
ACCURACY OF NONINVASIVELY DETERMINED PULMONARY ARTERY PRESSURE IN DOGS WITH MYXOMATOUS MITRAL VALVE DISEASE

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ACADEMIC ABSTRACT

Development of pulmonary hypertension is an independent predictor of poor outcome in dogs affected by myxomatous valvular degeneration (MMVD). Systolic pulmonary arterial pressure is routinely estimated by Doppler echocardiography applying the simplified Bernoulli equation to the velocity of tricuspid regurgitation (sPAP_D). The accuracy of this estimation is unknown in dogs with MMVD, but experimental studies suggest that the method is imperfect. In order to fill this knowledge gap we prospectively enrolled dogs affected by MMVD and cardiac remodeling - American College of Veterinary Internal Medicine (ACVIM) stages B2 and C MMVD for which treatment had been unchanged for at least one month. A flow-directed thermodilution monitoring catheter was percutaneously placed in the right jugular vein and advanced to the main pulmonary artery. Pulmonary arterial systolic pressure was recorded through this catheter connected to a pressure-transducer and data acquisition-analysis system (sPAP_C). A second operator simultaneously acquired tricuspid regurgitant velocity spectra to calculate sPAP_D. Each operator was blinded to the result of the other technique. Twenty dogs were enrolled. Technical difficulties prevented catheterization in 2 dogs. Eighteen measurement pairs were therefore used for comparison of sPAP_C and sPAP_D through Bland-Altman analysis and linear regression. A statistically significant bias between sPAP_C and sPAP_D (mean difference=0.5mmHg; Confidence interval: -6.5mmHg, +7.5mmHg) was not detected. The limits of agreement between the techniques were wide (-

27.3mmHg, +28.2mmHg). Regression analysis failed to identify a significant linear association between the two techniques ($r=0.11$, $p=0.17$). In conclusion, sPAP_D poorly agrees with sPAP_C measurement in dogs affected by MMVD in ACVIM stages B2 and C. In these dogs, sPAP_D could under- or over-estimate sPAP_C by more than 20mmHg, and therefore caution should be used when interpreting PASP_D.

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GENERAL AUDIENCE ABSTRACT

The most common heart disease of dogs is myxomatous mitral valve disease (MMVD). In many affected dogs, this disease can be complicated by the development of high pressure in the vessels of the lungs, a condition called pulmonary hypertension (PH). On average, dogs with MMVD and PH have shorter survival compared to dogs affected solely by MMVD. The pulmonary pressure in dogs is usually estimated using cardiac ultrasound (echocardiography). This technique has the advantage of being “non-invasive” but it is not a direct measurement of pressure, therefore it may not be accurate. In order to evaluate the accuracy of echocardiography in measuring pulmonary pressure, in this study we compared direct measurements of pulmonary pressure obtained through cardiac catheterization to the measurements estimated using echocardiography, in dogs affected by MMVD. We performed this on 18 dogs affected by MMVD, with one person performing the direct measurements and another performing the echocardiographic ones; the two people were not aware of the measurements obtained with the other technique. We found that the echocardiographic estimated pressures can be very different from the real pressures measured with cardiac catheterization. Particularly, echocardiography resulted both in relevant over- and under-estimation of the real pressure, in an unpredictable way. This study therefore suggests that pulmonary pressures estimated by echocardiography should be interpreted cautiously in dogs affected by MMVD.

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LIST OF ABBREVIATIONS

Abbreviation	Definition
ACVIM	American College of Veterinary Internal Medicine
CI	Cardiac index
CO	Cardiac output
dPAP	Diastolic pulmonary arterial pressure
dPAP_C	Diastolic pulmonary arterial pressure obtained by right heart catheterization
LA	Left atrium
MMVD	Myxomatous mitral valve disease
mPAP	Mean pulmonary arterial pressure
mPAP_C	Mean pulmonary arterial pressure obtained by right heart catheterization
PA	Pulmonary artery
PAP	Pulmonary arterial pressure
PCWP	Pulmonary capillary wedge pressure
PHT	Pulmonary hypertension
PI	Pulmonary valve insufficiency
PVR	Pulmonary vascular resistance
RA	Right atrium
RAm	Mean right atrial pressure
RHC	Right heart catheterization
RV	Right ventricle
RVs	Systolic right ventricular pressure
sPAP_C	Systolic pulmonary arterial pressure obtained by right heart catheterization
TPG	Transpulmonary gradient

TR Tricuspid regurgitation
σ Standard deviation

INTRODUCTION

Pulmonary hypertension (PHT) is a recognized cause of morbidity and mortality in dogs.¹⁻⁴ Several different causes of PHT have been identified in dogs, with left-sided heart disease, and notably myxomatous mitral valve disease (MMVD), being the most commonly identified cause.^{1,4-10} Although not all dogs affected by MMVD develop PHT, this condition carries prognostic and therapeutic implications.^{3,9,11} Consequently, it is crucial to evaluate the accuracy of measurements of pulmonary arterial pressure (PAP) in these patients.

The “gold standard” method for evaluation of PAP is right heart catheterization (RHC) and direct measurement of PAP.¹² This technique however requires special equipment, it is invasive, and time-consuming; for these reasons, it is not frequently used in veterinary medicine. Instead, PHT is most commonly diagnosed by estimating PAP using Doppler findings, notably the speed of tricuspid regurgitation (TR) or pulmonary valve insufficiency (PI) jets.^{13,14} The accuracy of Doppler derived PAP however has been deemed poor in human medicine, across patients with different diseases.¹⁵⁻¹⁹ In veterinary medicine however, only one study has investigated the accuracy of Doppler derived PAP compared to RHC,²⁰ with results indicating inconsistent predictive value of individual echocardiographic measurements to predict PAP. Nonetheless, this study utilized an acute model of PHT and tightly controlled study settings, therefore results are not necessarily translatable to clinical patients. For these reasons, we designed a prospective double-blind clinical study aimed at evaluating the accuracy of Doppler echocardiography in estimating PAP measured by RHC in dogs affected by myxomatous mitral valve disease (MMVD). Our hypothesis was that Doppler estimate of systolic PAP (sPAP_D) would not be an accurate predictor of systolic PAP obtained by RHC (sPAP_C).

REVIEW OF LITERATURE

PULMONARY HYPERTENSION IN MYXOMATOUS MITRAL VALVE DISEASE

DEFINITIONS

Pulmonary hypertension is a well-defined condition in people. The widely accepted clinical definition was established by a panel of experts in 1973 during the 1st World Symposium on Pulmonary Hypertension organized by the World Health Organization – A mean PAP (mPAP) ≥ 25 mmHg at rest.²¹ This was “[...]empirically and arbitrarily defined”, but clearly based on measurements obtained through RHC,²² although the panel did recognize the importance of researching non-invasive indicators of the condition.²¹ More recently, a literature review which included RHC data from 1,187 healthy people from 47 studies identified a more conservative resting value for mPAP of 20mmHg,²³ and this data may influence future human guidelines and definitions.^{12,24}

A precise and widely accepted definition of PHT is somewhat lacking in veterinary medicine. The most recently published guidelines broadly define PHT as an “abnormally increased pressure within the pulmonary vasculature”, also stating that “definitive diagnosis of PH requires RHC.”²⁵ Historically however, a diverse array of echocardiographically estimated systolic and diastolic PAP cutoffs has been used for identification of PHT in the veterinary literature, with pressures of 30/19mmHg being the most widely accepted cutoffs.^{1,5-7,26,27}

Clearly in both human and veterinary medicine the term PHT is used to indicate a hemodynamic condition which can be caused by several different etiologic processes.

Most recently, a detailed and extensive classification scheme has been proposed.²⁵ This scheme stems from the most recent one developed during the fourth and fifth world symposia on pulmonary hypertension,^{28,29} and identifies 6 clinical classification groups:

1. Pulmonary arterial hypertension

2. PHT due to left heart disease
3. PHT due to respiratory disease/hypoxia
4. Thromboembolic PHT
5. Parasitic disease
6. PHT with multifactorial and/or unclear mechanisms

Group 2 PHT is further subclassified in different classes based on the type of left heart disease; PHT secondary to myxomatous valvular disease is identified as Group 2b1a.

GROUP 2B1A - PHT SECONDARY TO MYXOMATOUS VALVULAR DISEASE

Left-sided heart disease is the most common cause of PHT in humans.³⁰⁻³² Pulmonary hypertension due to left heart disease is defined by the findings of mPAP ≥ 25 mmHg in association with pulmonary capillary wedge pressure (PCWP) ≥ 15 mmHg. In patients with left sided heart disease, the elevation of PAP may be caused by two mechanisms: passive retrograde transmission of left atrial pressure and reactive increase in precapillary vascular resistances^{25,33-35} – the latter defined by a transpulmonary gradient (TPG) ≥ 12 mmHg.³⁶ It has also been hypothesized that the changes in the pulmonary vasculature of these patients have a “reversible” phase – removal or attenuation of the primary cause for PHT may result in decrease of PAP – that can become irreversible with the chronicity of the condition.³⁷⁻³⁹ In dogs with MMVD a previous study identified a Doppler-determined cutoff of sPAP_D of 48mmHg to identify dogs whose PHT would respond to therapy or not, although it was recognized that this cutoff had only moderate sensitivity and specificity.¹¹

In people with heart failure with reduced ejection fraction the prevalence of PHT is reported to be 40-75%,⁴⁰⁻⁴² and a similar prevalence is noted in people with heart failure with preserved ejection fraction, 36-83%.⁴³⁻⁴⁵ Although the real prevalence of PHT is not known in veterinary medicine, data similar to what has been reported in people can be inferred from published literature. Particularly, studies that focused on PHT, found that the prevalence of MMVD in this population (assumed to be the cause of PHT) was between 30 and 74%,^{1,5-9,46,47} while the prevalence of PHT in dogs with

MMVD has been reported to be 14-66%.^{3,4,8-10,47-50} It is worth noticing that many of these studies used different cutoff values for defining PHT, and this may be at least in part responsible for the wide range in prevalence reported.

The importance of assessing and estimating right-sided hemodynamics in patients with predominantly left-sided heart disease has been well recognized in humans.^{42,51,52} More recently, elevation of Doppler estimated systolic PAP (sPAP) was found to be an independent predictor of survival also in dogs affected by MMVD,³ with estimated cutoff values very similar to what is known in humans.⁵³ Overall, it appears clear therefore how the assessment of PAP represents a key step of the assessment of dogs affected by MMVD.

THE BERNOULLI EQUATION – HEMODYNAMIC PERSPECTIVE

The non-invasive assessment of pressure gradients through echocardiography is routinely performed using the simplified Bernoulli equation [Eq. 1].^{54,55}

$$\Delta P = 4v^2 \text{ [Eq. 1]}$$

Where ΔP represents the instantaneous pressure difference across an orifice and v is the flow velocity across the orifice. The equation is based on *Bernoulli's Principle* (hydrodynamic perspective of the principle of conservation of energy): as a fluid's speed increases, this is accompanied by a simultaneous decrease in pressure (Fig. 1).⁵⁶

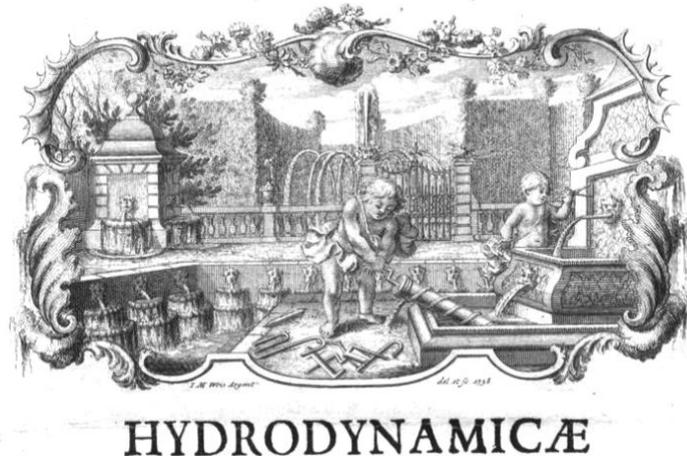


Fig. 1 Illustration on the first chapter of Daniel Bernoulli's *Hydrodinamica* where the author demonstrates what will later be known as the Bernoulli's principle. 1738 ETH-Bibliothek Zürich

The motion of viscous fluids is described in physics by a set of equations called Navier-Stokes equations. A particular form of Navier-Stokes equation that assumes non-viscous fluid and zero thermal conductivity is Euler's equation [Eq. 2].

$$\frac{\partial p}{\partial s} = -\rho \left[\frac{\partial v}{\partial t} + v \frac{\partial v}{\partial s} - g \right] \text{ [Eq. 2]}$$

Where p is instantaneous pressure, s is distance, ρ is density of the fluid, v is velocity, t is time, g is the gravitational constant. Integrating this equation along a streamline (from point 1 to point 2), we obtain the **unsteady Bernoulli equation** [Eq. 3], the precursor of the simplified [Eq. 1].

$$\Delta p = \frac{1}{2} \rho (v_2^2 [t] - v_1^2 [t]) + \rho \int_1^2 \frac{\partial v [s,t]}{\partial t} \partial s + g \text{ [Eq. 3]}$$

In order for Eq. 3 to be simplified into Eq. 1, several biological assumptions are made. In order to discuss these assumptions, it is useful to break down Eq. 3 into its main components by rewriting it as [Eq. 4]

$$\Delta p(t) = \frac{1}{2} \rho (v_2^2 - v_1^2) + M \frac{\partial v}{\partial t} + R + G \text{ [Eq. 4]}$$

Equation component: ■=convective ■=inertial ■=viscous ■=gravitational

From this form, it appears clear how only the first half of the equation is utilized in the simplified version. This portion of the equation is known as “convective” term, and is the one that explains the fall in pressure consequent to an increase in kinetic energy (flow velocity). The inertial component (inertance) accounts for the energy required to accelerate the fluid. Although this term may become significant when considering large orifices, this is usually considered negligible for restrictive ones.⁵⁷ Furthermore, its calculation would require the integration of changes in velocities across a streamline, which is usually not performed in clinical applications where the gradient is assumed to be calculated at steady state, when the rate of change in velocity is considered to be 0.⁵⁸⁻⁶⁰ The pressure drop caused by the viscous (resistance) component can be calculated by Poiseuille equation [Eq. 5]

$$\Delta P_{visc} = \frac{8 \times v \times L \times \mu}{r^2} \text{ [Eq. 5]}$$

Where ΔP_{visc} is the change in pressure due to viscous resistance, v is the speed of flow, L is the length along which flow is investigated, μ is blood viscosity and r is the radius of the orifice. By plugging in empirical values from common clinical scenarios in which the Bernoulli formula is used, it is clear that this term is mostly negligible for flow across restrictive orifices.⁵⁹

Based on all these assumptions, sPAP and diastolic PAP (dPAP) are commonly estimated in veterinary practice by applying Eq. 1 to TR or PI. Applying Eq.1 to TR therefore estimates right ventricular (RV) to right atrial (RA) gradient; in absence of right ventricular outflow tract obstructions,

peak right ventricular (RV) systolic pressure substantially equals sPAP. Theoretically, right atrial pressure can be estimated by echocardiogram or physical examination and its value may be added to the estimated RV-to-RA gradient in order to obtain sPAP_D.⁶¹ However, non-invasive estimation of right atrial pressure have often been found to be inaccurate, and can therefore constitute a further source of error.^{13,20,49,62,63} Therefore, adding estimated RA pressures to Doppler-derived gradients is currently recommended against both in human and veterinary guidelines.^{12,25}

INVASIVE HEMODYNAMIC MONITORING

PRESSURES MEASUREMENT

The first cardiac catheterization was performed by Stephen Hales in 1711 (Fig. 2).



Fig. 2 First documented cardiac catheterization, right and left-heart study performed by Hales (left) in 1711 From Mueller RL, Sanborn TA. Am Heart J. 1995.

The importance of invasive hemodynamic monitoring has long been well recognized. Described as the “[...] key in the lock” by Andre Courand in 1956 when along with Drs Richards and Forssmann received the Nobel Prize for Medicine or Physiology,⁶⁴ cardiac catheterization was the main mean for understanding physiology and pathophysiology of cardiovascular disease during 1950s and 1960s, and it became widespread during the 1970’s when H.J.C. Swan and W. Ganz, inspired by sailboats on Santa Monica Bay, developed the first balloon-tipped catheter.⁶⁵⁻⁶⁷ Despite the proof of concept was indeed obtained in a dog, the practice of bedside pressure monitoring received exponentially increasing attention in human medicine, while has had limited application in the veterinary field. Several different systems exist for the measurement of invasive hemodynamics.⁶⁸ The most commonly used configuration however uses a fluid-filled catheter system attached to a strain gauge pressure transducer. The main concept of a fluid-filled catheter system does not differ substantially from what illustrated in Fig. 2. In terms of pressure measurement, the catheter works as a mere conduit for a fluid column. The pressure applied to the fluid column is then sensed by a transducer.

The most common pressure transducers make use of a Wheatstone bridge circuit in order to convert pressure in electrical signal (Fig. 3).

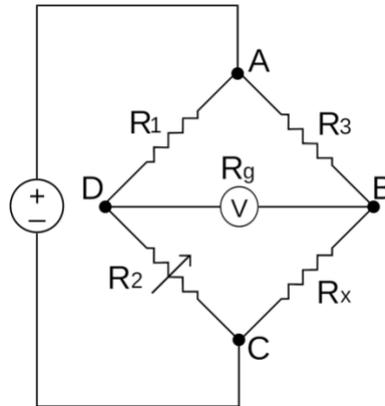


Fig. 3 Wheatstone bridge circuit used by most pressure transducers. ©Creative Commons

In this configuration when no pressure is applied R_1 , R_2 , R_3 , R_4 are all equal resistances, therefore no current flows between D to B. When pressure is applied, this is transmitted to a thin membrane which in turn stretches one arm of the circuit, causing a change and unbalance in the resistance and consequent flow of current. The electrical output derived, is then usually amplified and interpreted by a computer system. It can be inferred from the description of the system how the pressure measured are indeed “changes in pressure” applied to the fluid column. Therefore, correct “zeroing” of the system is extremely important and improperly zeroed system may result in significant measurement errors.⁶⁹

CARDIAC OUTPUT

Invasive measurement of cardiac output (CO) is another important parameter that can be obtained through invasive monitoring. Measurement of CO can be performed using several different methods, most notably Fick oxygen method, indicator dilution method, and thermodilution method. All these methods can be seen as different applications of the Fick’s principle, in which an indicator is tracked through the cardiovascular system. The thermodilution method was first described in 1954,⁷⁰ but became widely used in clinical practice after the work of Ganz et al.^{71,72} In this method, the indicator

is a cold injectate (5% dextrose or saline), and the variable measured is the change in temperature occurring at a thermistor distal to the injection. The cardiac output is therefore calculated using the formula in [Eq. 6].

$$CO = \frac{\left(V_I(T_B - T_I)\left(\frac{S_I C_I}{S_B C_B}\right)60sec \cdot min^{-1}\right)}{\int_0^{\infty} (\Delta T_B)(t) dt} \text{ [Eq. 6]}$$

Where V_I is the volume of injectate, T_B and T_I are the temperature of blood and of the injectate, respectively, S_I and C_I are the specific gravity and specific heat of the injectate, and S_B and C_B are the specific gravity and heat of blood. This computation is usually automatically performed by a cardiac output computer.

VASCULAR RESISTANCE

Once the pressure difference between two sites is measured and CO is calculated, resistance can be calculated by the hemodynamic version of Ohm's Law [Eq. 7].

$$R = \frac{\Delta P}{Q} \text{ [Eq. 7]}$$

Where R is the vascular resistance, ΔP is the pressure difference across the vascular bed investigated and Q is the flow through that vascular bed (in absence of shunts CO is used as a surrogate of Q). Many of the assumptions related to the use of this law are violated by physiologic vascular beds. Nonetheless, the values calculated by this method have assumed empirical important value in clinical settings. By using the conventional unit of measurement used during hemodynamic studies, resistance will be expressed in mmHg/L/min, often referred to as "Hybrid Resistance Units" or "Wood units". Converting mmHg to dynes/cm², and L/min to cm³/sec, result in resistance expressed as dynes · sec · cm⁻⁵.

In the context of identification and classification of PHT, pulmonary vascular resistance (PVR) is measured by calculating the pressures across the pulmonary vascular bed, i.e. the TPG divided by

the CO. The TPG is in turn defined as the difference between mPAP and left atrial (LA) pressure, the latter estimated by PCWP [Eq. 8]

$$PVR = \frac{TPG}{CO} = \frac{mPAP - PCWP}{CO} \text{ [Eq. 8]}$$

ACCURACY OF DOPPLER IN ESTIMATING PULMONARY ARTERIAL PRESSURE

The accuracy of Doppler echocardiography in estimating PAP has long been debated.

Early studies showed promising results.^{61,63} However, it is often debated that these studies have several limitations among which being performed in controlled study settings, and showing correlation between techniques, rather than agreement. Most recent studies and systematic reviews instead, show that Doppler estimated PAP have at best modest correlation with pressures measured by RHC, that the two techniques often present unacceptably wide limits of agreement, and that Doppler estimated PAP often leads to PHT misclassification, with both over and underestimation occurring.^{15,16,62,73-75} The sources of error have not been clearly identified. However, the most commonly suggested ones relate to the innate imprecision of ultrasound examinations (i.e. misalignment of Doppler with regurgitant jet, Doppler trace measurement errors, artifacts), and validity of the assumptions posed in using the simplified Bernoulli equation. For these reasons, current guidelines recommend the use of echocardiography simply as a screening tool to identify individual at risk of PHT, while RHC is required to confirm the diagnosis.^{12,76}

In veterinary medicine, diagnostic RHC is not commonly performed. Therefore, extensive data about the accuracy of Doppler echocardiography in estimating PAP is lacking. A single study by Soydan et al. has assessed the accuracy of Doppler echocardiographic estimates of PAP in a canine model of PHT.²⁰ The authors found results similar to the most recent human literature:

- only moderate correlation and wide confidence intervals between the two techniques
- negligible effect of adding estimated RA pressures to estimated sPAP_D or dPAP
- both under- and over-estimation of sPAP and dPAP occur by Doppler echocardiography
- RA pressure estimated by echocardiography is mostly inaccurate

The main limitations of this study however are the not simultaneous RHC echocardiographic assessment, and the use of a well-controlled study setting in which beagle dogs were used as an embolization model of PHT.⁷⁷ For these reason, in order to evaluate the accuracy of Doppler echocardiography in estimating PAP in dogs affected by MMVD, the study object of this thesis was designed and performed.

EXPERIMENT

MATERIALS AND METHODS

This was a prospective study performed at the veterinary teaching hospital of the Virginia-Maryland College of Veterinary Medicine.

ANIMALS

At the time of enrollment, the procedures illustrated in Fig. 4 were performed:

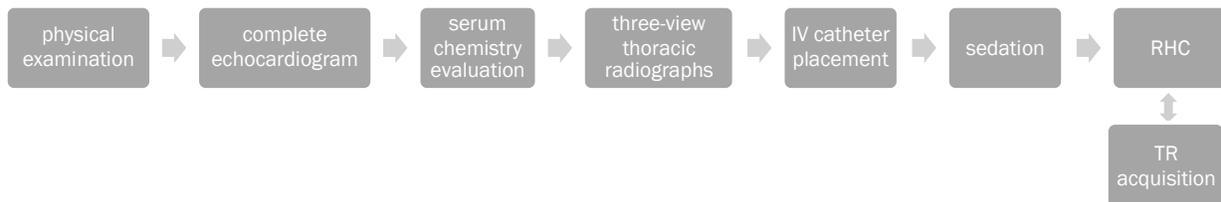


Fig. 4 Flowchart illustrating the procedures performed upon enrollment.

Client-owned dogs with echocardiographic evidence of TR affected by MMVD American College of Veterinary Internal Medicine (ACVIM) Stages B2 and C based on 2009 ACVIM consensus statement were eligible for this study.⁷⁸ Particularly, MMVD was defined as echocardiographic evidence of thickening and/or prolapse of the mitral valve leaflets associated with color Doppler evidence of mitral valve regurgitation. Stage B2 was defined as echocardiographic evidence of LA and left ventricular enlargement in absence of clinical signs or pulmonary infiltrates compatible with congestive heart failure. Dogs were classified as affected by MMVD Stage C if they had previous diagnoses of MMVD and cardiogenic pulmonary edema. Dogs with concurrent co-morbidities identified by thoracic radiographs, echocardiogram, and blood work were not eligible for enrollment. Current administration of cardiac medications (any combination of ace-inhibitors, pimobendan, furosemide) did not constitute an exclusion criterion. However, dogs had to be stable in their

treatment regimen without any change in type or dosage of medications for at least 30 days before being enrolled in the study.

ECHOCARDIOGRAPHY

A complete echocardiography was performed in every dog at enrollment prior to sedation with acquisition of standard image planes.⁷⁹ The echocardiograms were performed using one of the two available ultrasound units (Artida, Toshiba Medical Systems, Tokyo, Japan; Aplio i900, Canon Medical Systems USA, Tustin, CA) and a transducer selected as appropriate for the dog's size based on ultrasonographer preference (PST-30BT and PST50BT, Toshiba Medical Systems, Tokyo, Japan). All echocardiographic examinations were performed by a single operator, blinded to the RHC measurements. Transvalvular flows were interrogated by means of color, pulsed-wave and continuous-wave Doppler as appropriate. Left atrial enlargement was defined as a ratio between left atrial diameter to aortic root diameter >1.5 , as measured by the "Swedish Method".⁸⁰ Briefly, a right parasternal short axis view of the heart base at the level of the aorta was obtained.⁷⁹ The aortic diameter was measured by a line starting at the midpoint of the convex curvature of the wall of the right aortic sinus extending through the center of the aorta to the junction of noncoronary and left coronary cusps. The left atrial diameter was measured by a line virtually extending the one used for the aortic measurement starting from the blood-tissue interface of the left atrial wall in proximity to the aortic root extending to the blood-tissue interface of the left atrial wall posteriorly. Both measurements were performed using inner-edge-to-inner-edge technique. Attention was placed to exclude pulmonary veins in the left atrial diameter measurement by approximating the position of the left atrial wall by proximity, when needed.

Left ventricular enlargement was defined as M-Mode derived left ventricular end-diastolic diameter exceeding the 97.5 percentile of the prediction interval based on allometric scaling of the measurement.⁸¹

Tricuspid valve regurgitation peak velocity was obtained by using continuous-wave Doppler from a left-parasternal view optimized for obtaining the best possible alignment with the TR jet. The Doppler sample line was directed by color Doppler. The spectrogram of minimum three consecutive TR jets was recorded and stored. The evaluation of TR was then repeated in the same fashion after sedation and simultaneously to RHC. The simplified Bernoulli equation [Eq. 1] was applied to the average peak velocity of 3 consecutive TR spectrograms in order to obtain sPAP_D.

RIGHT HEART CATHETERIZATION

The dogs were sedated with IV administration of acepromazine (0.03 mg/kg) and buprenorphine (0.007 mg/kg). Due to a temporary shortage of buprenorphine, this drug was substituted by IV butorphanol (0.2mg/kg) in one dog. Had the dog required additional sedation during the procedure, an extra dose of 0.02mg/kg of acepromazine was administered. Cefazolin (22 mg/kg) was administered IV immediately before and 90 minutes after catheterization. The dog was positioned in left lateral recumbency and after infiltration of the subcutis with 1mL of lidocaine, a 5F sheath-introducer system (Fast Cath™, Abbott Vascular, Santa Clara, CA) was aseptically placed into the right external jugular vein using the modified Seldinger technique. A 5F multi-lumen, flow-directed thermodilution catheter (Arrow © Thermodilution Catheters, Teleflex, Wayne, PA) was connected to a fluid-filled pressure transducer system (TSD104A, BioPac Systems, Goleta, CA). Atmospheric pressure, with the transducer positioned at the level of the right atrium (RA), was taken as zero-pressure reference. The analogue pressure signal was amplified and subject to digital conversion by a data acquisition and analysis system (MP150 – BIOPAC) prior to storage on a laptop computer. A simultaneously recorded electrocardiogram was also digitally stored. The tip of the catheter was sequentially advanced to the RA, right ventricle (RV), pulmonary artery (PA) and then to “wedge” position. Catheter manipulations were guided by observation of the distinctive pressure contours of the relevant chambers and vessels. However, one dog required fluoroscopy to guide catheter placement. A commercially available software (Acknowledge – BIOPAC) was used to view and

analyze the digitally stored data. The following hemodynamic variables were measured by an operator blinded to the echocardiographic results: mean RA pressure (RAm), systolic RV pressure (RVs), systolic, end-diastolic and mean PAP (sPAP_C, dPAP, mPAP) as well as the pulmonary capillary wedge pressure (PCWP). The catheter was then attached to a dedicated CO computer (Baxter Edwards Com-2, Baxter Healthcare Corporation) for determination of CO by thermodilution by injection of 5mL iced 5% dextrose in water. Three to five CO measurements were performed, values exceeding a range of 20% were discarded, and the average of the remaining measurements used for further analyses. Cardiac index (CI) was calculated as CO/Body surface area. Body surface area was calculated using the formula $(0.101 \times \text{weight}^{2/3})$.^{82,83} Transpulmonary gradient and PVR were calculated as in Eq.8. Following measurement of CO, the thermodilution catheter and sheath introducer were removed and hemostasis was achieved by manual compression of the site for 15min. An oral dose of Cephalexin (~22mg/kg) was dispensed and owners were instructed to administer it 8 hours post-procedure.

STATISTICAL ANALYSIS

All continuous numerical data was analyzed for normality of distribution by visual assessment of normal probability plots. Normally distributed data is presented as mean \pm standard deviation, while not normally distributed data is presented as median (25th percentile - 75th percentile). The effect of sedation on sPAP_D was evaluated by Wilcoxon Signed Rank Test. The effect of clinical variables and treatment received on PCWP was evaluated by Student's t-test. Presence of linear association between variables was evaluated by Pearson's correlations coefficient. A p-value <0.05 was considered significant for all tests.

The agreement between variables was evaluated by Bland-Altman analysis.⁸⁴ Briefly, the distribution of differences was visually assessed for the assumption of normality. The difference between paired measurements was plotted against the mean of the two measurements. The plot was visually assessed for trends of the data. As suggested by Bland and Altman, since both the mean bias and

the limits of agreements are point estimates, 95% confidence intervals were created for these estimates. The mean difference (mean bias) and its standard deviation (σ) were calculated. The standard error of the mean difference was calculated as $\sqrt{\frac{\sigma^2}{n}}$ where “n” is the number of paired observations. The 95% confidence interval of the mean bias was therefore calculated as [(mean bias) $\pm t \times$ (standard error of the mean bias)], where “t” is the value of the t distribution with n-1 degrees of freedom. The mean bias was considered significant if the 95% confidence interval of the mean bias did not contain 0. The 95% limits of agreement were calculated as [mean bias $\pm 1.96 \times \sigma$]. The standard error of the limits of agreement was calculated as $\sqrt{\frac{3\sigma^2}{n}}$. The 95% confidence interval of the limits of agreement was therefore calculated as [(95% confidence interval) $\pm t \times$ (standard error of the limits of agreement)].⁸⁵

The distribution of differences between sPAP_D and sPAP_C was also assessed in order to identify clinically relevant differences (arbitrarily defined as ± 10 mmHg).

RESULTS

Twenty dogs were enrolled in the study. In one dog jugular vein catheterization was unsuccessful. One other dog was excluded from data analysis because after review of pressure tracings and CO data it was concluded that the catheter was most likely looped in the RV, rather than advanced in the PA. The data presented therefore was obtained from the 18 dogs with successful RHC.

DEMOGRAPHICS

Data regarding the dogs' age, body weight, sex, ACVIM stage, treatment received at the time of enrollment (indicated as number of dogs receiving a certain drug), and symptoms presented at time of enrollment is presented in Table 1.

Table 1 Demographics of the study population

Age (y)	10.6 ± 3.4
Weight (Kg)	9.7 (7.4 - 13.8)
Sex (M/F)	11/7
ACVIM (B2/C)	11/7
Furosemide (n)	8
Pimobendan (n)	8
Ace-inhibitor (n)	5
Cough (Y/N)	10/8
Syncope (Y/N)	4/14
Exerc. intolerance (Y/N)	6/12

Four dogs were Cavalier King Charles Spaniels, 3 mixed breed, 2 whippet and one each of the following breeds: Bichon Frisé, Border Collie, Chihuahua, Cocker Spaniel, Dachshund, Miniature Schnauzer, Shetland Sheepdog, Shih-Tzu, Yorkshire Terrier.

HEMODYNAMIC DATA

The study population's hemodynamic data is presented in Table 2.

Table 2 Summary of hemodynamic data obtained from the study population

HR (bpm)	86.3	(62.6 - 97.7)
CO (L/min)	1.0	(0.8 - 1.5)
CI (L/min/m ²)	2.1	(1.8 - 2.5)
sPAP_C (mmHg)	22.9	(18.6 - 28.9)
RAm (mmHg)	1.1	(0.6 - 2.9)
PCWP (mmHg)	6.3 ± 3.3	
RVs (mmHg)	25.7 ± 11.7	
PAd (mmHg)	10.8 ± 4.9	
PAm (mmHg)	16.2 ± 5.9	
TPG (mmHg)	9.9 ± 5.5	
PVR (dynes • sec • cm ⁻⁵)	905.1 ± 700.8	

In order to assess a possible association between the dog's therapy or clinical signs with their cardiac status, the PCWP was compared between dogs showing a specific clinical sign or not, receiving a specific drug or not and their ACVIM class. Data regarding this comparison are reported in Table 3. No difference in PCWP was found between dogs presenting different clinical signs or receiving certain medications.

Table 3 Comparison of pulmonary capillary wedge pressures (expressed in mmHg) between dogs presenting with different clinical signs and receiving different medications.

	PCWP		P-value
	Y	N	
Cough	6.3 ± 2.7 mmHg	6.4 ± 4.2 mmHg	0.936
Syncope	6.4 ± 2.6 mmHg	6.3 ± 3.6 mmHg	0.935
Ex Int	6.9 ± 3.4 mmHg	6.1 ± 3.4 mmHg	0.621
Pimobendan	6.2 ± 2.1 mmHg	6.5 ± 4.2 mmHg	0.838
ACE-i	4.4 ± 2.6 mmHg	7.1 ± 3.4 mmHg	0.110
Furosemide	6.6 ± 4.6 mmHg	6.1 ± 2.2 mmHg	0.789

ACVIM B2 6.5 ± 2.4 mmHg 6.1 ± 4.7 mmHg 0.845

EFFECT OF SEDATION ON DOPPLER ESTIMATED GRADIENT

The sPAP_D was significantly lower post-sedation, compared to the value estimated prior to sedation ($p=0.003$) as illustrated in Fig. 5. The estimated gradient decreased in 16 out of 18 dogs. The sPAP_D decreased by a median of 9.8mmHg (5.8 – 15.2 mmHg).

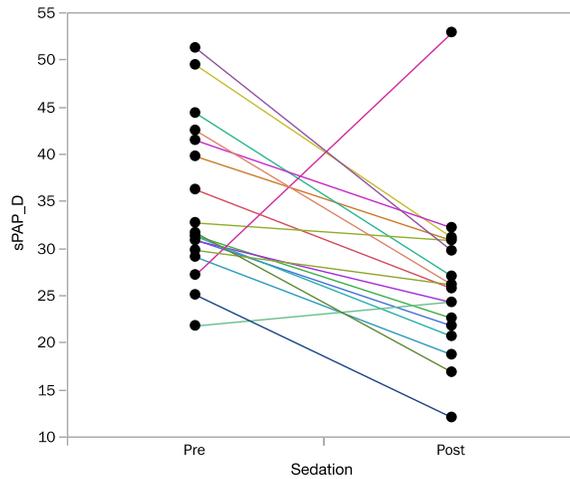


Fig. 5 Effect of sedation on Doppler estimated systolic pulmonary arterial pressure (sPAP_D).

CORRELATION BETWEEN TECHNIQUES

There was no significant correlation between sPAP_D and sPAP_C ($r=0.149$, $p=0.554$) as illustrated by Fig. 6.

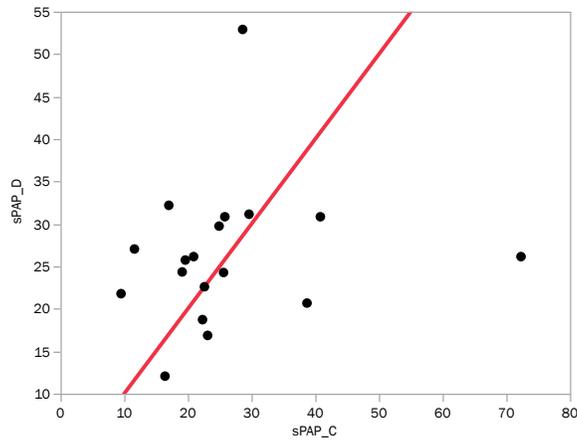


Fig. 6 Correlation between Doppler estimated systolic pulmonary arterial pressure (sPAP_D) and systolic pulmonary arterial pressure obtained by right heart catheterization (sPAP_C). The red line represents the line of equality.

By observation of the data points, two extreme values are identified. Therefore, the correlation analysis was attempted also by excluding those two points (Fig. 7). However, no significant correlation was identified ($r=0.229$, $p=0.394$).

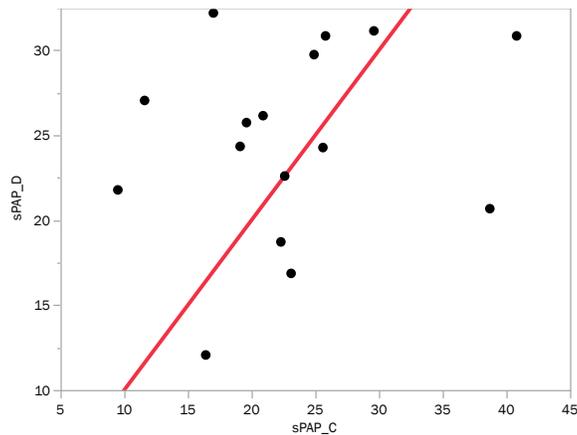


Fig. 7 Correlation between Doppler estimated systolic pulmonary arterial pressure (sPAP_D) and systolic pulmonary arterial pressure obtained by right heart catheterization (sPAP_C) performed excluding the previously identified outliers. The red line represents the line of equality.

In order to test whether adding RA pressure to sPAP_D would have improved the measurements correlation a hybrid variable was created by adding the Ram to sPAP_D. The correlation between this hybrid variable and sPAP_C was found not statistically significant ($r=0.158$, $p=0.544$) and is illustrated in Fig. 8.

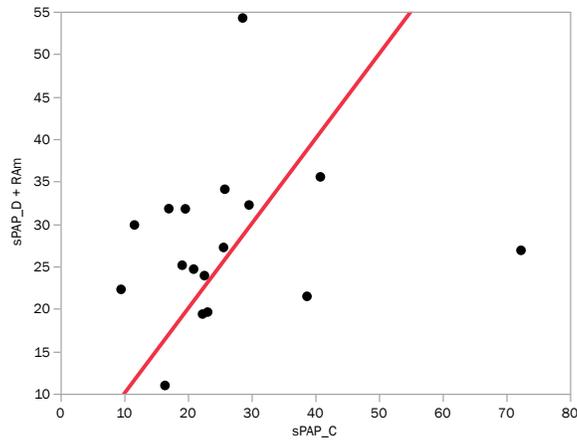


Fig. 8 Correlation between a hybrid variable [created by adding the mean right atrial pressure (RAM) measured by right heart catheterization to the estimated systolic pulmonary arterial pressure (sPAP_D)] and the systolic pulmonary arterial pressure measured by right heart catheterization (sPAP_C). The red line represents the line of equality.

Furthermore, the correlation between sPAP_D and the difference between RVs and RAM was evaluated, but no significant linear relationship was found between the two variables ($r=0.233$, $p=0.424$) – Fig. 9.

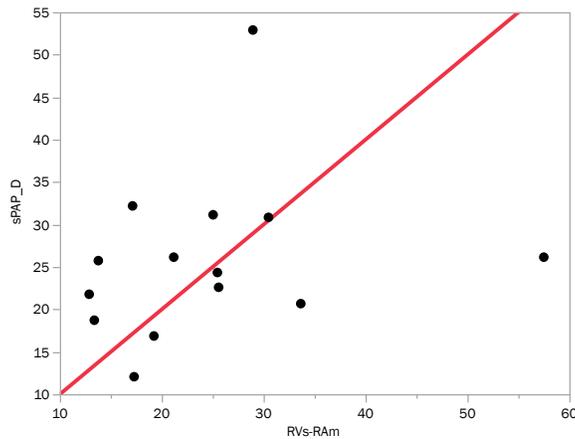


Fig. 9 Correlation between Doppler estimated systolic pulmonary arterial pressure (sPAP_D) and the difference between systolic right ventricular pressure (RVs) and mean right atrial pressure (RAM). The red line represents the line of equality.

AGREEMENT BETWEEN TECHNIQUES

The difference distribution of differences between sPAP_C and sPAP_D was considered following a normal distribution.

The Bland-Altman plot of sPAP_D vs. sPAP_C is presented in Fig. 10.

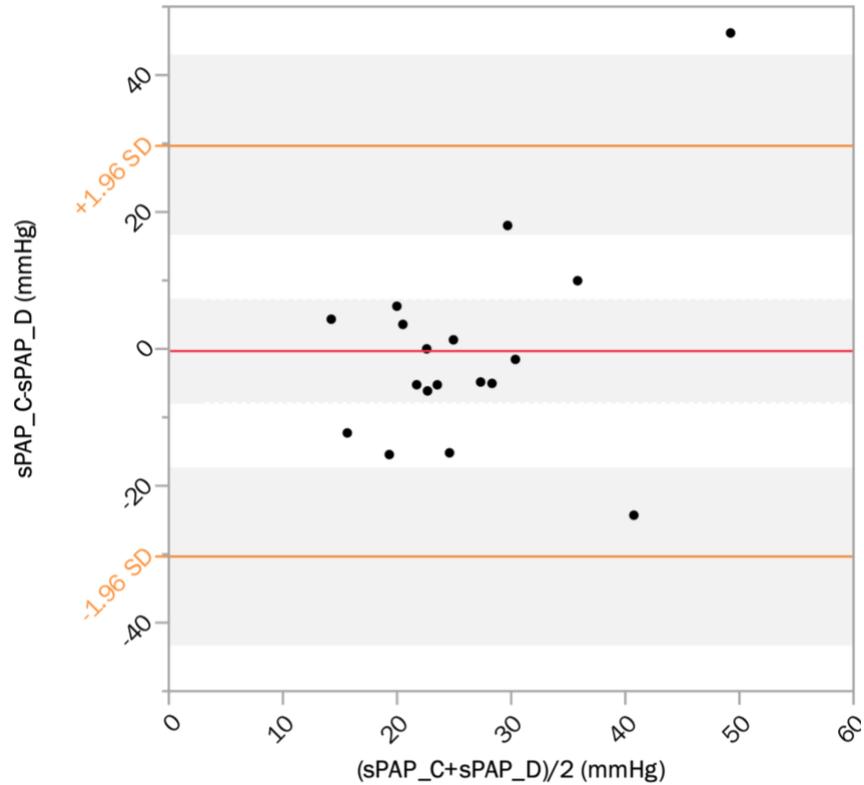


Fig. 10 Bland-Altman plot of systolic pulmonary arterial pressure measured by right heart catheterization (sPAP_C) and estimated by Doppler (sPAP_D). The red line represents the mean bias, while the orange lines represent the 95% limits of agreement. The areas shaded in grey are the 95% confidence intervals of the mean bias and of the limits of agreement.

Data relative to this plot is tabulated in Table 4.

Table 4 Values from Bland-Altman analysis of systolic pulmonary arterial pressure measured by right heart catheterization and estimated by Doppler. All values are expressed in mmHg.

	Mean Bias	Lower 95% limit of agreement	Upper 95% limit of agreement
	-0.31	-30.3	29.68
95% Confidence interval	(-7.92 ; 7.30)	(-43.47 ; -17.12)	(16.50 ; 42.85)

As it can be inferred from the plot and numerical data in Table 4, no data trend or significant bias was identified. The 95% limits of agreement were wide and clinically relevant.

One observation was noted to have an extreme deviation from the overall trend, therefore in attempt to evaluate the possible effect of this single measurement on the overall data, the analysis

was repeated excluding this observation. The Bland-Altman plot relative to this comparison is presented in Fig. 11.

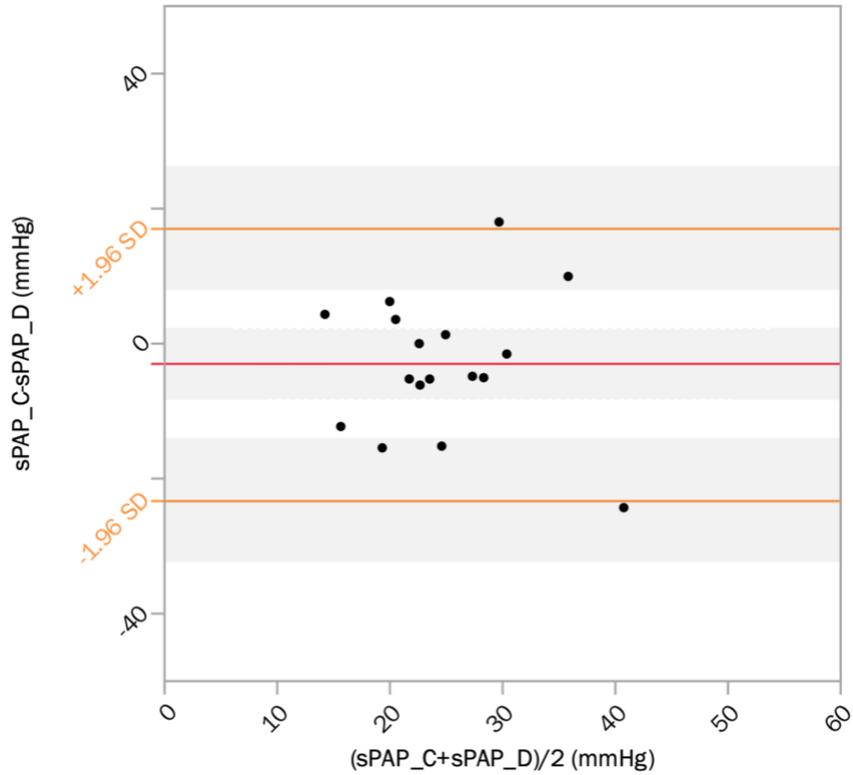


Fig. 11 Bland-Altman plot of systolic pulmonary arterial pressure measured by right heart catheterization (sPAP_C) and estimated by Doppler (sPAP_D), excluding the previously identified outlier. The red line represents the mean bias, while the orange lines represent the 95% limits of agreement. The areas shaded in grey are the 95% confidence intervals of the mean bias and of the limits of agreement.

Data relative to this plot is tabulated in Table 5.

Table 5 Values from Bland-Altman analysis of systolic pulmonary arterial pressure measured by right heart catheterization and estimated by Doppler, excluding the previously identified outlier. All values are expressed in mmHg.

	Mean Bias	Lower 95% limit of agreement	Upper 95% limit of agreement
	-3.05	-23.21	17.12
95% Confidence interval	(-8.33 ; 2.24)	(-32.27 ; -14.05)	(7.96 ; 26.28)

As it can be inferred from the plot and numerical data in Table 5 no data trend or significant bias was identified. The 95% limits of agreement were still wide and clinically relevant.

In order to express the agreement between techniques in terms of a percentage of the measurement, a third Bland-Altman plot was built by plotting the differences between sPAP_C and sPAP_D expressed as percentages of their mean, and the average between the two measurement. This Bland-Altman plot is presented in Fig. 12, and the relative data is presented in Table 6.

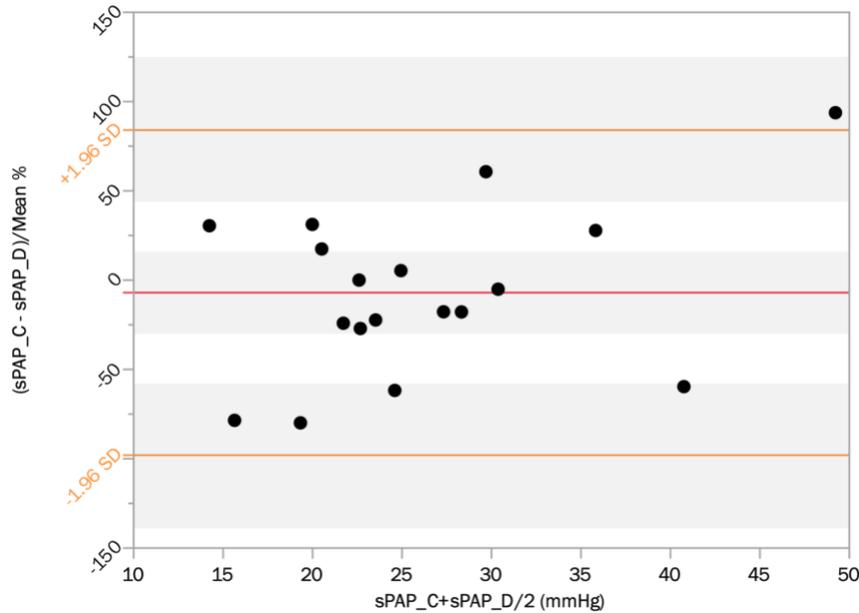


Fig. 12 Bland-Altman plot of systolic pulmonary arterial pressure measured by right heart catheterization (sPAP_C) and estimated by Doppler (sPAP_D) expressed as percentages of their mean. The red line represents the mean bias, while the orange lines represent the 95% limits of agreement. The areas shaded in grey are the 95% confidence intervals of the mean bias and of the limits of agreement.

Table 6 Values from Bland-Altman analysis of systolic pulmonary arterial pressure measured by right heart catheterization and estimated by Doppler expressed as percentages of their mean, excluding the previously identified outlier. All values are expressed in percentages.

	Mean Bias	Lower 95% limit of agreement	Upper 95% limit of agreement
	-7.06	-98.47	84.35
95% Confidence interval	(-30.25 ; 16.13)	(-138.65 ; -58.30)	(44.18 ; 124.52)

Even based this analysis no significant bias or data trend is identified. The 95% limits of agreement are wide and clinically relevant.

The agreement between sPAP_D and the difference between RVs and RA_m was also investigated (Fig. 13 – Table 7).

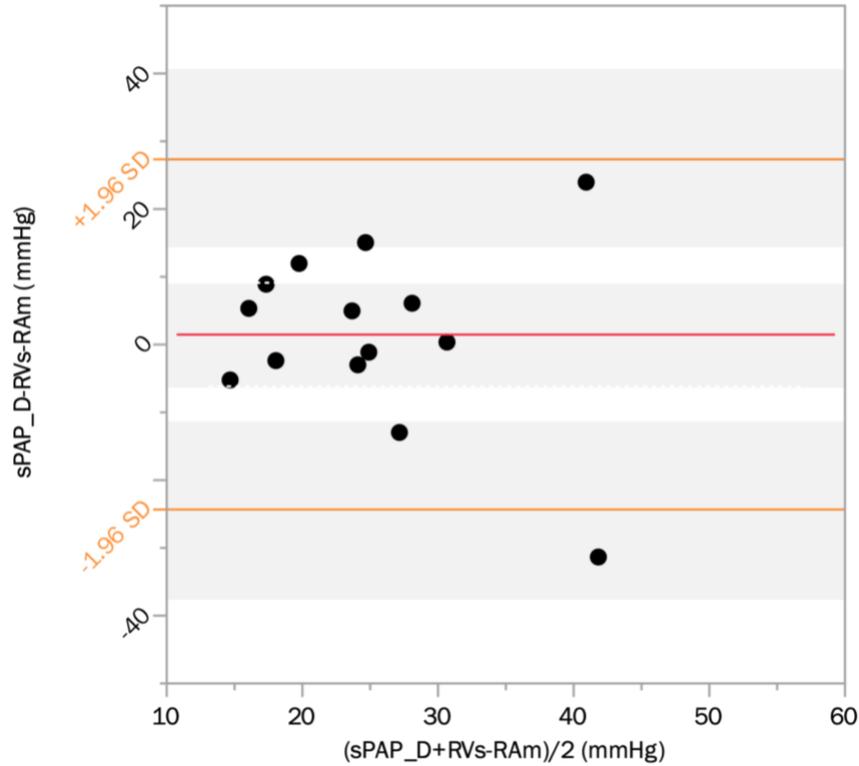


Fig. 13 Bland-Altman plot of systolic pulmonary arterial pressure estimated by Doppler (sPAP_D) and the difference between right ventricular systolic pressure (RVs) and right atrial mean pressure (RA_m) measured by right heart catheterization. The red line represents the mean bias, while the orange lines represent the 95% limits of agreement. The areas shaded in grey are the 95% confidence intervals of the mean bias and of the limits of agreement.

Table 7 Values from Bland-Altman analysis of systolic pulmonary arterial pressure estimated by Doppler (sPAP_D) and the difference between right ventricular systolic pressure (RVs) and right atrial mean pressure (RA_m) measured by right heart catheterization. All values are expressed in mmHg.

	Mean Bias	Lower 95% limit of agreement	Upper 95% limit of agreement
	1.47	-24.47	27.41
95% Confidence interval	(-6.17 ; 9.12)	(-37.70 ; -11.23)	(14.18 ; 40.65)

DISTRIBUTION OF DIFFERENCES

The differences between sPAP_C and sPAP_D are plotted in Fig. 14.

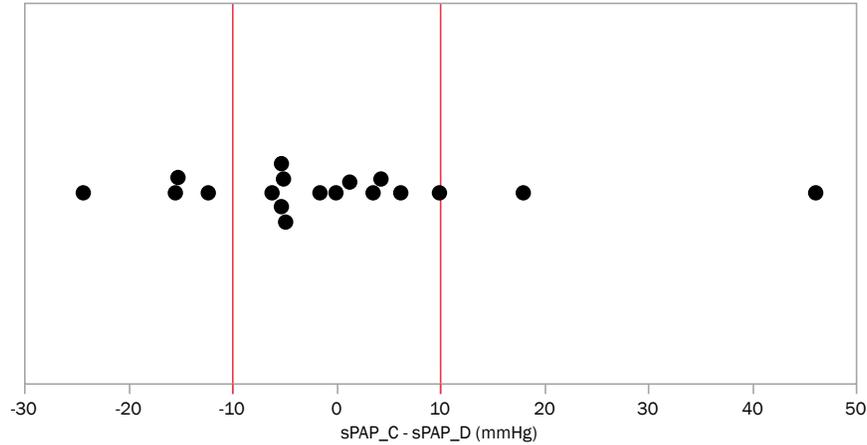


Fig. 14 Distribution of differences between systolic pulmonary arterial pressure obtained by right heart catheterization (sPAP_C) and Doppler (sPAP_D).

It can be noted that sPAP_D overestimated sPAP_C by ≥ 10 mmHg in 4/18 (22%) of dogs, while it underestimated sPAP_C by ≥ 10 mmHg in 3/18 (17%) of the study dogs. Therefore, overall sPAP_D provided an estimate of sPAP_C wrong by ± 10 mmHg in 7/18 (39%) of dogs.

Furthermore, in order to visually assess the possible clinical misclassification of the study subjects, the Pam and sPAP_D for each subject were plotted (Fig. 15).

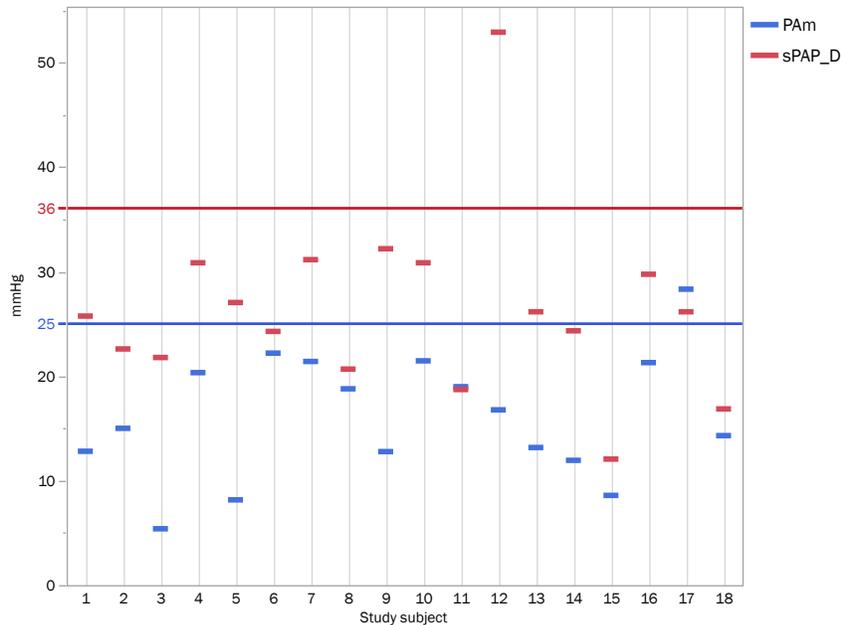


Fig. 15 Pulmonary arterial mean pressure (PAm) and systolic pulmonary arterial pressure obtained by Doppler (sPAP_D) for each study subject. The red line and the blue line represent cutoff values that have been commonly used as definition of pulmonary hypertension by Doppler echocardiography and right heart catheterization, respectively.

By using the most widely accepted definition of PHT, i.e. $PAm \geq 25\text{mmHg}$, only one study subject (n. 17) was diagnosed with PHT; the sPAP_D for this subject however was below the cutoff value commonly used by Doppler echocardiography to diagnose PHT (i.e. 36mmHg). Conversely, one study subject (n. 12) would have been falsely diagnosed with PHT by the Doppler definition, while its PAm was within normal range.

DISCUSSION

First, in this study we demonstrated that RHC is feasible in sedated dogs affected by stable MMVD ACVIM Stage B2 and C.

We confirmed our hypothesis that Doppler estimate of sPAP would not be an accurate predictor of sPAP obtained by RHC in dogs affected by MMVD. Indeed, we found that sPAP_D could over or underestimate sPAP_C by approximately 30mmHg, and more than 1/3 of the dogs in our study had a discrepancy between techniques >10 mmHg. Given the range of PAP expected in both healthy dogs and dogs affected by PHT, these represent clinically relevant difference. Our findings are mostly in agreement with the most recent literature in humans affected with a variety of clinical conditions. Particularly, the absence of a significant bias and the 95% limits of agreement found in the present study, are almost identical to what has been found by Attaran et al, Fisher et al, Rich et al, and Kim et al,^{16,17,62,86} while they are wider than what previously reported in a dog model of PHT.²⁰

Furthermore, we also found lack of correlation between the techniques, indicating that Doppler echocardiography should not be used to estimate PAP in dogs with MMVD. In order to justify these discrepancies, several sources of error should be considered. The inaccuracy in estimating RA pressure is often mentioned as a source of inaccuracy. Overall in fact, applying the simplified Bernoulli equation to TR should only provide the RV to RA gradient, rather than the systolic PAP, and for this reason the RA pressure should be added to the calculated trans-tricuspid gradient. However, in this study we rejected this hypothesis by calculating correlation and agreement between sPAP_C and (sPAP_D + RAm), and also sPAP_D and (RVs-RAm). In fact, despite using the measured value of RAm rather than an estimate, we found no correlation and wide limits of agreement. This further supports the recommendation to avoid adding right atrial pressure to the echocardiographically determined sPAP_D.^{12,25} Further error may arise from the intrinsic limitations of Doppler echocardiography. Indeed, less than optimal alignment with the investigated flow will inevitably result in underestimation of peak velocities.^{54,55} In this study, effort was placed in order to obtain the

best possible alignment with the TR jet, but in agreement with real-world clinical scenarios, perfect alignment was not always feasible. However, if this would have represented the main source of error, we would have observed a significant bias indicating constant underestimation of sPAP_C by sPAP_D, which was not the case. Doppler envelope measurement could also represent another source of error. For being included in this study, dogs had to have TR, independently from jet size and Doppler spectrogram quality. It is possible therefore that some dogs had less than optimal quality of imaging which could have resulted in imprecise TR peak velocity measurements, representing a scenario more similar to the one found in clinical practice. It must be also considered that although RHC is considered the “gold-standard” method for evaluation of cardiac and vascular pressures, this technique is not free from technical limitations. The use of fluid-filled catheters for example is not as accurate as micromanometer systems by presenting relatively low frequency response.^{68,87} Nonetheless, fluid-filled systems are widely used in clinical applications because of their reduced cost and versatility provided by the multi-lumen configuration. Furthermore, although particular care was taken in zeroing the transducer system at the level of the right atrium, even small calibration errors could have resulted in significant measurement errors in the pressure ranges investigated in this study.

Our finding of decrease of sPAP_D following sedation is in contrast with what was previously reported by Rhinehart et al.⁴⁹ However, the sedation protocol differed significantly between the two studies, therefore a direct comparison cannot be made. On the other hand, our findings are concordant with data from Stepien et al.⁸⁸ in which sedation with acepromazine significantly decreased sPAP_C, mPAP, and diastolic PAP. However, it must be recognized that there are relevant differences between our study and the one from Stepien et al. in which invasive pressure measurements before and after sedation were performed, and a significantly higher dose of acepromazine was administered. Nonetheless, our study indicates that the effect of sedation on sPAP_D should be taken into account when making clinical assessments of Doppler signals.

Although it was not a primary goal of this study, interesting findings arise also from the invasive hemodynamic data obtained from the 18 dogs affected by MMVD. Of particular interest is the low PCWP in all subject, independently from the presence of clinical signs, therapeutic regimen or ACVIM classification. This may indicate that dogs with MMVD may not have left atrial hypertension when stable in their disease status. In fact, the PCWP measured in the dogs of this study are compatible with what has been reported in healthy dogs sedated with a very similar protocol.⁸⁸ However, it is also possible that in this subset of patients, PCWP may not be a good representation of LA pressure.⁸⁹

The main limitation of this study is represented by the limited number of dogs enrolled. The perceived invasiveness of RHC discouraged many owners and therefore significantly slowed enrollment of cases. Nonetheless, our results are extremely similar to the one obtained in several human studies with hundreds of patients enrolled. Another limitation of this study is the narrow interval of pulmonary pressures investigated, with only one dog that would have fulfilled the definition of PHT reported by human guidelines. Therefore, we cannot infer whether the agreement between the two techniques is higher or lower at increased values of PAP. At the same time because of this limitation we can only make very limited inferences regarding the ability of sPAP_D to correctly diagnose PHT in patients affected by MMVD.

CONCLUSIONS

In conclusion, this study showed that Doppler echocardiographic estimates of PAP are inaccurate and present wide limits of agreement compared to invasively measured PAP in dogs affected by MMVD ACVIM stage B2 and C stable in their treatment regimen. This study supports the recommendations provided in the most recent guidelines published in veterinary medicine and human medicine, in which it is specified that a non-invasive diagnosis of PHT should not rely merely on TR velocities. Furthermore, a common sedation protocol used for patients with cardiac disease significantly reduces Doppler estimates of PAP, therefore the effect of sedation on this variable should be taken into account when performing clinical evaluations.

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