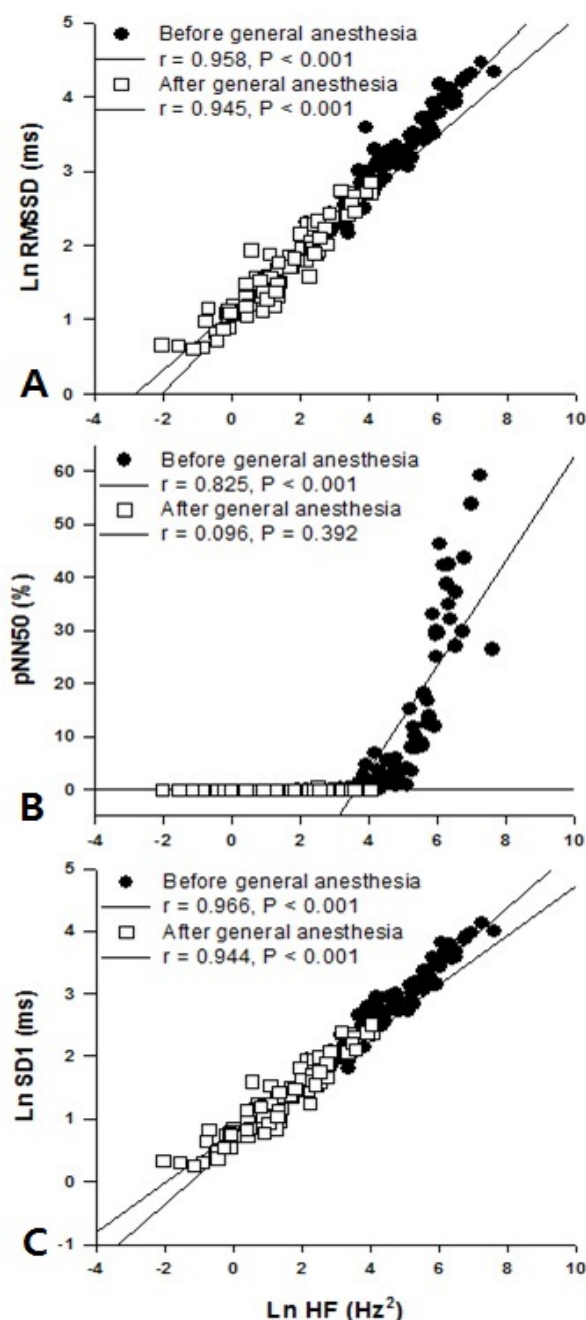


Fig. 2. Correlations between RMSSD, pNN50, SD1 and LnHF. The effect of general anesthesia. Ln RMSSD and Ln SD1 are significantly correlated with Ln HF at before and after general anesthesia state. Note that pNN50 is not correlated with Ln HF during general anesthesia. Ln HF (ms^2): natural logarithmic transformed of high frequency power of heart rate variability, Ln RMSSD (ms): natural logarithmic transformed of root mean square of successive differences of RR intervals, pNN50 (%): proportion of successive RR intervals differences > 50 ms in relation to the total RR intervals, Ln SD1 (ms): natural logarithmic transformed of standard deviation 1 from the Poincaré analysis.

< 0.001 before anesthesia, but the correlation disappeared during anesthesia and similar results were obtained with Ln SD1 ($r = 0.861, P < 0.001$ before anesthesia, $r = 0.134, P = 0.229$ after anesthesia). There was no correlation between pNNx and each factor in NN 47-50 under general anesthesia and the correlation coefficient was over 0.4 in NN less than 30 ms (pNN30 - Ln HF $r^2 = 0.35$, pNN30 - RMSSD and SD1 $r^2 = 0.41$) (Fig. 3).

DISCUSSION

The purpose of this study was to investigate the correlation of various factors reflecting cardiac vagal activity by general anesthesia and to evaluate the usefulness of pNN50 to reflect vagal activity. As a result, pNN50 is rarely observed in general anesthesia, unlike other factors that show parasympathetic activity. Moreover, the correlation between HF, RMSSD, and SD1 and pNN50 of frequency variation is lost post-anesthesia, unlike pre-anesthesia. That is, from pNN50 to pNN47 there was no correlation with each factor, suggesting that pNN50 does not exhibit vagal activity during anesthesia.

HRV is used as a basis for evaluating cardiovascular morbidity not only in healthy adults but also in patients with diabetes and heart failure. HRV is known to be closely related to the renin-angiotensin system, body temperature, and respiration in addition to the autonomic nervous

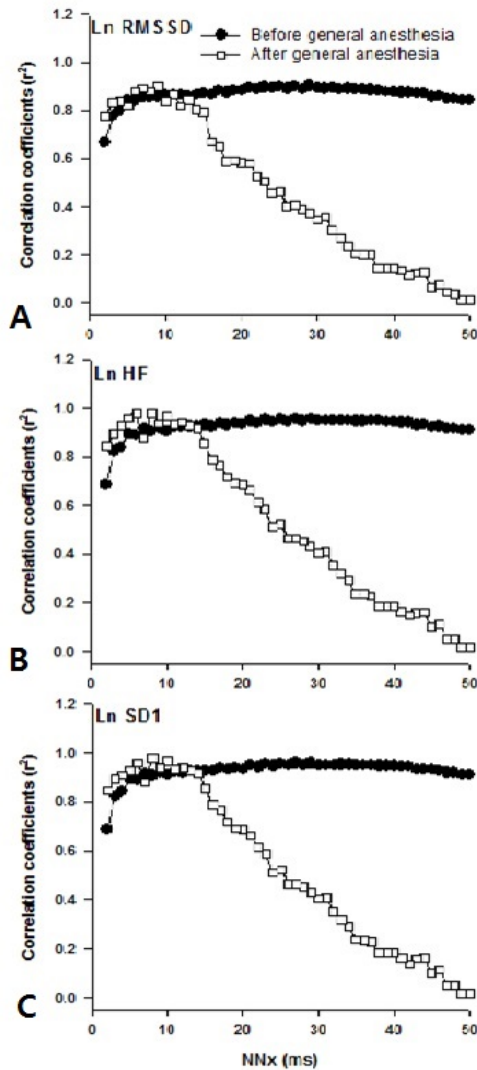


Fig. 3. Correlations of Ln RMSSD (A), LnHF (B), and Ln SD1 (C) for pNNx. pNN values greater than 47 ms are not correlated with other three parameters. Ln RMSSD (ms): natural logarithmic transformed of root mean square of successive differences of RR intervals, LnHF (ms²): natural logarithmic transformed high frequency power of heart rate variability, Ln SD1 (ms): natural logarithmic transformed of standard deviation 1 from the Poincaré analysis, NNx (ms): successive RR intervals differences $> x$ ms ($x = 2 - 50$ ms).

system.^{1,21}

The parasympathetic nervous system is the reaction of a fast sinus nodule in a short period of time and has been associated with respiratory sinus arrhythmia (RSA). The sympathetic nerve

has a 10-second cycle of Mayer waves and the renin-angiotensin system has a slower and longer cycle time affecting the heart rate.²² As previously mentioned, the evaluation method of HRV uses methods such as time and frequency domain and analysis of the Poincaré plot through the distributed shape of the non-linear consecutive RRI.^{1,19}

Autonomic insufficiency of the sinus nodule can cause sudden death in patients with heart failure. Parasympathetic dysfunction, in particular, increases the incidence of death or life-threatening ventricular arrhythmia in patients with heart failure. It has also been reported that the activation of vagal nerves in these patients stops ventricular tachycardia.^{14,15,23} Because the autonomic nervous system hypofunction, including parasympathetic nerves, is present during anesthesia, an accurate assessment of HRV can be used as an indicator of the balance or failure of the autonomic nervous system during anesthesia.²⁴

In this study, general anesthesia showed a significant decrease in HRV factors such as HF, RMSSD, pNN50, and SD1 except for nuHF, which can be explained by a decline in autonomic nervous system functionality. All inhaled anesthetics have a dose-dependent decrease in HRV. They are also known to affect the autonomic nervous system in addition to anesthetics during general anesthesia, as well as the operation itself and the body temperature.^{12,13,25} All of the HRV factors ex-

cept nuHF were significantly decreased during isoflurane and sevoflurane anesthesia. The pNN50 value was not measured in both groups except for 1 patient,¹⁶ which was consistent with the study results. The increase of nuHF during general anesthesia seems to be less than that of LF due to mechanical ventilation rather than an increase of vagal nerve activity. Considering the effect of the administered drugs on HRV, the use of thiopental resulted in a decrease in all frequency domain values of HRV. This was especially apparent in HF rather than LF, which is due to the vagal suppression effect of barbiturate.¹³ Though related to the parasympathomimetic effect, primarily on the parasympathetic nerves, fentanyl does not change the balance of the autonomic nervous system.^{26,27} Midazolam 0.3 mg/kg reduced all spectra of HRV and dose-dependently had great resistance from sympathetic nerves at lower doses and from parasympathetic nerves at higher doses.²⁸ In this study, pNN50 appeared in only 62 patients before the anesthesia, which is thought to be the effect of midazolam prior to operation.¹³ In this study, concurrent use of midazolam and sevoflurane seemed to deliver a synergistic effect of autonomic nervous suppression. However, because the same anesthetic agent was used in the same environment, there was no difference in HRV value due to the anesthetic agent.

The HRV measurements reflecting the parasympathetic nervous system are known as HF in the frequency domain, RMSSD in the time domain,

pNN50, and SD1 in Poincaré plot.^{1,19} These factors are useful for evaluating cardiac vagal activity by HRV in normal conditions and are known to show a significant correlation with each other. In addition, Bigger et al.²¹ reported that pNN50, RMSSD, and HF of HRV were correlated with each other by 0.9 or higher after acute myocardial infarction. However, no studies have investigated whether there is any correlation between the factors that reflect these vagal activities during anesthesia. pNN50 is known to reflect the activity of cardiac-vagal nerves as the HRV factor in the time domain as calculated from the differences in successive RRI.¹⁷ This was first cited by Ewing et al.⁵, and is reported facilitate in the diagnosis and prognosis of patients with various clinical conditions.^{1,21} However, Mietus et al.⁹ reported that by using the RRI difference of less than 950 ms, information on the heart rhythm dynamics in the pathological state, as well as in normal conditions, can be obtained, which is closely related to the control of parasympathetic nerves. However, Hutchinson²⁹ reported no significant difference in the ability to distinguish between pNN20 and pNN50. Further, there was no evidence that pNN20 was more beneficial. With respect to the controversial result of the present study, pNN50 did not appear in most patients and the correlation with HF was lost in general anesthesia using inhalation anesthetics. Moreover, using a threshold value of less than 47 ms showed a significant correlation with HF, RMSSD, and SD1, and Ln HF and r2 were 0.35 at 30 ms, and

0.41 for RMSSD and SD1, respectively. The values were measured in 20% or more patients, and the correlation coefficients were 0.58 and 0.69 for 20 ms, respectively. The values were measured in 46% of patients. Thus, during general anesthesia, pNN50 appears to be insignificant in reflecting cardiac vagal activity. However, further studies are needed to determine whether the clinical significance of using intervals other than that of 50 ms or other specified values is most useful.

Unlike other studies, one limitation of this study was that an electrocardiogram of 5 minutes was analyzed. Although a 24-hour electrocardiogram analysis is used to compare HRV parameters, similar information is often presented in short-term records, and short-term records are used in most studies.^{4,21} Perkiomaki et al.³⁰ reported that short-term records reflect the results of long-term records even in non-linear HRV analysis, such as Poincaré plot analysis. Therefore, an electrocardiogram of 5 minutes also provides important information in HRV analysis. It is also necessary to increase the vagal activity during anesthesia by administering a drug such as pyridostigmine, which suppresses cholinesterase and stimulates cholinergic. This method enables the observance of the correlation between the changes of each factor reflecting the vagal activity including pNN50 in the future. It is possible to inhibit ventricular tachycardia induced by electrical stimulation through cholinergic stimulation. For pa-

tients with heart failure, administration of 30 mg pyridostigmine 3 times daily for 2 days reduces the heart rate and the frequency of ventricular tachycardia in a short period of time and increases pNN50 of HRV and RMSSD. The results, however, did not differ from the placebo effects in the function on echocardiography. In conclusion, pNN50 is not observed in most patients during anesthesia and it is difficult to determine whether pNN50 reflects the activity of cardiac parasympathetic nerves under general anesthesia because the correlation between HRV and HF is lost. According to the results of this study, using a value of less than 47 ms improves the correlation with other HRV variables. However, it is still necessary to investigate the clinical significance of the numerical use of less than 47 ms.

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