Relationship between right heart echocardiographic parameters and invasive pulmonary artery pressures in canine models of chronic embolic pulmonary hypertension

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ABSTRACT. Basic information related to the association between right heart echocardiographic parameters and invasive pulmonary artery pressure (PAP) in dogs with pulmonary hypertension (PH) is scarce. The aim of this study was to examine the association between conventional right heart echocardiographic parameters and invasive PAP by right heart catheterization (RHC) before and after PH. Five female beagle dogs regarded as clinically healthy were used. Echocardiography and RHC were conducted before and after creating chronic embolic pulmonary hypertension (CEPH) models. The acceleration time to ejection time ratio in pulmonary artery flow profile (AT/ET), the ratio of the pulmonary artery and aortic diameter in diastole (PA/Ao), the right pulmonary artery distensibility index by M-mode method (RPAD M-mode), the normalized right ventricular internal diameter in diastole (RVIDdn), and the normalized tricuspid annular plane systolic excursion (TAPSEn) were correlated with the invasive systolic PAP (sPAP), mean PAP (mPAP) and diastolic PAP (dPAP). Multiple linear regression analysis identified AT/ET and RVIDdn as independent predictors of sPAP, PA/Ao and RPAD M-mode as independent predictors of mPAP, and PA/Ao and RPAD M-mode as independent predictors of dPAP. AT/ET and PA/Ao had high sensitivity and specificity for predicting CEPH. In conclusion, AT/ET, PA/Ao, RPAD M-mode, RVIDdn and TAPSEn were significantly correlated with invasive PAP and alterations in PA/Ao or AT/ET might enable clinicians to predict PH, even if tricuspid regurgitation is not observed.

KEY WORDS: catheterization, dog, echocardiography, microsphere, pulmonary hypertension

Pulmonary hypertension (PH) is a progressive disorder characterized by the elevation of pulmonary artery pressure (PAP), often leading to right heart failure and poor prognosis [3, 10]. PH can be classified as pre- or post-capillary PH, or it can be identified based on the disease process that is causing it. There are generally five categories of this condition: 1) pulmonary arterial hypertension, 2) pulmonary venous hypertension, 3) pulmonary disease or hypoxia, 4) thromboembolic disease, and 5) miscellaneous disease [1, 6, 8, 11, 16, 19].

In veterinary medicine right heart catheterization (RHC) remains the gold standard for detecting PH, generally using the following parameters: systolic PAP [sPAP] >30 mmHg and mean PAP [mPAP] >20 mm Hg [22]. However, this approach is not often utilized in clinical medicine due to its cost, as well as the high level of invasiveness and the complications it can generate [22]. Therefore, PH is usually diagnosed based on a tricuspid regurgitation pressure gradient, with an entry point of (TRPG) >30 mmHg, calculated from the measurement of TR by echocardiography and using the modified Bernoulli equation. TRPG is used to classify PH severity in three levels—mild: 31.4–50 mmHg, moderate: 50–75 mmHg, and severe: greater than 75 mmHg [9]. However, even in the absence of PH, TR can be caused by several diseases such as valve degeneration, ruptured chordae tendinae, or pulmonary stenosis. In addition, TR is not always detectable in patients with PH [14]. Furthermore, a previous study has reported that TRPG rather imperfectly estimated sPAP with wide variation in canine models of acute PH [21]. Thus, there is clearly a need for a simpler screening tool to identify dogs with PH.

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Recently, several conventional echocardiographic parameters related to the right ventricle (RV) or pulmonary artery (PA) have been evaluated regarding their ability to identify and characterize PH in dogs. Six of these include the main PA and aorta ratio (PA/Ao), the tricuspid annular plane systolic excursion (TAPSE), the pulmonary artery flow profiles (i.e., acceleration time to ejection time ratio [AT/ET]), the RV fractional area change (RVFAC), and the right pulmonary artery distensibility index (RPAD), as well as the RV internal diameter in diastole (RVIdD) [19, 26–28]. However, although the usefulness of these parameters has been validated in dogs with PH diagnosed by TRPG, at present there are unfortunately very few studies examining the relationship between these echocardiographic parameters and invasive PAP measurements by RHC.

Therefore, using chronic models, the current study was performed to examine the association between conventional echocardiographic parameters related to right heart and invasive PAP before and after PH.

MATERIALS AND METHODS

Animals

This analytical study was performed using five female beagle dogs (2–7 years, 8.3–10.5 kg) that were regarded as being clinically healthy, based on a physical examination, complete blood count, biochemistry, echocardiography, electrocardiography, and systemic blood pressure measured using oscillometric methods. The dogs were housed individually in cages and fed a commercial dry food diet twice daily with free access to water. We followed the Guidelines for Institutional Laboratory Animal Care and Use at the Nippon Veterinary and Life Science University (Approval Number 30S-36).

Echocardiography

Before and after creating the models, echocardiographic and RHC measurements were performed. Echocardiography was performed prior to RHC. A single observer (S.S.) conducted all examinations using the Xario SSA-660A ultrasound system (Canon Medical Systems, Tochigi, Japan), with a 5.0 MHz sector transducer. The observer was not aware of RHC findings at the time of echocardiographic measurements. The dogs were unsedated and restrained in right or left lateral recumbency during these examinations.

Conventional echocardiographic parameters related to right heart dimension and function were measured during the phase of expiration monitoring respiratory status in a resting state, and three consecutive readings were averaged for each parameter, except for the velocities, where the highest recorded measurement was used. The PA/Ao ratio was obtained using the right parasternal short-axis view. The PA diameter at the end-diastole was measured right under the closed pulmonary valve, and the aortic diameter was measured on the same view. Then the PA/Ao was calculated [19]. The RVIdD was measured using the right parasternal short-axis view at the chordae tendineae level by the M–mode method [2]. The TAPSE was obtained by measuring the maximal longitudinal displacement of the tricuspid valve annulus toward the RV apex using the left parasternal apical four chamber view [27]. Using the M–mode method, the cursor was aligned as parallel as possible to the motion of the RV free wall. Measurements of the RV area for RVFAC determination were obtained by tracing the RV endocardial border at the end-diastole (RVEDA) and the end-systole (RVESA) using the left parasternal apical four chamber view as previously reported [27]. We calculated the RVFAC using the following formula: RVFAC (%)=[(RVEDA–RVESA)/RVEDA] × 100. RPAD was measured using both B-mode and M-mode methods. In the B-mode approach, the minimum diastolic (RPAd; at the beginning of Q wave) and maximum systolic (RPAs; around the largest T wave) internal diameters of the RPA were measured using the right parasternal short-axis view optimized for the main PA and RPA [28]. The internal diameters of the RPA were measured as perpendicular as possible to its internal borders. By contrast, in the M–mode method, the RPAd and RPAs were measured using the right parasternal long-axis view optimized for the left atrium, together with the pulmonary vein and the right PA [26]. RPAD was calculated using the following formula: RPAD (%)=[(RPAs–RPAd)/RPAs] × 100. The normalized TAPSE (TAPSEn) and RVIdD (RVIdDn) were calculated using the following formula according to a previous report: [a] TAPSEn=TAPSE (mm)/body weight ([BW], kg)0.33 and [b] RVIdDn=RVIdD (mm)/BW0.33 [5]. To assess the load to the RV, an eccentricity index (EI) was measured in systole and diastole, respectively. This EI was obtained by calculating the ratio of the two minor axes of the left ventricle (LV) in the right parasternal short-axis view at the chordae tendineae level using the following formula: EI=D2/D1, where D1 represents the LV minor-axis perpendicular to the ventricular septum and D2 represents the LV minor-axis parallel to the ventricular septum [2].

Doppler measurements of the PA outflow were obtained with the sample volume positioned right under the opened pulmonary valve in the right parasternal short-axis view. Then, ET and the pulmonary artery flow (PAF) velocity were measured. Additionally, AT was measured from the onset of PAF to the time corresponding to peak flow velocity, leading to the calculation of the AT/ET ratio [2]. In the left parasternal long-axis view, the tricuspid early diastolic flow (E wave) and late diastolic flow (A wave) velocities were measured, and the E/A ratio was calculated. The velocity–time integral (VTI) was obtained by tracing the PAF profile, and the RV cardiac output (CO) was calculated as CO=VTI × pulmonary artery cross sectional area × heart rate [23]. The tricuspid valve was interrogated from multiple viewpoints and a continuous wave Doppler was used to measure the peak TR velocity. The pulmonary valve was similarly assessed, and peak as well as end PR jet velocities were measured. Care was taken to align the regurgitation jet as parallel as possible to the ultrasound interrogation beam. The modified Bernoulli equation (PG=4 × [regurgitation velocity]²) was used to calculate the different PG values (i.e., TRPG, peak PRPG and end PRPG) using each regurgitation jet.
Right heart catheterization and creation of chronic embolic pulmonary hypertension (CEPH)

The dogs were sedated with butorphanol tartrate (0.2 mg/kg, IV) and midazolam hydrochloride (0.2 mg/kg, IV). They were then anesthetized with propofol (6 mg/kg, IV) and intubated. Anesthesia was maintained using a continuous rate infusion of propofol (0.1–0.2 mg/kg/min, IV) and 100% oxygen. The anesthetized dogs were positioned in left or right lateral recumbency. A 4-Fr introducer sheath (Medikit, Tokyo, Japan) was percutaneously inserted through the right or left jugular vein, and then a 4-Fr balloon wedge pressure catheter (Harmac Medical Products, Buffalo, NY, U.S.A.) was inserted and advanced into the PA. The fluid–filled catheter was connected to pressure transducers to allow the monitoring of PA pressure. The transducer was zeroed at the level of the right heart and recalibrated before each set of measurements. The location of the catheter was confirmed by detection of the typical pressure wave of this artery. After advancing the catheter into the PA, infusion of propofol was stopped. The dogs were allowed to recover from the anesthesia and sufficient recovery time (60–90 min) was allowed until they could walk without assistance. Then, the conscious dogs were again restrained in left or right lateral recumbency. sPAP, mPAP, and diastolic PA pressure (dPAP) were measured at end-expiration monitoring respiratory status in a resting state by another observer (R.A.). The values of the echocardiographic parameters were not revealed until the statistical analysis was conducted.

CEPH was induced according to a modified version of a previously described method [17, 20]. Briefly, 300 µm microspheres (GE Healthcare, Chicago, IL, U.S.A.) were injected into the pulmonary artery through the 4-Fr catheter monitoring PAP. The number of microspheres infused in each treatment was adjusted to increase sPAP to 40–50 mmHg. After each procedure, the sheath and catheter were removed from the jugular vein. Because partial recovery of PAP between each injection day described previously was observed consistently, this treatment was repeated approximately once a week until the sPAP and mPAP values prior to microsphere injection exceeded 30 and 20 mmHg, respectively [17]. Then, this condition (i.e. sPAP >30 mmHg and mPAP >20 mmHg) was maintained for at least 3 months.

Assessment of variability

Intraobserver variability was assessed using 1 dog at baseline and at CEPH, respectively. This dog was examined three times on a given day by a single observer (SS). All variables were measured on each of the 3 acquisitions for the dog and the resulting mean values and standard deviations (SD) were used to determine the coefficient of variation (CV). CV was calculated as: CV (%)=(SD/mean) × 100.

Statistical analysis

A commercial software package, SPSS ver.24 for Windows (SPSS Co., Ltd., Tokyo, Japan), was used for all statistical analyses. Data are represented as medians (min–max). The normality of the data was assessed using the Shapiro–Wilks test. A paired t-test or Wilcoxon’s signed-rank test was used to compare each parameter between baseline (BL) and CEPH. Partial correlation analysis adjusting for the effect of dogs was used to determine the relationship between each PAP and echocardiographic parameter. Dog or Wilcoxon’s signed-rank test was used to compare each parameter between baseline (BL) and CEPH. Partial correlation analysis adjusting for the effect of dogs was used to determine the relationship between each PAP and echocardiographic parameter. Dog was treated as a categorical factor using a dummy variable with four degrees of freedom. Then, using the statistically significant parameters, multiple linear regression analyses with forward-backward stepwise selection (p<0.05) adjusting for the effect of dogs were conducted to determine the independent predictor of each PAP. Additionally, coefficient of determination adjusted for the degrees of freedom and Durbin-Watson ratio were calculated to evaluate goodness of fit and residual error. Receiver operating characteristic (ROC) curves were generated for selected echocardiographic parameters to investigate their sensitivity and specificity for predicting CEPH. The curves were also used to investigate noninvasive cut-off values. Cut-off value was determined based on the minimum distance to the upper left corner in the ROC curve. P<0.05 was considered statistically significant.

RESULTS

Creation of CEPH models

We needed 6–11 months (21–35 injection times) to create our preferred CEPH model. The medians (min–max) of sPAP increased from 20.0 (15.7–22.9) mmHg at BL to 38.4 (33.2–45.8) mmHg at CEPH. Similarly, mPAP increased from 13.9 (12.2–16.4) mmHg at BL to 26.8 (22.0–31.9) mmHg at CEPH. No dogs showed significant clinical signs such as syncope, dyspnea, lethargy, and abdominal distension.

Changes in hemodynamic and echocardiographic parameters

Table 1 shows the difference of each parameter at BL and CEPH. In the CEPH models, PA/Ao was increased, and AT/ET was decreased significantly compared to BL. Although TR were observed by color Doppler imaging and measured (median 3.09 m/sec) via continuous wave Doppler in all dogs with CEPH, the regurgitant jets were quite small and limited to the tip of the tricuspid valve leaflets, so we could not obtain a complete TR profile. Similarly, PR was detectable in four dogs with CEPH (peak PR; median 2.45 m/sec, end PR; median 1.34 m/sec) but the related regurgitant jets were also small, making it difficult to obtain complete PR profiles.

Partial correlation analysis between echocardiographic parameters and invasive PA pressures

Partial correlations adjusting for the effect of dogs between each PAP and echocardiographic parameter are shown in Table 2. AT/ET, PA/Ao, RPAD_M, M, RVIDdn and TAPSe were correlated with each PAP. In this study, we did not detect a significant correlation between TRPG or PRPG and each PAP.

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Multiple linear regression analysis

In Table 3, multiple linear regression analysis revealed that AT/ET and RVIDdn were independent predictors of sPAP, and that PA/Ao and RVIDdn were independent predictors of mPAP. In addition, PA/Ao and RPAD M-mode were selected as independent predictors of dPAP.

ROC analysis

ROC analysis was used to determine the noninvasive cut-off values for predicting the presence of CEPH (sPAP >30 mmHg and mPAP >20 mmHg). Table 4 shows the result of our ROC analyses. We note that two metrics had high sensitivity and specificity to be able to predict CEPH: 1) AT/ET (cut-off value: 0.45), 2) PA/Ao (cut-off value: 0.97).
Assessment of variability showed that all CV mean values were below 10%, except for RVFAC and RPAD B-mode at CEPH, which was 27.1 and 14.9%, respectively.

**DISCUSSION**

In this study we showed that RVIDdn was correlated positively with each PAP, and identified it as an independent predictor of sPAP and mPAP via multiple linear regression analysis. Therefore, routine measurement of this parameter may bring a significant benefit to clinicians in being able to predict the elevation of PAP.

We found that PA/Ao was positively correlated with each PAP, and that it was also identified as an independent predictor of both mPAP and dPAP by multiple linear regression analysis. Previous studies have reported that PA/Ao is related to invasive sPAP, and our findings are consistent with those from earlier investigations [7, 19, 28]. In addition, clinical cases with pre–capillary PH showed elevation of this parameter in several studies, and this finding confirms our results [13, 24]. Furthermore, PA/Ao with a cut-off value of 0.97 could predict mild CEPH with high sensitivity and specificity in this study. Therefore, this parameter might be useful for the identification of mild elevation of PAP with or without significant TR.

We also found that TAPSEn was correlated with each PAP, although it was not selected as an independent variable regarding each PAP by multiple linear regression analysis. In contrast to our results, TAPSEn had no correlation with TRPG in an earlier study that included dogs with PH secondary to spontaneous myxomatous mitral valve disease (MMVD) [25]. Additionally, another study revealed that TAPSEn was not significantly different across dogs in varying MMVD or PH severity groups [15]. In an effort to characterize TAPSE, a previous study, which examined the effect of LV systolic function on TAPSE in human patients with PH, showed that this parameter was dependent on not only the RV systolic function but also the LV systolic function [12]. Though there is one study showing the tendency for lower TAPSE in patients with PH secondary to MMVD [4], TAPSE might have failed to reflect impaired RV systolic function in dogs with PH secondary to MMVD because increased LV systolic function could have influenced the RV systolic function due to the increment of preload and decrement of afterload resulting from mitral insufficiency. However, previous study including patients with pre–capillary PH indicated a decrease in TAPSEn [13]. Therefore, a decrease in TAPSEn over time might reflect impaired RV systolic function in PH caused not by post-capillary, but by pre-capillary pathogeneses.

A number of previous reports have showed that AT/ET is negatively correlated with PAP and TRPG [7, 17–19, 28], and we also confirmed these relationships in our current study. In addition, CEPH models with mildly elevated PAP in our investigation showed a decreased AT/ET value compared to BL, and this parameter could predict PH with excellent sensitivity and specificity. In clinical practice, previous study including patients with pre-capillary PH indicated a decrease in AT/ET [4, 24]. Furthermore, AT/ET with a cut-off value of 0.45 obtained in the current study is similar to that (0.44) in a previous report where TRPG was used to diagnose PH [19]. Thus, it fortunately appears that an impaired AT/ET value can predict the presence of PH in patients regardless of the presence or absence of TR.

| Dependent variable | Independent variable | Partial regression coefficient (95% CI) | Standard partial regression coefficient | P-value |
|--------------------|----------------------|----------------------------------------|----------------------------------------|---------|
| sPAP (mmHg)        | AT/ET                | −161.0 (−263.6–58.3)                   | −0.57                                  | <0.05   |
|                    | RVIDdn               | 5.6 (1.5–9.7)                          | 0.50                                   | <0.05   |
| mPAP (mmHg)        | PA/Ao                | 89.0 (55.3–122.8)                      | 0.69                                   | <0.01   |
|                    | RVIDdn               | 4.0 (1.9–6.0)                          | 0.51                                   | <0.01   |
| dPAP (mmHg)        | PA/Ao                | 76.2 (43.8–108.7)                      | 0.77                                   | <0.01   |
|                    | RPAD M-mode          | −0.4 (−0.6–−0.1)                       | −0.43                                  | <0.05   |

sPAP, systolic pulmonary artery pressure; mPAP, mean pulmonary artery pressure; dPAP, diastolic pulmonary artery pressure; AT/ET, acceleration time to ejection time ratio in pulmonary artery flow profile; RVIDdn, normalized right ventricular internal diameter in diastole; PA/Ao, the ratio of the pulmonary artery and aortic diameter in diastole; RPAD, the right pulmonary artery distensibility index; CI, confidence interval.

| Cut-off value | Sensitivity (%) | Specificity (%) | AUC (95% CI) | P-value |
|---------------|-----------------|-----------------|--------------|---------|
| AT/ET         | 0.45            | 100             | 100          | 1.00 (1.00–1.00) | <0.01   |
| PA/Ao         | 0.97            | 100             | 80           | 0.92 (0.74–1.00) | <0.05   |

AT/ET, acceleration time to ejection time ratio in pulmonary artery flow profile; PA/Ao, the ratio of the pulmonary artery and aortic diameter in diastole; AUC, area under the curve; CI, confidence interval.
We also showed that RPAD M-mode was negatively correlated with each PAP, and that it was also identified as an independent predictor of dPAP by multiple linear regression analysis. This finding is consistent with previous study showing significant correlation between RPAD M-mode and PAP obtained invasively by RHC in heartworm-infected dogs [26]. Therefore, this parameter might be useful for the identification of mild elevation of PAP in dogs with pre-capillary PH.

In the present study a significant correlation between our estimated PG and each PAP value was not observed, although TR and PR were confirmed and measured in some dogs with CEPH. We tried to interrogate TR and PR using multiple views, but this was less than fully successful, leading to unclear TR and PR profiles. This outcome supports the theory that TR and PR are not necessarily apparent in dogs with PH. In addition, a recent study in human medicine reported a lack of TR in patients with PH [9]. Furthermore, a previous report by Soydan et al. showed that TRPG and PRPG imperfectly estimated each PAP with wide variation in canine models of acute PH [14]. Consequently, patients with the type of altered echocardiographic parameters mentioned above may have PH, even if TR or PR are not observed. Indeed, noninvasive estimation by these regurgitation velocities may be better utilized as part of a comprehensive assessment in canine patients with PH.

There are several limitations in this study. First, the number of dogs included here was small. Second, we used CEPH models without significant TR and/or PR, and the elevation of PAP was mild. Thus, our findings in this study might not be applied to patients with significant TR and/or PR suggesting high PAP, or patients with pre–capillary PH secondary to other pathogeneses. In addition, it remains unclear whether the echocardiographic parameters we regarded as useful are tightly correlated with PAPs measured in patients with spontaneous PH, especially in cases secondary to MMVD. Further studies are warranted to investigate the relationship between echocardiographic parameters and invasive PAP in patients with spontaneous PH. Thirdly, we conducted echocardiography and RHC using conscious dogs, even though there is a risk that sedative and anesthetic agents might have influenced their hemodynamics. To help prevent this possibility we allowed sufficient recovery time so that the dogs could walk without assistance. In addition, we could not conduct echocardiography and RHC simultaneously, which might have influenced analysis results. Finally, in this study, observer of echocardiography could guess whether the dog were healthy or had CEPH because we needed long period of time to create our preferred model. Thus, it might have influenced echocardiographic measurements.

In conclusion, AT/ET, PA/Ao, RPAD M-mode, RVIDdn and TAPSEn were correlated with invasive PAP, and alterations in PA/Ao or AT/ET might suggest PH, even if TR is not observed.

CONFLICT OF INTEREST. We have no conflicts of interest.

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