Research Article

Utility of the amplitude of RV$_1$+SV$_{5/6}$ in assessment of pulmonary hypertension

Sachiyo Igata$^1$*, Nobuhiro Tahara$^1$*, Yoichi Sugiyama$^1$, Munehisa Bekki$^1$, Jun Kumanomido$^1$, Atsuko Tahara$^1$, Akihiro Honda$^1$, Shoko Maeda$^1$, Kazutaka Nashiki$^2$, Tomohisa Nakamura$^1$, Jiahui Sun$^1$, Toshi Abe$^2$, Yoshihiro Fukumoto$^1$

1 Division of Cardiovascular Medicine, Department of Medicine, Kurume University School of Medicine, Kurume, Japan, 2 Department of Radiology and Center for Diagnostic Imaging, Kurume University School of Medicine, Kurume, Japan

* sachyoiga@gmail.com (SI); ntahara@kurume-u.ac.jp (NT).

Abstract

Electrocardiogram (ECG) has been widely used for assessment of right ventricular (RV) hypertrophy (RVH) in patients with pulmonary hypertension (PH). However, it still remains unclear which ECG criteria of RVH are useful to predict for the severity of PH. The aim of our study was to examine the utility of ECG findings of RVH in PH patients. A total of 53 patients (42 women, mean age; 57.6 ± 16.4 years) with pre-capillary PH, who were diagnosed by right heart catheterization, underwent blood sampling, ECG, and cardiac magnetic resonance within a week before the right heart catheterization. We assessed the traditional ECG criteria of RVH in PH patients, and compared to age- and gender-matched control subjects without PH confirmed by 2-dimensional echocardiography ($n = 42$, mean age 55.3 ± 15.9 years). We also analyzed the clinical variables associated with ECG findings in patients with PH. Mean pulmonary arterial pressure (mPAP), cardiac index, and pulmonary vascular resistance (PVR) in PH patients were 35.3 ± 11.9 mmHg, 2.82 (2.09–3.45) L/min/m$^2$, and 576 ± 376 dyne·sec·cm$^{-5}$, respectively. The prevalence of right axis deviation (43.4%), R:S ratio $V_1 > 1$ (32.1%), and RV$_1$+SV$_{5/6}$ > 10.5 mm (69.8%) in PH patients was greater than those in control subjects ($p < 0.001$). In univariate analysis, mPAP, PVR, RV wall thickness, RV mass index, RV volume, and RV ejection fraction (EF) (inversely) were significantly correlated with the amplitude of RV$_1$+SV$_{5/6}$. Multiple regression analysis revealed that mPAP and RVEF (inversely) were independently associated with the amplitude of RV$_1$+SV$_{5/6}$ ($R^2 = 0.282$). Also, we performed the survival analysis among pre-capillary PH patients. During a mean follow-up of 3.7 years, patients with $\geq 16.4$ mm of RV$_1$+SV$_{5/6}$ had worse prognosis than those with $< 16.4$ mm (Log rank $p = 0.015$). In conclusion, the amplitude of SV$_1$+RV$_{5/6}$ could be the most useful factor reflected for RV remodeling, hemodynamics and survival in patients with pre-capillary PH.
Introduction

Pre-capillary pulmonary hypertension (PH) is a progressive disease characterized by increased pulmonary vascular resistance (PVR), which causes right ventricular (RV) remodeling such as hypertrophy and/or enlargement [1, 2], ultimately resulting in right heart failure and death [3, 4]. Therefore, accurate assessment of RV remodeling is important to evaluate the disease severity in patients with pre-capillary PH.

Although a 10-year follow-up study in patients with pre-capillary PH demonstrated that patients with mean PAP (mPAP) \( \geq 42.5 \) mmHg showed worse survival rates than those with mPAP < 42.5 mmHg [5], patients with pre-capillary PH can present the different courses of RV remodeling according to the disease severity; one is adaptive remodeling, and the other is maladaptive. RV hypertrophy (RVH) is initially an adaptive physiological response to increased overload. Adaptive remodeling is characterized by the increased RV wall thickness/mass and the preserved RV function, whereas maladaptation is related to the enlarged RV and the reduced RV function [6]. If the overload persistently continues, adaptive remodeling transitions to maladaptive remodeling. RV function has a significant impact on the prognosis of PH [7]. Reduced RV ejection fraction (RVEF) less than 25–35% is a prognostic factor for worse outcome among the PH patients [8, 9]. Especially, RVH is one of the triggers of RV dysfunction in PH. Although echocardiography and cardiovascular magnetic resonance (CMR) imaging are established for assessment of the RVH and RV function [10, 11], a low-cost equipment is required.

Guidelines from the American Heart Association, the American College of Cardiology Foundation and the Heart Rhythm Society had indicated ECG criteria for diagnosis of RVH [12]. Although ECG criteria have been widely used for screening of RVH, they often remain challenging to precisely evaluate RV remodeling in PH [13, 14]. Though there are many ECG studies on PH, ECG criteria of RVH have low sensitivity and low specificity for diagnosis of PH [12, 15]. The 2015 European Society of Cardiology/European Respiratory Society guidelines recommend the utility of right heart catheterization (RHC) and CMR for diagnosis and severity of PH [3]. However, the association between ECG findings and clinical variables including both RHC and CMR in PH are not fully investigated yet. Also, it is unknown whether the ECG parameter in PH patients can be a prognostic factor. Therefore, in the current study, we aimed to examine the utility of ECG findings of RVH in assessment of PH.

Materials and methods

Participants

This study included 53 consecutive patients with pre-capillary PH, who were diagnosed by right heart catheterization in Kurume University Hospital from January 2013 to February 2016. Pre-capillary PH was defined as a mPAP \( \geq 25 \) mmHg, pulmonary arterial wedge pressure (PAWP) \( \leq 15 \) mmHg, and PVR \( \geq 240 \) dyne·sec·cm\(^{-5}\) at rest. All patients underwent blood sampling, ECG, and CMR within a week before the right heart catheterization. The endpoint for survival analyses was defined as all cause of death until July 2018. During a follow-up period, lung transplantation and death were defined as all cause of death. Forty-two age- and gender-matched control subjects without PH confirmed by 2-dimensional echocardiography, who received blood sampling and ECG due to non-fatal arrhythmias, were also enrolled. The study was conducted in accordance with ethics guidelines introduced by the Declaration of Helsinki and was approved by the Ethics Committee of Kurume University. All subjects provided written informed consent.
Electrocardiography

A 12-lead ECG (10 mm = 1 mV, 25 mm/s) was acquired in a supine position during quiet respiration (ECG-1550; NIHON KOHDEN, Fukuoka, Japan). ECG findings including heart rate, frontal QRS axis, and amplitude of P, R, and S waves were assessed. Amplitude of P, R, and S waves was averaged from 3 consecutive cardiac cycles. The interpreter for ECG findings was blind to patients’ clinical information. We decided 10 ECG criteria of RVH based on the 2009 AHA/ACCF/HRS guideline and Murphy’s study [12, 15].

Hemodynamic measurements

The diagnosis of pre-capillary PH was confirmed by hemodynamic evaluation with right heart catheterization at rest. Hemodynamic measurements were performed with a Swan-Ganz catheter (Baxter Healthcare Corporation, Santa Ana, CA, USA) in the recumbent position. Cardiac output (CO) was determined using the Fick’s method. The cardiac index (CI) was derived by normalization of CO with the body surface area (BSA): CI = CO/BSA. PVR was calculated from the transpulmonary gradient and CO: PVR = 80 × [mPAP–PAWP] ÷ CO.

Blood sampling

After overnight fast, peripheral blood was drawn from the antecubital vein for measurements of blood cell counts, lipid profiles, liver and renal function markers, glycemic parameters, uric acid, and N-terminal pro-brain natriuretic peptide (NT-pro-BNP). These chemistries were measured at a commercially available laboratory in Kurume University Hospital.

CMR imaging

ECG-gated CMR imaging was performed with a standardized clinical protocol on a 3.0-T system (MAGNETOM Skyra; Siemens, Erlangen, Germany). To quantify ventricular end-systolic volume, end-diastolic volume, stroke volume, mass index and ejection fraction (EF), two experienced radiologists semi-automatically traced the ventricular endocardial and epicardial contours in the end-systolic and end-diastolic frames of transaxial slices using dedicated software. Cardiac volume was corrected for BSA.

Echocardiography

Echocardiogram was performed using the commercially available ultrasound units, General Electric Vivid 7 (GE Medical Systems, Milwaukee, WI) by Japanese registered sonographer. All echocardiographic parameters were calculated according to the American society of echocardiography guideline [16].

Statistical analysis

Data were presented as mean ± standard deviation or medians with the interquartile range. The Shapiro-Wilk test was performed to evaluate the assumption of normality. Statistical analysis was performed by means of appropriate parametric and nonparametric methods. Unpaired Student t test was performed for comparisons between PH patients and control subjects. Chi-square test was used for categorical variables. Pearson correlations were used to compare between ECG findings and clinical factors. The determinants for RVH criteria were identified by multivariate regression analysis. Receiver-operator characteristics plotting was performed to identify the cut-off value of RV1+SV56 or RV1 for severe PH with mPAP ≥ 42.5 mmHg and/or RVEF < 35%, which are associated with poor outcome. Survival analyses were performed using the Kaplan-Meier method and the log-rank test. The relationship between
survival and selected variables was analyzed with the Cox proportional hazards model for all-cause mortality adjusted by the age and gender. Values of \( p < 0.05 \) were considered to indicate statistical significance. All statistical analyses were performed with the use of the SPSS system (IBM, Chicago, IL, USA).

**Results**

**Patient characteristics**

Table 1 presents clinical characteristics of 53 patients with pre-capillary PH and 42 control subjects. Thirty-five (66.0%) with pulmonary arterial hypertension (PAH), 12 (22.6%) with PAH coexisting pulmonary disease, and 5 (9.4%) with CTEPH patients were enrolled in the present study. The mean age was 57.6 ± 16.4 years and female predominance (79.2%) was observed in PH patients. Patients with pre-capillary PH had been treated with home oxygen therapy [26 (49.1%)], anticoagulation therapy [27 (50.9%)], diuretics [23 (43.4%)], and/or PH specific therapies consisting of prostacyclin analogs [15 (28.3%)], phosphodiesterase type 5 inhibitors (PDE5-Is) [21 (39.6%)], endothelin receptor antagonists (ERAs) [21 (39.6%)], and soluble guanylate cyclase stimulators [1 (1.9%)]. Under these treatments, WHO functional classification was predominantly class II (26.4%) and class III (62.3%). Systolic pulmonary artery pressure (sPAP), mPAP, cardiac index, PVR, and mean right atrial pressure were presented with 57.6 ± 20.8 mmHg, 35.3 ± 11.9 mmHg, 2.82 (2.09–3.45) L/min/m², 576 ± 376 dyne·sec·cm⁻⁵, and 5.0 (3.0–8.0) mmHg, respectively. CMR revealed RV free wall thickness of 3.7 (3.1–4.4) mm and RVEF of 36.9 ± 9.6%. LV wall thickness and LVEF were not significantly different in both groups (\( p > 0.05 \)).

**ECG findings**

All PH patients and control subjects were in sinus rhythm with an average heart rate of 72.4 ± 14.3 beats/min. Patients with pre-capillary PH had a significantly greater prevalence of high R wave amplitude in lead V₁, deep S wave amplitude in lead V₅, high R:S ratio in lead V₁, low R:S ratio in lead V₅, right axis deviation, and high R wave amplitude in lead aVR than control subjects (Table 2).

Table 3 shows the prevalence of ECG criteria for RVH in PH patients and control subjects. RVH criteria such as RV₁+SV₅/₆ ≥ 10.5 mm, SV₅ ≥ 7 mm, QRS axis ≥ 90˚, and RV₁ ≥ 7 mm were frequently observed [37 (69.8%), 28 (52.8%), 23 (43.4%), and 18 (34.0%), respectively] in our PH patients. However, each ECG criterion of RVH was not enough to diagnose the RVH in pre-capillary PH. Of all ECG changes of RVH, the amplitude of R wave in lead V₁ plus S wave in lead V₅/₆ was the most frequent finding in our PH patients.

**Determinants of clinical variables for ECG findings**

Table 4 shows correlations between the clinical variables and ECG parameters. The QRS axis was significantly correlated with NT-pro-BNP (\( r = 0.316, p < 0.05 \)). There was no significant correlation between uric acid and ECG findings. In univariate analyses, the amplitude of R in lead V₁ and RV₁ + SV₅/₆, R in lead aVR, or QRS axis was significantly correlated with pulmonary hemodynamics (\( p < 0.01 \)). Also, the amplitude of R in lead V₁ or RV₁ + SV₅/₆ was correlated with CMR parameters including RV free wall thickness (RVWT) and RV mass index (RVMI) (\( p < 0.05 \)), RV end-systolic volume index (ESVI) (\( p < 0.05 \)), RV end-diastolic volume index (EDVI) (\( p < 0.05 \)), RVEDVI/left ventricular EDVI (\( p < 0.05 \)), and RVEF (inversely, \( p < 0.05 \)). Because these significant parameters could be closely correlated with each other, we performed multiple stepwise regression analyses to determine independent associates of the
Table 1. Characteristics of study population.

| Parameters                | Control subjects | Patients with pre-capillary PH | p-value |
|---------------------------|------------------|-------------------------------|---------|
| Number                    | 42               | 53                            |         |
| Age                       | 55.3 ± 15.9      | 57.6 ± 16.4                   | 0.493   |
| Female, n (%)             | 29 (69.0%)       | 42 (79.2%)                    | 0.256   |
| Cause of PH               |                  |                               |         |
| IPAH                      | -                | 12 (22.6%)                    |         |
| CTD-PAH                   | -                | 15 (28.3%)                    |         |
| CHD-PAH                   | -                | 4 (7.5%)                      |         |
| Pulmonary disease         | -                | 12 (22.6%)                    |         |
| CTEPH                     | -                | 5 (9.4%)                      |         |
| Others                    | -                | 5 (9.4%)                      |         |
| Systolic blood pressure, mmHg | 128.8 ± 22.8 | 118.7 ± 21.0                 | 0.030   |
| Heart rate, bpm           | 65.9 ± 10.6      | 77.5 ± 14.8                   | < 0.001 |
| Pulmonary hemodynamics    |                  |                               |         |
| sPAP, mmHg                | -                | 57.6 ± 20.8                   |         |
| mPAP, mmHg                | -                | 35.3 ± 11.9                   |         |
| PVR, dyne sec cm⁻³        | -                | 576 ± 376                     |         |
| CI, L/min/m²              | -                | 2.82 (2.09-3.45)              |         |
| mRAP, mmHg                | -                | 5.0 (3.0-8.0)                 |         |
| 6MWD, m                   | -                | 374 ± 142                     |         |
| CMR                       |                  |                               |         |
| RVEDVI⁻¹, mL/m²           | -                | 63.9 (51.8-93.5)              |         |
| RVESVI⁻¹, mL/m²           | -                | 38.7 (29.0-55.2)              |         |
| RVMI, g/m²                | -                | 36.5 (27.9-41.8)              |         |
| RVEF, %                   | -                | 36.9 ± 9.6                    |         |
| RVWT⁻¹, mm                | -                | 3.7 (3.1-4.4)                 |         |
| LVESVI⁻¹, mL/m²           | -                | 51.0 (41.8-64.8)              |         |
| LVMI, g/m²                | -                | 22.1 (17.7-30.1)              |         |
| Echocardiographic data    |                  |                               |         |
| IVST, mm                  | 8.9 ± 1.2        | 8.4 ± 1.5                     | 0.101   |
| PWT, mm                   | 8.9 ± 1.2        | 8.6 ± 1.1                     | 0.163   |
| LVIDm, mm                 | 44.6 ± 4.4       | 41.8 ± 6.5                    | 0.013   |
| LVETs, mm                 | 27.6 ± 3.7       | 25.5 ± 4.6                    | 0.017   |
| LVEF, %                   | 68.3 ± 5.8       | 69.4 ± 7.1                    | 0.409   |
| NT-pro-BNP², pg/mL        | 60.9 (28.9–102.7)| 219.3 (71.2–1233.4)          | < 0.001 |
| Uric acid, mg/dL          | 4.7 ± 1.3        | 5.8 ± 2.1                     | 0.003   |
| eGFR, mL/min/1.73m²       | 85.6 ± 19.6      | 80.6 ± 32.2                   | 0.376   |
| Comorbidity, n (%)        |                  |                               |         |
| Diabetes mellitus         | 2 (4.8%)         | 8 (15.1%)                     | 0.177   |
| Hypertension              | 15 (35.7%)       | 15 (28.3%)                    | 0.508   |
| Dyslipidemia              | 11 (26.2%)       | 16 (30.2%)                    | 0.819   |

Values are number (%), mean ± SD, or median (interquartile range).

Bold indicates statistically significant data.

n, number; IPAH, idiopathic pulmonary arterial hypertension; HPAH, heritable pulmonary arterial hypertension; CTD-PAH, pulmonary arterial hypertension associated with connective tissue disease; CHD-PAH, pulmonary arterial hypertension associated with congenital heart disease; CTEPH, chronic thromboembolic pulmonary hypertension; sPAP, systolic pulmonary arterial pressure; mPAP, mean pulmonary arterial pressure; PVR, pulmonary vascular resistance; CI, cardiac index; 6MWD, 6-minute walk distance; CMR, cardiovascular magnetic resonance; RVEDVI, right ventricular end-diastolic volume index; RVESVI, right ventricular end-systolic volume index; RVMI, right ventricular mass index; RVEF, right ventricular ejection fraction; RVWT, right ventricular free wall thickness; LVESVI, left ventricular end-systolic volume index; LVIDm, left ventricular mass index; IVST, interventricular septum thickness; PWT, posterior wall thickness; LVDD, left ventricular end-diastolic dimension; LVIDs, left ventricular end-systolic dimension; LVMI, left ventricular mass index; NT-pro-BNP, N-terminal pro-brain natriuretic peptide; eGFR, estimated glomerular filtration rate.

https://doi.org/10.1371/journal.pone.0206856.t001
amplitude of RV₁+SV₅/₆. Multiple regression analyses revealed that mPAP (p = 0.002) and RVEF (p = 0.007) were independently associated with the amplitude of RV₁+SV₅/₆ ($R^2 = 0.282$) (Table 5). Fig 1 shows representative ECG traces in a control subject without PH, and patients with mild PH and severe PH. To seek the cut-off value of RV₁+RV₅ for severe PH, we performed ROC analysis and compared criteria for severe PH (mPAP $\geq 42.5$ mmHg and/or RVEF $< 35\%$) (Fig 2A–2C). The largest area under curve (AUC) of the ROC curve was shown in combination with mPAP $\geq 42.5$ mmHg and RVEF $< 35\%$ (AUC: 0.811, confidence interval 0.633–0.988, p = 0.009), and defined the cut-off value of 16.4 mm in RV₁+SV₅/₆ (sensitivity 85.7%, specificity 76.1%) (Fig 2C). We examined all-cause mortality in our patients with pre-capillary PH. During a mean follow-up of 3.7 years, patients with $\geq 16.4$ mm of RV₁+SV₅/₆ had worse prognosis than those with $< 16.4$ mm (log-rank test, p = 0.015) (Fig 3A). No significant difference of survival was observed between the low amplitude of RV₁ and the high one (Fig 3B). Moreover, the high amplitude of RV₁+SV₅/₆ was significantly associated with all

Table 2. ECG findings of control subjects and pre-capillary PH patients.

| ECG findings          | Control subjects | Patients with pre-capillary PH | p-value |
|-----------------------|------------------|--------------------------------|---------|
| RV₁, mm               | 2.3 (1.5–3.6)    | 3.7 (2.1–8.9)                 | 0.013   |
| RV₅, mm               | 14.9 (11.5–18.2) | 11.8 (8.4–16.8)               | 0.008   |
| SV₁, mm               | 8.9 (6.5–12.8)   | 5.0 (2.1–6.8)                 | < 0.001 |
| SV₅, mm               | 3.3 (1.5–4.8)    | 7.1 (4.8–10.6)                | < 0.001 |
| V₁, R:S ratio         | 0.3 ± 0.2        | 3.8 ± 13.9                    | 0.014   |
| V₅, R:S ratio         | 6.6 ± 6.3        | 2.3 ± 2.2                     | < 0.001 |
| RV₁+SV₅/₆, mm         | 5.7 (4.2–8.4)    | 11.5 (8.2–18.2)               | < 0.001 |
| QRS axis              | 45.6 ± 30.2      | 81.1 ± 43.9                   | < 0.001 |
| R in aVR, mm          | 1.0 ± 0.8        | 1.6 ± 1.6                     | 0.010   |
| P in lead II, mm      | 1.2 ± 0.4        | 1.4 ± 68.4                    | 0.395   |

Values are number (%), mean ± SD, or median (interquartile range). Bold indicates statistically significant data.

https://doi.org/10.1371/journal.pone.0206856.t002

amplitude of RV₁+SV₅/₆. Multiple regression analyses revealed that mPAP (p = 0.002) and RVEF (p = 0.007) were independently associated with the amplitude of RV₁+SV₅/₆ ($R^2 = 0.282$) (Table 5). Fig 1 shows representative ECG traces in a control subject without PH, and patients with mild PH and severe PH. To seek the cut-off value of RV₁+RV₅ for severe PH, we performed ROC analysis and compared criteria for severe PH (mPAP $\geq 42.5$ mmHg and/or RVEF $< 35\%$) (Fig 2A–2C). The largest area under curve (AUC) of the ROC curve was shown in combination with mPAP $\geq 42.5$ mmHg and RVEF $< 35\%$ (AUC: 0.811, confidence interval 0.633–0.988, p = 0.009), and defined the cut-off value of 16.4 mm in RV₁+SV₅/₆ (sensitivity 85.7%, specificity 76.1%) (Fig 2C). We examined all-cause mortality in our patients with pre-capillary PH. During a mean follow-up of 3.7 years, patients with $\geq 16.4$ mm of RV₁+SV₅/₆ had worse prognosis than those with $< 16.4$ mm (log-rank test, p = 0.015) (Fig 3A). No significant difference of survival was observed between the low amplitude of RV₁ and the high one (Fig 3B). Moreover, the high amplitude of RV₁+SV₅/₆ was significantly associated with all

Table 3. Traditional ECG criteria of RVH in control subjects and with pre-capillary PH patients.

| ECG criteria of RVH | Control subjects | Patients with pre-capillary PH | p-value |
|--------------------|------------------|--------------------------------|---------|
| RV₁ $\geq$ 7 mm    | 4 (9.5%)         | 18 (34.0%)                    | 0.005   |
| SV₅ $> 7$ mm       | 4 (9.5%)         | 28 (52.8%)                    | < 0.001 |
| RV₅ $< 5$ mm       | 0 (0%)           | 1 (1.9%)                      | 0.371   |
| RV₁+SV₅/₆ $> 10.5$ mm | 6 (14.3%)    | 37 (69.8%)                    | < 0.001 |
| V₁, R:S $> 1$      | 0 (0%)           | 17 (32.1%)                    | < 0.001 |
| V₅, R:S $< 1$      | 0 (0%)           | 11 (20.8%)                    | 0.002   |
| V₁, R:S $> 1$ with R $> 5$ mm | 0 (0%)      | 11 (20.8%)                    | 0.002   |
| SV₁ $\leq$ 2mm with RV₅ $\geq 4$ mm | 0 (0%)    | 12 (22.6%)                    | < 0.001 |
| QRS axis $> 90^\circ$ | 1 (2.4%)     | 23 (43.4%)                    | < 0.001 |
| P in lead II $> 2.5$ mm | 0 (0%)       | 3 (5.7%)                      | 0.117   |

Values are number (%). Bold indicates statistically significant data.

https://doi.org/10.1371/journal.pone.0206856.t003
cause of death in the Cox proportional hazards regression model with adjustment for age and gender (p = 0.044) (Table 6). There was no lung transplantation in this study population.

**Discussion**

In this study, we examined the utility of ECG findings of RVH in the assessment of pre-capillary PH. Our study demonstrated that the amplitude of RV1 + SV5/6 was the most frequent finding as a traditional ECG criterion of RVH. Further, mPAP and RVEF was independently associated with the amplitude of RV1 + SV5/6. This is the first study to examine the association between ECG findings and the clinical variables evaluated by both RHC and CMR, including ECG parameter as a prognostic factor. A significant finding was that RV1 + SV5/6 is a powerful predictor of prognostic factors; cut-off value of over 16.4 mm in RV1 + SV5/6 derived from our study could predict the worse prognosis in patients with pre-capillary PH.

**Table 4. Correlations between clinical parameters and ECG findings.**

| Parameters | RV1* | RV5* | SV1* | SV5* | RV/S V1 | RV/S V5 | RV1+SV5/6* | QRS axis | R in aVR | P in lead II |
|------------|------|------|------|------|---------|---------|------------|----------|----------|-------------|
| sPAP       | .406** | -.099 | -.288* | .257 | .229 | -.201 | .511** | .459** | .376** | .026 |
| mPAP       | .338* | -.153 | -.312* | .247 | .208 | -.226 | .446** | .469** | .423** | .075 |
| PVR        | .231 | -.371** | -.330* | .314* | .262 | -.349* | .409** | .514** | .478** | .202 |
| CI*        | -.096 | .345* | .076 | -.085 | -.161 | .189 | -.082 | -.263 | -.309* | -.085 |
| RVWT*      | .297* | -.117 | .099 | .072 | .229 | .067 | .411** | .369** | .192 | .132 |
| RVEDVI*    | .316* | -.153 | -.065 | .222 | -.038 | -.198 | .403** | .242 | .100 | .029 |
| RVESVI*    | .347* | .109 | -.101 | -.223 | .006 | -.199 | .472** | .192 | .111 | .026 |
| RVMI*      | .383* | .205 | -.090 | .203 | .162 | -.028 | .552** | .307* | .228 | .055 |
| RVEF       | -.272* | .041 | .119 | -.064 | -.122 | .034 | -.407** | .068 | -.074 | .016 |
| RVEDV/LVEDV | .303* | -.119 | -.081 | .103 | -.047 | -.159 | .311* | .196 | .048 | .107 |
| 6MWD       | .147 | .220 | -.098 | -.137 | .109 | .081 | -.032 | -.177 | .179 | -.119 |
| NT-pro-BNP* | -.170 | -.098 | .093 | -.248 | -.040 | -.144 | .158 | .316* | -.128 | .013 |
| Uric acid  | .028 | -.050 | .138 | .018 | -.154 | -.009 | .016 | .211 | -.025 | -.038 |

*Log-transformed value was used. Values are regression coefficients. p < 0.05 **p < 0.01

Abbreviations as in Table 1.

https://doi.org/10.1371/journal.pone.0206856.t004

**Table 5. Univariate and multiple stepwise regression analyses for associates of the amplitude of RV1 + SV5/6.**

| Parameters | Univariate | Multivariate |
|------------|------------|--------------|
| mPAP       | .0446      | .001         | .385         | .002         |
| PVR        | .409       | .002         | -            | -            |
| RVWT*      | .411       | .002         | -            | -            |
| RVEF       | -.407      | .002         | -.338        | .007         |
| RVEDVI/LVEDVI | .311    | .024         | -            | -            |

R² | - | 0.282

*Log-transformed value was used. Bold indicates statistically significant data. Abbreviations as in Table 1.
Pre-capillary PH is diagnosed by the standard right heart catheterization [3]. RV volume, RVEF and RV free wall thickness can be accurately assessed by CMR [17]. Thus, we assessed pulmonary hemodynamics by right heart catheterization and RV remodeling by CMR. However, it is difficult for outpatients to constantly perform these examinations within follow-up periods. Non-invasive, low-cost ECG can be repeatedly performed in patients with PH. Indeed, traditional ECG criteria have been used for screening of RVH in clinical practice [12]. The relation between traditional ECG criteria of RVH and PH has been published previously [13, 14, 18, 19]. The sensitivity and specificity for RVH differently depends on the study subjects. In particular, ECG criteria of RVH were determined from patients with congenital heart disease (CHD) [12, 20]. Whereas CHD such as atrial septal defect yields the right heart volume

Fig 1. Representative electrocardiogram traces obtained from a control subject without pulmonary hypertension (PH). (A), and patients with mild PH (B) and severe PH (C).

Fig 2. Receiver operating characteristic plotings of the RV +SV₅₆ for predicting the severity of PH. mPAP ≥ 42.5 mmHg (A), RVEF < 35% (B) and mPAP < 42.5 mmHg + RVEF < 35% (C). AUC, area under curve; CI, confidence interval.
overload, pre-capillary PH shows the RV pressure overload. Therefore, it is difficult to divide RVH and RV enlargement using ECG criteria of RVH.

Pre-capillary PH is characterized by elevated PVR leading to RV pressure overload, which leads to RV deformity including ventricular hypertrophy and/or enlargement [3, 21, 22]. RV enlargement (RVEDVI ≥ 84 mL/m²) and reduced stroke volume (SVI ≤ 25 mL/m²) have been shown as poor prognostic factors in patients with pulmonary arterial hypertension (PAH). Moreover, heart failure and reduced RVEF in PH patients decrease survival rate [21]. Both mPAP ≥ 42.5 mmHg and RVEF < 35% are predictive factors for poor outcome in patients with PH [5, 8]. Therefore, assessment of the hemodynamics, RV structure, and RV function are essential for prediction of prognosis in patients with PH. When we sought the non-invasive ECG criteria associated with pulmonary hemodynamics and RV remodeling in pre-capillary PH, the amplitude of RV₁+SV₅/₆ was the most frequent ECG criterion of RVH.

Further, mPAP and RVEF was independently associated with the amplitude of RV₁+SV₅/₆. Obviously, RVEDV and RVESV leading to RVEF were correlated with the amplitude of RV₁+SV₅/₆. Moreover, pulmonary hemodynamic parameters were related to RVEDVI and RVESVI (p < 0.01). RVEF was negatively correlated with PVR (r = -0.296, p < 0.05). These findings suggest that the amplitude of RV₁+SV₅/₆ may be a useful variable associated with hemodynamics and RV remodeling in patients with pre-capillary PH.

Kopec et al. examined the ECG criteria for predicting RVH and increased RV volume, RV₁+SV₅/₆ had a correlation with the RV mass index (RVMI: r = 0.54, p = 0.008) but not RV volume (r = 0.05, p = 0.82), RV₁+SV₅/₆ had a significant AUC with high sensitivity and specificity for predicting the RVH (AUC = 0.78, p = 0.03, sensitivity 81%, specificity 57%) [19]. In
our study, RV1+SV5/6 had a correlation with both RVMI and RV volume (RVMI: $r = 0.552$, $p < 0.001$, RVEDVI: $r = 0.403$, $p = 0.003$, RVESVI: $r = 0.472$, $p < 0.001$). While diagnostic accuracy of RV1+SV5/6 for RVH was lower than Kopec’s study (AUC = 0.697, sensitivity 54.5%, specificity 88.9%, the cut-off value of RV1+SV5/6: 12.7 mm), RV1+SV5/6 predicted the worse prognosis with mPAP $\geq$ 42.5 mmHg and/or RVEF < 35%.

The amplitude of R in lead V1 characterized by RV pressure overload is widely used for assessment of the RVH severity. It is well known that increased R amplitude in lead V1 is common in adolescent subjects. Therefore, we compared the electrocardiographic RVH parameters between patients with PH and age-matched control subjects, and found that the R amplitude in lead V1 was greater in PH patients than in controls. It has shown that the amplitude of R in lead V1 is correlated with RV mass index by CMR [19]. Cheng XL et al. reported that the amplitude of RV1 or SV6 was correlated with mPAP [23]. Sato S et al. showed that decrease in RV1 predicted the better survival in patients with PAH [24]. As with the Cheng’s study, the amplitude of RV1 or SV5/6 was correlated with mPAP in our study (RV1, $r = 0.338$; SV5/6, $r = 0.334$; $p < 0.05$, respectively). Also, the amplitude of RV1+SV5/6 was significantly associated with PAP and CMR parameters including RV size, function, wall thickness and mass (Table 4). The cut-off value of 16.4 mm in RV1+SV5/6 predicted prognostic factors such as mPAP $\geq$ 42.5 mmHg and RVEF < 35% (AUC 0.811, $p = 0.009$, sensitivity 85.7%, specificity 76.1%). Although RV1 presented a high AUC (AUC 0.795, $p = 0.013$) similar to RV1+SV5/6, specificity of the cut-off value of 2.5 mm in RV1 was inferior to RV1+SV5/6 (sensitivity 100%, specificity 37%).

As widely known, ECG changes depend on left ventricular volume [25]. Therefore, we excluded patients with post-capillary secondary PH such as hypertrophic/dilated cardiomyopathy, ischemic heart disease, and valvular disease. In the current study, the amplitudes of RV1+SV5/6 and RV1 were significantly correlated with RVEDVI to LVEDVI ratio ($p < 0.05$).

| Parameters | Univariate |
|------------|------------|
|            | HR         | 95% CI      | p-value  |
| sPAP       | 1.035      | 0.997–1.074 | 0.069     |
| mPAP       | 1.046      | 0.987–1.110 | 0.131     |
| PVR        | 1.151      | 1.013–1.309 | 0.031     |
| CI*        | 0.133      | 0.009–2.005 | 0.145     |
| RVWT       | 1.526      | 1.018–2.285 | 0.040     |
| RVEDVI*    | 2.695      | 0.764–9.511 | 0.123     |
| RVESVI*    | 2.572      | 0.954–6.937 | 0.062     |
| RVMI*      | 8.920      | 1.357–58.654| 0.023     |
| RVEF       | 0.954      | 0.904–1.007 | 0.087     |
| LVEF       | 0.989      | 0.926–1.057 | 0.744     |
| 6MWD       | 0.993      | 0.986–1.000 | 0.048     |
| NT-pro-BNP*| 1.467      | 1.014–2.121 | 0.042     |
| Uric acid  | 1.357      | 0.997–1.847 | 0.052     |
| RV1+SV5/6* | 3.718      | 1.038–13.313| 0.044     |
| RV1*       | 1.252      | 0.629–2.492 | 0.522     |

*Log-transformed value was used.

Bold indicates statistically significant data.

HR, hazard ratio; 95% CI, 95% confidence interval.

Other abbreviations as in Table 1.

https://doi.org/10.1371/journal.pone.0206856.t006
Ogawa et al reported that mPAP ≥ 42.5 mmHg showed worse survival rate in patients with idiopathic/heritable PAH [5]. Also, RVEF < 35% is associated with a poor outcome regardless of PVR values [8]. In our study, the cut-off value of 16.4 mm in RV1+SV5/6 predicts the both mPAP ≥ 42.5 mmHg and RVEF < 35%. Also, we performed the survival analysis among pre-capillary PH patients. During a mean follow-up of 3.7 years, patients with ≥ 16.4 mm of RV1+SV5/6 had worse prognosis than those with < 16.4 mm (Fig 3A). Moreover, the amplitude of RV1+SV5/6 was significantly associated with all cause of death in the Cox proportional hazards analysis (Table 6). On one hand, the amplitude of RV1 did not predict the survival in this study (Fig 3B). Thus, the amplitude of RV1+SV5/6 could be a predictor for prognosis in patients with pre-capillary PH.

NT-pro-BNP is well recognized as a biomarker of disease severity in post-capillary PH. Also, elevated NT-pro-BNP is associated with worse prognosis in pre-capillary PH [26–30]. We found that NT-pro-BNP was associated with PVR, RV volume, 6MWD, QRS axis, and S amplitude in lead V5. The amplitude of RV1+SV5 might include the factors derived from not only right ventricle, but also left ventricle.

Study limitations
Several limitations should be mentioned for the present study. First, the small study size limits our interpretation and discussion. Also, our PH patients consisted of different etiologies. It was difficult to enroll many patients with a homogeneous etiology, because PH is a rare disease and has a poor prognosis. A previous study by Nagai et al. demonstrated that ECG findings for RVH predict the presence of RV systolic dysfunction assessed by CMR in patients with pre-capillary PH [31]. Also, Nishiyama et al. reported that therapeutic improvement of ECG findings for RVH after balloon pulmonary angioplasty was correlated to that of hemodynamics in patients with CTEPH [32]. However, there have been no ECG studies assessed for the severity and the prognosis of pre-capillary PH using both CMR and RHC. Second, we did not perform RHC and CMR in control subjects. In the study, all control subjects were confirmed without significant organic heart disease by 2-dimensional echocardiography. Third, we did not consider the negative T-wave in the limb and precordial leads in our patients. Negative T wave is relatively common in acute pulmonary embolism, and is not correlated with RV free wall thickness [33]. As T wave change and R amplitude in lead V1 depend on age of the patients, we compared our patients with age-matched control subjects. Fourth, low voltage in the limb leads was frequently present in patients with pulmonary disease owing to increased lung volume. There were 12 patients with pulmonary disease in our study. Although we did not include to analyze the amplitude in limb leads except aVR as electrocardiographic RVH parameters in this study, we can not deny that lung volume may affect our results. Finally, our data lack clinical outcomes to support a utility of ECG findings of RVH in patients with pre-capillary PH. Accordingly, further longitudinal studies are needed to clarify whether the ECG findings of RVH are useful to predict clinical outcomes.

Conclusion
In conclusion, our study demonstrated that the amplitude of SV1+RV5/6 could be the most useful factor reflected for RV remodeling, hemodynamics and survival in patients with pre-capillary PH.

Acknowledgments
We thank Mami Nakayama, Miho Nakao-Kogure, Katsue Shiramizu, Miyuki Nishikata and Makiko Kiyohiro, Division of Cardiovascular Medicine, Department of Medicine, Kurume
University School of Medicine. This work was supported in part by research grants from the Kimura Memorial Foundation (to A.T. and T.N.); the Mitsui Life Social Welfare Foundation (to N.T.); and the Grant-in-Aid for Scientific Research (Grant Number 17K09564 to N.T., Grant Number 17K16030 to Y.S., and Grant Number 17K16031 to T.N.) from the Japan Society for the Promotion of Science (JSPS KAKENHI), Tokyo, Japan.

Author Contributions
Conceptualization: Sachiyo Igata, Nobuhiro Tahara.

Data curation: Sachiyo Igata, Yoichi Sugiyama, Munehisa Bekki, Jun Kumanomido, Atsuko Tahara, Akihiro Honda, Tomohisa Nakamura, Jiahui Sun.

Formal analysis: Shoko Maeda, Kazutaka Nashiki, Toshi Abe.

Funding acquisition: Nobuhiro Tahara.

Investigation: Sachiyo Igata.

Methodology: Jun Kumanomido.

Software: Kazutaka Nashiki, Toshi Abe.

Supervision: Nobuhiro Tahara, Yoshihiro Fukumoto.

Writing – original draft: Sachiyo Igata, Nobuhiro Tahara.

Writing – review & editing: Nobuhiro Tahara, Yoshihiro Fukumoto.

References
1. Ryan JJ, Archer SL. The right ventricle in pulmonary arterial hypertension: disorders of metabolism, angiogenesis and adrenergic signaling in right ventricular failure. Circ Res. 2014; 115(1):176–188. https://doi.org/10.1161/CIRCRESAHA.113.301129 PMID: 24951766.

2. Pietra GG, Capron F, Stewart S, Leone O, Humbert M, Robbins IM, et al. Pathologic assessment of vasculopathies in pulmonary hypertension. J Am Coll Cardiol. 2004; 43(12 Suppl S):25S–32S. https://doi.org/10.1016/j.jacc.2004.02.033 PMID: 15194175.

3. Galie N, Humbert M, Vachiery JL, Gibbs S, Lang I, Torbicki A, et al. 2015 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension: The Joint Task Force for the Diagnosis and Treatment of Pulmonary Hypertension of the European Society of Cardiology (ESC) and the European Respiratory Society (ERS): Endorsed by: Association for European Paediatric and Congenital Cardiology (AEPC), International Society for Heart and Lung Transplantation (ISHLT). Eur Heart J. 2016; 37 (1):67–119. Epub 2015/08/29. https://doi.org/10.1093/eurheartj/ehv317 PMID: 26320113.

4. Badano LP, Ginghina C, Easaw J, Muraru D, Grillo MT, Lancellotti P, et al. Right ventricle in pulmonary arterial hypertension: haemodynamics, structural changes, imaging, and proposal of a study protocol aimed to assess remodelling and treatment effects. Eur J Echocardiogr. 2010; 11(1):27–37. Epub 2009/10/7. https://doi.org/10.1093/ejechocard/jep152 PMID: 19815539.

5. Ogawa A, Ejiri K, Matsubara H. Long-term patient survival with idiopathic/heritable pulmonary arterial hypertension treated at a single center in Japan. Life Sci. 2014; 118(2):414–419. Epub 2014/02/11. https://doi.org/10.1016/j.lfs.2014.01.077 PMID: 24530872.

6. Vonk-Noordegraaf A, Haddad F, Chin KM, Forfla PR, Kawut SM, Lumens J, et al. Right heart adaptation to pulmonary arterial hypertension: physiology and pathobiology. J Am Coll Cardiol. 2013; 62(25 Suppl):D22–33. https://doi.org/10.1016/j.jacc.2013.10.027 PMID: 24355638.

7. Amsallem M, Sweat AJ, Aymami MC, Kuznetsova T, Selez M, Lu H, et al. Right Heart End-Systolic Remodeling Index Strongly Predicts Outcomes in Pulmonary Arterial Hypertension. Circ Cardiovasc Imaging. 2017; 10:e005771. https://doi.org/10.1161/CIRCIMAGING.116.005771 PMID: 28592589.

8. van de Veerdonk MC, Kind T, Marcus JT, Mauritz GJ, Heymans MW, Bogaard HJ, et al. Progressive right ventricular dysfunction in patients with pulmonary arterial hypertension responding to therapy. J Am Coll Cardiol. 2011; 58(24):2511–2592. https://doi.org/10.1016/j.jacc.2011.06.068 PMID: 22133851.
9. Courand PY, Pina Jomir G, Khouatra C, Scheiber C, Turquier S, Glerand JC, et al. Prognostic value of right ventricular ejection fraction in pulmonary arterial hypertension. Eur Respir J. 2015; 45(1):139–149. Epub 2014/12/23. https://doi.org/10.1183/09031936.00158014 PMID: 25537560.

10. Maceira AM, Prasad SK, Khan M, Pennell DJ. Reference right ventricular systolic and diastolic function normalized to age, gender and body surface area from steady-state free precession cardiovascular magnetic resonance. Eur Heart J. 2006; 27(23):2879–2888. Epub 2006/11/16. https://doi.org/10.1093/eurheartj/ehi336 PMID: 17088316.

11. Tsugu T, Murata M, Kawakami T, Yasuda R, Tokuda H, Minakata Y, et al. Significance of echocardiographic assessment for right ventricular function after balloon pulmonary angioplasty in patients with chronic thromboembolic induced pulmonary hypertension. Am J Cardiol. 2015; 115(2):256–261. Epub 2014/10/31. https://doi.org/10.1016/j.amjcard.2014.10.034 PMID: 25476559.

12. Hancock EW, Deal BJ, Mirvis DM, Okin P, Kligfield P, Gettes LS, et al. AHA/ACC/HRS recommendations for the standardization and interpretation of the electrocardiogram: part V: electrocardiogram changes associated with cardiac chamber hypertrophy: a scientific statement from the American Heart Association Electrocardiography and Arrhythmias Committee, Council on Clinical Cardiology; the American College of Cardiology Foundation; and the Heart Rhythm Society. Endorsed by the International Society for Computerized Electrocardiology. J Am Coll Cardiol. 2009; 53(11):992–1002. https://doi.org/10.1016/j.jacc.2008.12.015 PMID: 19281932.

13. Sokolow M, Lyon TP. The ventricular complex in right ventricular hypertrophy as obtained by unipolar precordial and limb leads. Am Heart J. 1949; 38(2):273–294. PMID: 18133559.

14. Myers GB, Klein HA, Stofer BE. The electrocardiographic diagnosis of right ventricular hypertrophy. Am Heart J. 1948; 35(1):1–40. PMID: 18919647.

15. Murphy ML, Thenabadu PN, de Soya N, Doherty JE, Meade J, Baker BJ, et al. Reevaluation of electrocardiographic criteria for left, right and combined cardiac ventricular hypertrophy. Am J Cardiol. 1984; 53(8):1140–1147. PMID: 6230928.

16. Lang RM, Badano LP, Mor-Avi V, Afilalo J, Armstrong A, Ernande L, et al. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. J Am Soc Echocardiogr. 2015; 28(1):1–39.e14. https://doi.org/10.1016/j.echo.2014.10.003 PMID: 25559473.

17. Katz J, Whang J, Boxt LM, Barst RJ. Estimation of right ventricular mass in normal subjects and in patients with primary pulmonary hypertension by nuclear magnetic resonance imaging. J Am Coll Cardiol. 1993; 21:1475–1481. PMID: 8473659.

18. Ahearn GS, Tapson VF, Rebeiz A, Greenfield JC Jr. Electrocardiography to Define Clinical Status in Primary Pulmonary Hypertension and Pulmonary Arterial Hypertension Secondary to Collagen Vascular Disease. Chest. 2002; 122(2):524–527. PMID: 12171826.

19. Kopec G, Tyrka A, Miszalski-Jamka T, Sobien M, Waligora M, Brozda M, et al. Electrocardiography for the Diagnosis of Right Ventricular Hypertrophy and Dilation in Idiopathic Pulmonary Arterial Hypertension. Circ J. 2012; 76(7):1744–1749. PMID: 22498658.

20. Milnor WR. Electrocardiogram and Vectorcardiogram in Right Ventricular Hypertrophy and Right Bundle-Branch Block. Circulation. 1957; 16(3):348–367. PMID: 13461242.

21. van Wolferen SA, Marcus JT, Boonstra A, Marques KM, Bronzwaer JG, Spreeuwenberg MD, et al. Prognostic value of right ventricular mass, volume, and function in idiopathic pulmonary arterial hypertension. Eur Heart J. 2007; 28(10):1250–1257. Epub 2007/01/22. https://doi.org/10.1093/eurheartj/ehl477 PMID: 17242010.

22. Galie N, Palazzini M, Manes C. Pulmonary arterial hypertension: from the kingdom of the near-dead to multiple clinical trial meta-analyses. Eur Heart J. 2010; 31(17):2080–2086. Epub 2010/05/26. https://doi.org/10.1093/eurheartj/ehq152 PMID: 20504865.

23. Cheng XL, He JG, Liu ZH, Gu Q, N XH, Zhao ZH, et al. The Value of the Electrocardiogram for Evaluating Prognosis in Patients with Idiopathic Pulmonary Arterial Hypertension. Lung. 2017; 195(1):139–146. Epub 2016/11/25. https://doi.org/10.1007/s00408-016-9967-2 PMID: 27888398.

24. Sato S, Ogawa A, Matsubara H. Change in R wave in lead V1 predicts survival of patients with pulmonary arterial hypertension. Eur Respir J. 2015; 45(1):139–146. Epub 2015/04/25. https://doi.org/10.1177/0903193614525490 PMID: 25735629.

25. Devereux RB, Phillips MC, Casale PN, Eisenberg RR, Kligfield P. Geometric determinants of electrocardiographic left ventricular hypertrophy. Circulation. 1983; 67(4):907–911. PMID: 6218940.

26. Williams MH, Handler CE, Akram R, Smith CJ, Das C, Smea J, et al. Role of N-terminal brain natriuretic peptide (N-TproBNP) in scleroderma-associated pulmonary arterial hypertension. Eur Heart J. 2006; 27(12):1485–1494. Epub 2006/04/27. https://doi.org/10.1093/eurheartj/ehi891 PMID: 16682379.

27. Reesink HJ, Tulevski II, Marcus JT, Boomsma F, Kloeck JJ, Vonk Noordegraaf A, et al. Brain natriuretic peptide as noninvasive marker of the severity of right ventricular dysfunction in chronic thromboembolic
pulmonary hypertension. Ann Thorac Surg. 2007; 84(2):537–543. https://doi.org/10.1016/j.athoracsur.2007.04.006 PMID: 17643631.

28. Chi SY, Kim EY, Ban HJ, Oh JJ, Kwon YS, Kim KS, et al. Plasma N-terminal pro-brain natriuretic peptide: a prognostic marker in patients with chronic obstructive pulmonary disease. Lung. 2012; 190 (3):271–276. Epub 2012 Jan 14. https://doi.org/10.1007/s00408-011-9363-7 PMID: 22246552.

29. Fijalkowska A, Kurzyna M, Torbicki A, Szewczyk G, Florczyk M, Pruszczyk P, et al. Serum N-terminal brain natriuretic peptide as a prognostic parameter in patients with pulmonary hypertension. Chest. 2006; 129(5):1313–1321. https://doi.org/10.1378/chest.129.5.1313 PMID: 16685024.

30. Nagaya N, Nishikimi T, Uematsu M, Satoh T, Kyotani S, Sakamaki F, et al. Plasma brain natriuretic peptide as a prognostic indicator in patients with primary pulmonary hypertension. Circulation. 2000; 102 (8):865–870. PMID: 10952954.

31. Nagai T, Kohsaka S, Murata M, Okuda S, Anzai T, Fukuda K, et al. Significance of Electrocardiographic Right Ventricular Hypertrophy in Patients with Pulmonary Hypertension with or without Right Ventricular Systolic Dysfunction. Intern Med. 2012; 51(17):2277–2283. https://doi.org/10.2169/internalmedicine.51.7731 PMID: 22975535.

32. Nishiyama T, Takatsuki S, Kawakami T, Katsumata Y, Kimura T, Kataoka M, et al. Improvement in the electrocardiograms associated with right ventricular hypertrophy after balloon pulmonary angioplasty in chronic thromboembolic pulmonary hypertension. Int J Cardiol Heart Vasc. 2018; 19:75–82. https://doi.org/10.1016/j.ijcha.2018.05.003 PMID: 29892707.

33. Padmavati S, Raizada V. Electrocardiogram in chronic cor pulmonale. Br Heart J. 1972; 34(7):658–667. PMID: 4261215.