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STANDARD ARTICLE

Echocardiographic evaluation of velocity ratio, velocity time integral ratio, and pulmonary valve area in dogs with pulmonary valve stenosis

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Background: Velocity ratio, velocity time integral (VTI) ratio, and pulmonary valve area indexed to body surface area (iPVA) are methods of assessment of pulmonary valve stenosis (PS) severity that are less dependent on blood flow. Studies evaluating these methods are limited.

Objectives: To determine the effects of butorphanol, atenolol, and balloon valvuloplasty (BV) on velocity ratio, VTI ratio, iPVA, mean PG, and max PG.

Animals: Twenty-seven dogs with PS (max PG >50 mm Hg).

Methods: Prospective study. All dogs underwent an echocardiogram at baseline, 5-minutes after administration of butorphanol (0.2-0.25 mg/kg IV), and 2-to-4 weeks after atenolol (1-1.5 mg/kg q12h). Twenty-one of these were evaluated 24-hours after BV.

Results: There were no significant differences ($P > .05$) amongst any of the methods of assessment of PS severity after butorphanol. After atenolol, mean (SD) of mean (57.0 [21.0] mm Hg) and max PG (93.1 [33.8] mm Hg) were significantly decreased ($P \leq .047$) compared with baseline (65.2 [26.2] mm Hg and 108 [44.4] mm Hg, respectively). After atenolol, there were no significant ($P \geq .12$) differences in velocity ratio (0.29 [0.09]), VTI ratio (0.18 [0.05]), or iPVA (0.43 [0.16] cm^2/m^2) compared with baseline (0.30 [0.09], 0.19 [0.09], 0.44 [0.17] cm^2/m^2, respectively).

Conclusions and Clinical Importance: Atenolol might reduce mean and max PG but does not alter less flow-dependent methods of assessment of PS severity (velocity ratio, VTI ratio, and iPVA) in dogs with PS. Results support an integrative approach to assessment of PS severity that includes less flow-dependent methods, particularly in states of altered flow or right ventricular function.

KEYWORDS

canine, echocardiography, effective orifice area, pressure gradient, pulmonic stenosis, sedation

Abbreviations: 2D, two-dimensional; AoD, aortic valve diameter; AV, aortic valve; BV, balloon valvuloplasty; CI, cardiac index; CSA, cross-sectional area; PFO, patent foramen ovale; iPVA, pulmonary valve area indexed to body surface area; PV mean PG, pulmonary valve mean pressure gradient; PV max PG, pulmonary valve maximum pressure gradient; PS, pulmonary valve stenosis; PV, pulmonary valve; RV, right ventricle/ventricular; RV S', peak systolic RV myocardial velocity at the lateral tricuspid annulus; SV, stroke volume; iTAPSE, tricuspid annular plane systolic excursion indexed to body weight; V_{max}, maximum velocity; VTI_{AV}, velocity time integral of the aortic valve; VTI_{PV}, velocity time integral of the pulmonary valve

1 | INTRODUCTION

Pulmonary valve stenosis (PS) is one of the most commonly diagnosed congenital heart diseases in dogs.[1,2] Clinical management of dogs with PS is largely dependent on its severity and if clinical signs are evident. Although prospective controlled treatment studies are lacking, many clinicians agree that dogs with clinical signs or severe PS are likely to

benefit from balloon valvuloplasty (BV).[3–5] Treatment with beta-blockers such as atenolol might also be recommended depending on the severity of PS or the presence of clinical signs, tachyarrhythmias, or dynamic outflow tract obstruction. Assessment of PS severity plays a pivotal role in the clinical management of dogs with PS including, decisions on when to intervene with treatment, judging effectiveness of BV, and prognosis.[6,7]

The most common and only echocardiographic method mentioned in guidelines[8] published in the veterinary literature to determine the severity of PS is Doppler echocardiography-derived maximum velocity of blood flow across the stenotic pulmonary valve (PV). Maximum velocity is then converted to the PV maximum pressure gradient (PV max PG) using the simplified Bernoulli equation. Advantages of this method include its ease to acquire and body size-independence. However, in addition to the degree of stenosis, pressure gradients are largely influenced by transvalvular flow, as described by Gorlin's formula.[9] Reduced cardiac output caused by, for example, sedatives, anesthetics, negative inotropic drugs, or ventricular systolic dysfunction might underestimate stenosis severity.[9–11] Conversely, high cardiac output states such as stress/anxiety, anemia, heightened sympathetic tone, or after interventional states might overestimate stenosis severity.[9,10,12]

Current human guidelines[13] recommend an integrative approach for assessment of aortic valve (AV) stenosis severity, and do not recommend solely relying on maximum velocity or pressure gradient. In addition to mean PG and max PG, the continuity equation valve area is recommended for routine assessment.[13] Calculation of valve area is based on the principal that flow volume at different locations in a closed system are equal.[9,14] Hence, stroke volume (SV) through the PV is equal to SV through the AV, and SV represents the product of cross-sectional area (CSA) and the distance blood moves over the ejection period, called stroke distance or velocity time integral (VTI). This equation ($CSA_{AV} \times VTI_{AV} = CSA_{PV} \times VTI_{PV}$) can be rewritten to solve for pulmonary valve area (PVA) as: $PVA = (CSA_{AV} \times VTI_{AV}) \div VTI_{PV}$.[9,14] In theory, this method is less dependent on transvalvular flow, representing its major advantage. Disadvantages include the necessity to index to body size, its more time-consuming nature, and the large impact of small measurement errors in estimating CSA. To overcome these limitations, the equation can be simplified by eliminating CSA to determine the VTI ratio (VTI_{AV}/VTI_{PV}) or maximum velocity ratio ($Vmax_{AV}/Vmax_{PV}$).[13,15] Other than a single case example,[14] a comprehensive evaluation of PVA, VTI ratio, or velocity ratio has not been reported in dogs with PS.

The objective of our study was to determine the effects of butorphanol, atenolol, and BV on PVA, VTI ratio, velocity ratio, PV mean PG, and PV max PG. Butorphanol was selected because it is a commonly used sedative utilized to help facilitate echocardiographic examinations in dogs with PS. Because sedatives and negative inotropic drugs, such as atenolol, are expected to reduce transpulmonic flow, we hypothesized that these drugs would reduce the flow-dependent methods of assessment of PS severity (PV mean PG and PV max PG) but would not alter the less flow-dependent methods of assessment of PS severity (PVA, VTI ratio, velocity ratio). Lastly, as proof-of-principal, we expected PVA, VTI ratio, and velocity ratio to be significantly increased after BV.

2 | MATERIALS AND METHODS

2.1 | Animals

Study subjects were client owned dogs that presented to our hospital's cardiology service for evaluation of known or suspected cardiac disease or were referred for the purposes of our study. Owner consent was obtained for each dog before enrollment. Dogs were consecutively enrolled over a 15-month period if they were diagnosed with PS and had a max PG at the level of the PV >50 mm Hg that was verified by a board-certified cardiologist. This max PG was used to represent dogs with at least moderate PS according to the veterinary guidelines where moderate PS = 50–80 mm Hg, and severe PS = >80 mm Hg.[8] Dogs were excluded from the study if they had any additional cardiovascular disease, were suspected to be affected with systemic disease based on a history and physical examination, were diagnosed with congestive heart failure previously or during their evaluation for the study, had a sustained clinically important arrhythmia, such as atrial fibrillation, or were taking any medication(s) that affect the cardiovascular system. Dogs diagnosed with a patent foramen ovale (PFO) were not excluded provided the shunting was considered mild based on an agitated saline contrast study and their hematocrit was <55%. Dogs with mild regurgitation of the tricuspid, mitral and AV (assessed subjectively) were not excluded, provided valve morphology appeared normal.

2.2 | Study design

In this prospective clinical trial, all dogs underwent a cardiovascular examination and a baseline echocardiographic examination. After the baseline echocardiographic examination, dogs received butorphanol 0.2-0.25 mg/kg IV once. Approximately 5-minutes after administration of butorphanol, dogs underwent a second, identical, echocardiographic examination. At the end of the second echocardiogram, an agitated saline contrast study was performed in all dogs to determine if a right-to-left shunting PFO was present. Dogs were then discharged to their caretaker and instructed to be administered atenolol 1.0–1.5 mg/kg PO q12h. Owners were scheduled to return in 2–4 weeks, where a third echocardiogram was performed ∼2–4 hours after atenolol. At this visit, all dog owners were offered BV at the discretion of the attending clinician to be performed the next day. If BV was performed, dogs underwent a 4th echocardiographic examination the morning after BV ∼2–4 hours after atenolol. For the purpose of our study further follow-up was not performed. Eight dogs were selected (based on owner consent) to undergo within-day variability studies whereby each dog underwent an additional echocardiogram performed by the same operator 2–4 hours after the first echocardiogram and before any drug administration. An effort was made to have all of the echocardiographic examinations for each dog in our study performed by the same sonographer (operator) but was not always possible because of conflicting patient and clinician schedules. The sonographer's echocardiographic measurements (performed for clinical purposes) were not used for study purposes, as a single investigator performed all echocardiographic measurements and assessments.

2.3 | Echocardiographic assessment

2.3.1 | Image acquisition

All echocardiographic studies (Philips EPIQ 7C or iE33, Philips Healthcare, Andover, MA) were performed by a board-certified veterinary cardiologist or a cardiology resident under the direct supervision of a board-certified veterinary cardiologist. A variety of transducers were utilized for our study and were matched to the size of the dog to optimize 2D and Doppler image quality. A standardized echocardiographic imaging protocol and imaging planes[16] were used for each dog. A simultaneous ECG was recorded during each echocardiographic examination. The right parasternal long axis 5-chamber view optimized for the left ventricular outflow tract and ascending aorta was used to measure the aortic valve diameter (AoD). The zoom feature was frequently used to view the AV and aid measurement of the AoD. A right parasternal short axis basilar view optimized for the right ventricular (RV) outflow tract was used to acquire the maximum blood velocity atranspulmonic velocity using continuous-wave Doppler using 2D and color Doppler guidance. The subcostal view was used to acquire pulsed-wave Doppler profiles at the level of the AV. The sample volume (1–2 mm) was positioned at the level of the AV hinge points. A left apical 4-chamber view optimized for the RV was used to acquire M-mode recordings of tricuspid annular plane systolic excursion (TAPSE) and pulsed-wave tissue Doppler-derived peak systolic RV myocardial velocity at the lateral tricuspid annulus (RV S') as previously described.[17] For all spectral Doppler recordings, the baseline, scale, and gain were manually adjusted in an attempt to optimize the Doppler signal. Sweep speeds of at least 100 mm/s were used. Particular care was taken to avoid over-gained signals and to align the cursor as parallel as possible with high velocity blood flow through the RV and LV outflow tract.

2.3.2 | Echocardiographic measurements and calculations

All echocardiographic measurements were performed by a single cardiology resident at a digital off-cart workstation (Syngo Dynamic Workplace, Version 10.0.01_HF04_Rev5 [Build 2884], Siemens Medical Solutions, Inc, Malvern, Pennsylvania). This investigator was blinded to the drug status and sequence of the echocardiographic examinations, as all echocardiographic studies were coded and randomized. Values for each echocardiographic variable consisted of the average of 5 usually consecutive measurements. The average of 5 measurements was recorded because the common occurrence of sinus arrhythmias was anticipated after butorphanol and atenolol. We anticipated AoD to be less affected by a sinus arrhythmia and therefore the average of 3 measurements was recorded. The AoD (in centimeters) was measured in early systole between the hinge points of the maximally opened AV leaflets. The pulsed-wave Doppler-derived profile of the AV was determined by tracing the outer edge of the modal velocities (denser signals) throughout systole.[13] The software package then calculated the VTI of the AV (VTI_{AV}). Aortic valve maximum blood velocity ($Vmax_{AV}$) was also measured. To determine the PV mean pressure gradient (PV mean PG) and VTI of the PV (VTI_{PV}), the exact same technique was applied to the continuous-wave Doppler profile of the PV. The echocardiographic measurement software package provided the VTI and mean PG values after tracing Doppler profiles. Care was taken to trace the modal velocities and avoid including fine linear signals at the peak of the curve.[13] Pulmonary valve maximum pressure gradient was determined by measuring the peak of outer edge of the denser signal while avoiding measurement of fine linear signals.[13] Pulmonary valve maximum blood velocity ($Vmax_{PV}$) was converted to a pressure gradient using the simplified Bernoulli equation: pressure gradient = 4 × velocity $(m/s)^2$. Heart rate was recorded for each echocardiographic examination and represents the average of the heart rates recorded during each of the echocardiographic measurements acquired during that examination.

Velocity time integral ratio (VTI_{AV}/VTI_{PV}) was calculated as VTI_{AV} (cm) ÷ VTI_{PV} (cm). Velocity ratio was calculated as $Vmax_{AV}$ (m/s) ÷ $Vmax_{PV}$ (m/s). Cross sectional area of the AV (CSA_{AV}) was calculated as: $\pi \times (AoD \div 2)^2$. Stroke volume was calculated as $CSA_{AV} \times VTI_{AV}$, cardiac output (L/min) as heart rate (gathered from the time the VTI was recorded) × SV, and cardiac index (CI) $(L/min/m^2)$ as cardiac output ÷ body surface area. The continuity equation was used to estimate the PVA (ie, the effective orifice area) as follows: PVA $(cm^2) = (CSA_{AV} \times VTI_{AV}) \div VTI_{PV}$.[9,14] The PVA was then indexed to each dog's body surface area (iPVA) as follows: PVA ÷ body surface area (m^2). Tricuspid annular plane systolic excursion was indexed to bodyweight (iTAPSE) according to the formula: iTAPSE = TAPSE (mm) ÷ bodyweight $(kg)^{0.297}$.[17]

2.4 | Echocardiographic repeatability and measurement variability

Within-day repeatability was determined by having a single investigator measure the 2 echocardiographic examinations that were performed by the same sonographers from the 8 dogs selected to assess within-day repeatability. Measurements were performed at least 2 weeks apart with the investigator blinded to previous measurements. Intraobserver measurement variability was determined by having a single investigator measure 9 randomly selected echocardiographic studies (3 baseline, 3 after butorphanol, and 3 after atenolol) on 3 separate occasions. These measurements were separated by at least 1 week and the investigator was blinded to previous results. Interobserver measurement variability was determined by having 2 additional investigators (3 total) measure the echocardiographic variables from the same 9 randomly selected dogs. These investigators were blinded to the results of the previous measurements as well as the other investigators' measurements.

2.5 | Balloon valvuloplasty

Balloon valvuloplasty was performed in a standard fashion using fluoroscopic guidance.[18] Specific decisions on type of equipment used (eg, catheter type and size) were at the discretion of the attending clinician although the procedure was generally performed in a similar manner as follows. A vascular introducer was placed in the right jugular vein. After pressure assessment of the pulmonary artery and RV, a right ventriculogram was performed using a flow-directed angiographic catheter to allow for measurement of the PV annulus. Balloon size was based on multiplying the PV annulus diameter by a factor of

TABLE 1 Echocardiographic data from 27 dogs with PS at baseline, after butorphanol, and after atenolol

	Baseline	After butorphanol	After atenolol	Overall *P*-value
Heart rate (min^{-1})	131 (24)	106 (26)[a]	109 (20)[a]	**<.001**
CI ($L/min/m^2$)	4.2 (1.4)	3.6 (1.2)[a]	3.1 (0.8)[a,b]	**<.001**
PV mean PG (mm Hg)	65.2 (26.2)	63.2 (25.5)	57.0 (21.0)[a,b]	**.002**
PV max PG (mm Hg)	108 (44.4)	105 (41.1)	93.1 (33.8)[a,b]	**<.001**
$Vmax_{AV}/Vmax_{PV}$	0.30 (0.09)	0.30 (0.09)	0.29 (0.09)	.41
VTI_{AV}/VTI_{PV}	0.19 (0.06)	0.19 (0.06)	0.18 (0.05)	.12
iPVA (cm^2/m^2)	0.44 (0.17)	0.42 (0.16)	0.43 (0.16)	.42
iTAPSE	4.2 (0.7)	4.1 (1.2)	3.7 (0.9)[a]	**.035**
RV S' (cm/s)[#]	8.2 (6.5–10.1)	8.2 (6.1–10.2)	7.4 (5.0–10.3)[a]	**.019**

Abbreviations: AV, aortic valve; iPVA, pulmonary valve area indexed to body surface area; iTAPSE, tricuspid annular plane systolic exclusion indexed to body weight; PG, pressure gradient; PV, pulmonary valve; RV S', peak systolic RV myocardial velocity at the lateral tricuspid annulus; VTI, velocity time integral.

[#]Non-normally distributed, median (interquartile range) reported.

[a]Post-hoc comparison significantly different ($P < .05$) from baseline examination.

[b]Post-hoc comparison significantly different ($P < .05$) from examination after butorphanol.

Bolded values denote statistical significance.

1.3-1.5.[18] Each dog had at least 2 inflations but, if necessary, repeat inflations were performed until resolution of the stenotic waist was observed. All balloons were inflated to between nominal and rated burst pressure and held for 3–5 seconds. If there was concern for an anomalous prepulmonic coronary artery based on a levophase angiogram or computed tomography and angiography study before BV, it was not attempted.

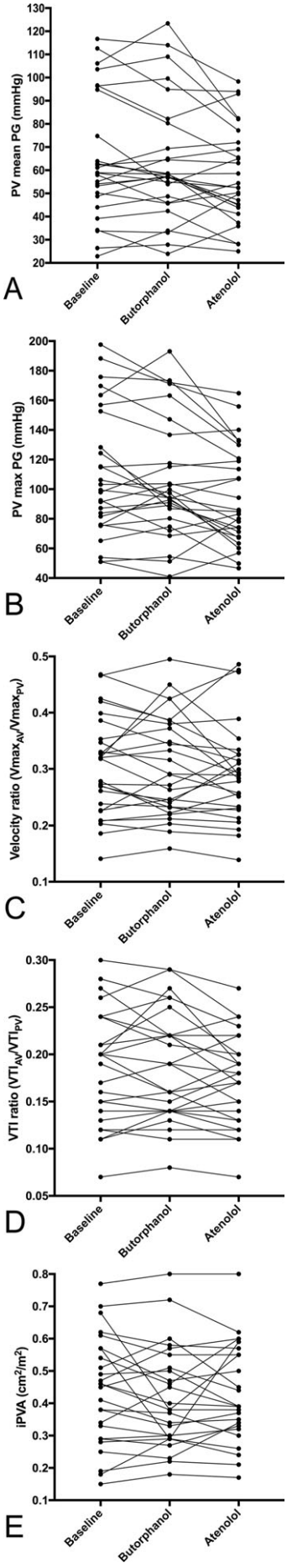
2.6 | Statistical analysis

All statistical analyses were performed using commercial software packages (Prism 7 for Mac OS X, Version 7.0d, GraphPad Software, Inc, La Jolla, CA and MedCalc Statistical Software for Windows 10, Version 18, MedCalc Software bvba, Ostend, Belgium). Descriptive statistics were generated and normality testing with the Shapiro-Wilk test was performed for all continuous data. Data are reported as mean (SD) unless otherwise stated. Comparisons of baseline, after butorphanol, after atenolol data were made with one-way repeated measures ANOVA with Tukey's post-hoc test or Friedman's test with Dunn's post-hoc test if non-normally distributed. Differences in before BV (echocardiographic studies after atenolol) versus after BV data were evaluated by a paired *t*-test or a Wilcoxon matched-pairs signed rank test if non-normally distributed. Pearson product-moment correlation analyses were used to determine the strength of association between the pressure gradient based methods (PV max PG and PV mean PG) and iPVA, VTI ratio, and velocity ratio from the baseline echocardiographic studies. Within-day repeatability, interobserver and intraobserver measurement variability were each quantified by a 2-way single-measure intraclass correlation coefficient (ICC) for absolute agreement and by the coefficient of variation (CV). Percent CV was calculated as follows: (within-subject standard deviation ÷ mean) × 100. For the purpose of our study, an ICC >0.80 and a CV <10% were defined to represent high agreement/low variability. A probability of $P < .05$ was considered statistically significant. No adjustment for multiple analyses was made.

3 | RESULTS

Twenty-nine dogs were enrolled in our study. Of these, 2 dogs were excluded; 1 because of failure to return for the after atenolol examination and one was being administered an incorrect dose of atenolol. Of the remaining 27 dogs, 21 underwent BV. Three dogs did not undergo BV because of a suspected anomalous prepulmonic coronary artery (all Bulldogs) and 3 because their owners elected not to have the procedure performed. Breeds included were mixed breeds (n = 9), pit bull terriers (n = 4), French bulldogs (n = 4), Bulldogs (n = 4), and one of each of the following breeds; American cocker spaniel, Entlebucher mountain dog, German shepherd dog, Basset hound, Manchester terrier, and Redbone coonhound. There were 13 females and 14 males. Mean (SD) age was 1.9 (1.8) years, and bodyweight was 15.0 (9.0) kg. Four dogs were diagnosed with a right-to-left shunting PFO, and their packed cell volumes ranged from 35 to 51%. Mean (SD) butorphanol dose was 0.22 (0.02) mg/kg and atenolol dose was 1.3 (0.2) mg/kg PO q12h. Mean (SD) time from baseline examination to after atenolol examination was 22 (10) days.

A summary of the echocardiographic data from 27 dogs with PS at baseline, after butorphanol, and after atenolol is presented in Table 1 and Figure 1. Aside from a significant reduction in heart rate ($P < .001$) and CI ($P = .025$), there were no significant differences (all $P > .05$) in methods of assessment of PS severity (PV mean PG, PV max PG, VTI ratio, velocity ratio, or iPVA) or indices of RV systolic function (iTAPSE and RV S') after butorphanol. After atenolol, PV mean PG and PV max PG were significantly reduced (all $P \leq .047$) compared with baseline and after butorphanol echocardiographic examinations. Whereas VTI ratio, velocity ratio, and iPVA did not demonstrate a significant difference (all $P \geq .12$) after atenolol compared with baseline or after butorphanol. Heart rate and RV function quantified by iTAPSE, and RV S' were significantly reduced (all $P \leq .022$) after atenolol compared with baseline. The max PG changed from >80 to <80 mm Hg in 8 of 27 dogs (30%) after atenolol whereas this occurred in 1 dog (4%) after butorphanol.



A summary of the echocardiographic data of the 21 dogs that underwent BV is presented in Table 2. Compared with before BV (ie, the echocardiographic examination after atenolol), heart rate, PV mean PG, and PV max PG were all significantly (all $P < .001$) decreased after BV. Additionally, CI, VTI ratio, velocity ratio, iPVA, iTAPSE, and RV S' were all significantly (all $P \leq .031$) increased after BV compared with before BV.

Significant linear correlations of PV max PG to iPVA (r = −0.63; $P < .001$), VTI ratio (r = −0.73; $P < .001$), and velocity ratio (r = −0.64; $P < .001$) were observed from the baseline echocardiographic studies (n = 27). Similar significant linear correlations of PV mean PG to iPVA (r = −0.62; $P < .001$), VTI ratio (r = −0.69; $P < .001$), and velocity ratio (r = −0.59; $P < .001$) were observed.

Table 3 shows the results of the echocardiographic variability studies for the indices of PS severity. With the exception of within-day repeatability of iPVA (CV = 10.5%), all echocardiographic methods for assessing severity of PS had low variability (ie, ICC > 0.80 and CV < 10%) with regard to within-day repeatability, intraobserver measurement variability, and interobserver measurement variability.

4 | DISCUSSION

Our results supported our hypothesis that atenolol causes a decrease in the flow-dependent methods of assessment of PS severity (PV mean PG and PV max PG) but the less flow-dependent methods, VTI ratio, velocity ratio, and iPVA, would be unaffected. In contrast to our hypothesis, the sedative butorphanol (0.2-0.25 mg/kg IV) did not significantly alter the flow-dependent methods of assessment of PS severity. As proof-of-principal we were able to demonstrate that iPVA, velocity ratio, and VTI ratio were all significantly increased after BV in addition to documenting significant decreases in PV mean PG and PV max PG after BV. Lastly, our results showed that the measurement variability and within-day repeatability of the 5 indices of assessment of PS severity (PV mean PG, PV max PG, iPVA, velocity ratio, and VTI ratio) were well within clinically acceptable limits (ICC > 0.80, CV < 10%) or close (iPVA within-day repeatability CV = 10.5%).

Applying the continuity equation valve area (also called effective orifice area) concept to the PV in dogs with PS has not been comprehensively evaluated. However, the concept has been introduced and its advantages and disadvantages discussed in the veterinary literature.[9,14] In humans with valvular aortic stenosis, AV area is routinely

FIGURE 1 Pulmonary valve mean pressure gradient (PV mean PG) (A), PV maximum pressure gradient (PV max PG) (B), Velocity ratio (C), VTI ratio (D), and PV area indexed to body surface area (iPVA) (E) at baseline, 5-minutes after butorphanol (0.2-0.25 mg/kg IV), and 2-to-4 weeks after atenolol (1-1.5 mg/kg PO q12h) in 27 dogs with PS. No significant differences ($P > .05$) for any of the methods of assessment of PS severity were noted when comparing baseline to after butorphanol. After atenolol, PV mean PG and PV max PG were significantly reduced (all $P \leq .047$) compared with baseline and after butorphanol. Velocity ratio, VTI ratio, and iPVA did not demonstrate a significant difference (all $P \geq .12$) after atenolol compared with baseline or after butorphanol. Vmax, maximum blood velocity; AV, aortic valve; PV, pulmonary valve

TABLE 2 Echocardiographic data from 21 dogs with PS before and after BV

	Before BV*	After BV	*P*-value
Heart rate (min^{-1})	108 (20)	87 (24)	**<.001**
CI (L/min/m^2)	3.1 (0.9)	3.7 (1.4)	**.023**
PV mean PG (mm Hg)	60.6 (19.6)	22.3 (11.0)	**<.001**
PV max PG (mm Hg)	99.3 (32.8)	40.5 (17.5)	**<.001**
$Vmax_{AV}/Vmax_{PV}$	0.28 (0.09)	0.45 (0.12)	**<.001**
VTI_{AV}/ VTI_{PV}	0.18 (0.05)	0.35 (0.15)	**<.001**
iPVA (cm^2/m^2)	0.39 (0.13)	0.88 (0.40)	**<.001**
iTAPSE	3.7 (0.9)	4.3 (1.0)	**.012**
RV S' (cm/s)#	6.7 (5.0–10.6)	8.2 (7.2–9.8)	**.031**

Abbreviation: BV, balloon valvuloplasty.
See Table 1 for the remainder of the key.
*Before BV represents the after atenolol echocardiographic data.
#Non-normally distributed, median (interquartile range).

estimated using this equation with the proximal or subvalvular region serving as the reference location or alternate anatomic location for which to calculate SV and solve for the unknown stenotic valve area.[13] To provide a frame of reference, one of the authors has generated reference values for iPVA from 70 healthy dogs using Clinical and Laboratory Standards Institute guidelines[19] and normal iPVA can be considered to be roughly 1.8-4.0 cm^2/m^2 (Visser LC, unpublished data). In addition, in humans, severe AV stenosis is considered to be an indexed AV area <0.6 cm^2/m^2.[13] In our study, we elected to use the alternate semilunar valve instead of the proximal RV outflow tract or subvalvular region due clinical experience suggesting it might be challenging to obtain reliable pulsed-wave Doppler profiles from the proximal RV outflow tract. Clinical experience also suggests it would be easier to obtain more reliable pulsed-wave Doppler profiles and measurement of the AV diameter compared with the proximal RV outflow tract. In addition, we hoped to avoid the potential complicating finding of a subvalvular lesion in some dogs with PS.[20–22] Further, a previous study has demonstrated a strong agreement of SV and cardiac output between the aortic and pulmonary root using Doppler echocardiography.[23]

The continuity equation valve area has been studied in dogs with subaortic stenosis[24,25] and in an experimental canine model of supravalvular aortic stenosis.[11] These studies evaluated the continuity equation valve area for the left ventricular outflow tract primarily in the context of its ability to predict adverse outcomes, development of subaortic stenosis in adulthood, and CSA over a wide range of hemodynamic conditions. Our study was designed to evaluate the continuity equation valve area applied to the PV (and VTI/velocity ratio). Specifically, our primary goal was to study the effects of altering transvalvular flow on the conventional flow-dependent pressure gradient-based methods and the less flow-dependent methods of assessment of PS severity in a clinically relevant manner. We anticipated that the sedative butorphanol, the beta-blocker atenolol, and BV would sufficiently alter transvalvular flow, and thus studying its effects on various methods of assessment of PS severity would provide clinically useful information. As expected, we documented that the less flow-dependent methods of assessment of PS severity (iPVA, VTI ratio, and velocity ratio) were unaffected by atenolol, whereas the flow-dependent methods of assessment of PS severity (PV mean PG and PV max PG) were significantly decreased. Pulmonary valve mean PG and PV max PG were almost certainly reduced because of reduced transvalvular flow secondary to the known cardiodepressant effects of atenolol versus a true change in valve area. The significant reduction in heart rate, RV systolic function (iTAPSE and RV S') and CI after atenolol strengthens this notion.

Our results suggest that PV mean PG and PV max PG are less accurate when evaluating dogs with PS on atenolol or in the setting of RV systolic dysfunction. The severity of PS might be underestimated in these clinical situations if only max (or mean) PG is used for assessment of severity. This is concerning when considering clinical management and prognosis assessment of dogs with PS is largely guided by max PG assessment (in addition to clinical signs).[4–7] For example, 30% of the dogs in our study transitioned from severe PS (max PG >80 mm Hg) to moderate PS (max PG = 50–80 mm Hg) when re-evaluated after atenolol. In addition to the statistically significant difference of max PG after atenolol, this helps highlight the clinical importance of our findings. Our results also highlight the importance of assessing RV systolic function in dogs with PS, as atenolol (1-1.5 mg/kg PO q12h) decreased RV systolic function. A decrease in TAPSE and RV S' has also been documented after a single oral dose of atenolol in healthy dogs.[26]

In contrast to our hypothesis, a single dose of butorphanol (0.2-0.25 mg/kg IV) did not significantly alter PV mean PG or PV max PG (or any of the other methods of assessment of PS severity), despite documenting a mild decrease in heart rate and CI. There are

TABLE 3 Echocardiographic within-day repeatability and intraobserver and interobserver measurement variability in dogs with PS

	Measurement variability (n = 9)					
	Intraobserver		Interobserver		Within-day repeatability (n = 8)	
	ICC	CV (%)	ICC	CV (%)	ICC	CV (%)
PV mean PG (mm Hg)	0.99	2.7	0.94	7.4	0.98	6.6
PV max PG (mm Hg)	0.99	2.4	0.95	6.8	0.98	5.8
$Vmax_{AV}/Vmax_{PV}$	0.99	2.8	0.96	6.8	0.96	7.8
VTI_{AV}/ VTI_{PV}	0.99	2.6	0.95	6.4	0.95	9.2
iPVA (cm^2/m^2)	0.99	4.1	0.98	5.3	0.96	10.5

Abbreviations: CV, coefficient of variation; ICC, intraclass correlation coefficient.
See Table 1 for the remainder for the key.

several potential explanations for this finding. First, the reduction in transvalvular flow as quantified by CI, although statistically significant, was not to the same degree when compared to the reduction in CI after atenolol, and CI after atenolol was significantly decreased beyond that after butorphanol. This could potentially be explained by the lack of effect of butorphanol on RV systolic function, as iTPASE and RV S' were not significantly different after butorphanol compared with baseline. This lack of effect of butorphanol on RV systolic function was also demonstrated in a recent study[27] evaluating the effect of butorphanol on Doppler echocardiography-derived tricuspid regurgitation pressure gradient in dogs with degenerative atrioventricular valve disease. Another study[28] showed that butorphanol in combination with acepromazine did not significantly alter peak right or left ventricular outflow velocity or left ventricular systolic function in healthy dogs. In addition, we elected to use a standard dose of butorphanol versus an attempt to titrate butorphanol to the desired level of sedation. Further, one must interpret these results with caution insomuch as each dog received butorphanol regardless if deemed clinically necessary. Clinically, butorphanol is likely to only be used if deemed necessary and one might expect a larger effect after a dog transitions from an agitated/anxious state to a sedated state. Estimating the level of sedation for the purposes of our study would have been largely subjective, complicating this approach and only recruiting agitated/anxious dogs would be impractical.

As proof-of-principal, the current study evaluated PS severity after BV. We demonstrated that, although iPVA, VTI ratio, and velocity ratio did not change after butorphanol or after atenolol, these indices were all significantly increased after BV as expected. In theory, there is strong potential value to evaluating PS severity after BV using iPVA (and VTI/velocity ratio). As previously opined in a discussion of the canine left ventricular outflow tract,[10] it is difficult to evaluate the success or failure of a balloon dilatation or surgery without understanding its effects on valve area. Balloon valvuloplasty, in addition to increasing valve area, is expected to increase transvalvular flow, in part, through improved ventricular systolic function (by reducing afterload).[9] This effect was illustrated in the current study with an increase in CI and RV systolic function noted after BV compared to before BV. Thus, favorable results after BV might be masked when solely assessed by PV mean or PV max PG.

The within-day repeatability and measurement variability studies suggest that all 5 indices of assessment of PS severity posses low variability (when assessed by CV) or high agreement (when assessed by ICC). Variability of within-day repeatability of iPVA was slightly above (CV = 10.5%) what was defined as low variability in the current study (CV < 10%). This is not an unexpected finding, as estimating valve area by the continuity equation inherently introduces an increased likelihood of measurement error.

Results of the current study might raise some questions regarding the clinical utility of the different less flow-dependent indices and, specifically, which might be best suited for clinical practice. Velocity ratio is appealing because it is quick and easy to measure, and like VTI ratio, fewer measurements, and calculations are necessary compared with iPVA. However, velocity ratio ignores the contour and flow duration of the flow profiles compared with VTI ratio and iPVA, which serves as a potential disadvantage.[13] Although iPVA, in theory, is more accurate, one must account for its cumbersome calculations, lower repeatability/higher measurement variability noted in our study, the greater potential for measurement error when determining CSA of the AV, and the necessity to index to body size. Thus, VTI ratio might serve as a compromise that is more appealing for routine clinical use for assessment of PS severity in dogs because of its potential advantages compared to velocity ratio and iPVA.

Perhaps the most obvious limitation of our study is that without a gold standard, we cannot comment on the accuracy of specific methods of assessment of severity (or lack thereof) and we cannot conclude that one method of assessment of PS severity is superior to another. This would require assessment by cardiac catheterization or advanced imaging that are unfortunately hindered by anesthesia, availability, and cost. However, our study design permits the evaluation of PS severity in a more clinically relevant manner by several indices of PS severity in non-anesthetized dogs after butorphanol, atenolol, and BV. The current study highlights a major disadvantage of PV mean and PV max PG assessment of PS, ie, their dependence on transvalvular flow. However, this should not be interpreted to suggest that we are advocating for the abandonment of pressure gradient-based methods of assessment of severity. Each of the 5 methods of assessment of PS severity studied have advantages and disadvantages, which are reviewed in more detail elsewhere.[9,13] Our results should be interpreted to highlight the benefits of not solely relying on a single measurement of PS severity, and this is particularly true in situations where one encounters altered transvalvular flow, eg, because of altered RV systolic function or after intervention.

Our study has additional limitations that should be addressed. We did not exclude dogs diagnosed with a PFO or mild valvular regurgitation. Shunts or valvular regurgitation can compromise the accuracy of the continuity valve area equation because of unequal SV through each outflow tract.[9] However, there was a lack of evidence of hemodynamic importance in the 4 dogs diagnosed with a PFO, as only mild shunting was observed with agitated saline contrast studies and a lack of erythrocytosis was noted. This coupled with only the mild degree of regurgitation tolerated suggests these findings were unlikely to have an important impact on our results. Another limitation is that a single sonographer did not perform all of the echocardiographic examinations in our study. Several cardiology residents or board-certified cardiologists performed echocardiographic examinations. This could compromise the reliability or consistency of the echocardiographic recordings. However, given our study design, we did prioritize having the same sonographer perform each dog's repeat examination, and the same investigator performed all measurements. This limitation mimics clinical practice in many facilities with multiple cardiologists. Recording or measurement error of pulsed-wave Doppler tracings at the level of the AV or, importantly, in the measurement AV diameter (measurement is squared for calculation of iPVA) can certainly occur and represents a limitation of the less flow-dependent indices. As with all Doppler echocardiographic recordings, Doppler profiles of the AV are subject to misalignment issues or might be over- or under-gained, which might skew measurements and assessments.

When studied in healthy dogs,[29] Doppler echocardiography-derived flow calculations of the right and left ventricular outflow tract (including aortic SV indexed body size) demonstrated considerable variability, which should be considered when performed the less flow-dependent methods of PS severity proposed in the current study.

We conclude by advocating for an integrative approach for the assessment of severity of canine PS. This approach should include the use of at least one less flow-dependent method of assessment of PS severity (iPVA, VTI ratio, velocity ratio) in addition to pressure gradient-based methods (PV mean PG and PV maxPG). Based on the results of our study, iPVA, VTI ratio, and velocity ratio are likely to provide clinically useful information for dogs with PS, particularly if RV systolic dysfunction is apparent, and during serial evaluations after atenolol, BV, or surgical intervention. Additional prospective studies on iPVA, VTI ratio, and velocity ratio in dogs with PS are warranted to provide cutoffs for guidance on treatment decisions and prognosis.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

All procedures in this study were approved by the IACUC at the University of California, Davis (protocol #19199).

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